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# The Hybrid Origin of Two Cultivars of *Crocus* (Iridaceae) Analysed by Molecular Cytogenetics including Genomic Southern and *in situ* Hybridization

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The origin of the two common cultivars of *Crocus*, *C.* 'Stellaris' ($2n = 2x = 10$) and *C.* '*Golden Yellow*' ($2n = 3x = 14$) was investigated by fluorescent *in situ* hybridization using both total genomic DNA and cloned DNA sequences as probes. The clear differentiation between the chromosomes after genomic *in situ* hybridization supports the proposals of a hybrid origin of the cultivars and shows that they have the same parental genomes originating from *C. flavus* ($2n = 8$) and *C. angustifolius* ($2n = 12$). *C.* 'Stellaris' has four chromosomes of *C. flavus* origin and six chromosomes of *C. angustifolius* origin. *C.* 'Golden Yellow' has eight chromosomes of *C. flavus* origin and six chromosomes of *C. angustifolius* origin. The number and location of 18S–5·8S–26S rRNA genes on the chromosomes of the hybrids and of the parental species agree with the results from the genomic probings. Hybridization to Southern membranes also supports the hybrid origin of *C.* 'Golden Yellow'. 

**Key words:** Taxonomy, cytology, rDNA sites, *in situ* hybridization, Southern hybridization, *Crocus*.

## INTRODUCTION

The complex origins of the widely cultivated yellow-flowered crocus (Iridaceae) have been considered a number of times using morphology, and chromosome analyses (e.g. Maw, 1886; Bowles, 1924, 1952; Karasawa, 1937, 1940, 1943; Burtt, 1952; Brighton, Scarlett and Mathew, 1980; Mathew, 1982). The group includes the species *Crocus angustifolius* West. (syn. *C. susianus* Ker-Gawl., known in gardens as 'Cloth of Gold'), *C. flavus* West. (syn. *C. aureus* Sibth. & Smith) and the two cultivars, *C.* 'Stellaris' (*C.* × *stellaris* Haw.), and *C.* 'Golden Yellow' (often referred to *C. flavus*) (Fig. 1). Extensive nomenclatural, taxonomical and historical notes may be found in the references cited above. For the sake of uniformity, the nomenclature used throughout this paper will follow that proposed in our conclusions.

Historical records indicate that the species *C. flavus* (originating from the Balkans and western Turkey) was sent to the Dutch botanist Clusius in 1579 from Belgrade (Clusius, 1601) [although Maw (1886) suggests an even earlier introduction], while *C. angustifolius* (from southwest Russia) was sent to Clusius in 1587 from Constantinople (Istanbul, Turkey). Since then, both species have been widely cultivated in northern and western Europe. A number of varieties (today we would use the term cultivars) mainly of garden origin were described soon after the introductions. As early as 1873, Baker suggested that *C.* 'Stellaris' was of hybrid origin (*C. flavus* × *C. angustifolius*) (Baker, 1873). However, Maw (1886) did not support the idea as no *Crocus* hybrids had been documented by then. More recently, Bowles (1924, 1952) and Mathew (1982) accepted the suggestion that *C.* 'Stellaris' was of hybrid origin. One of the most prolific and desirable forms of *Crocus* found in cultivation is *C.* 'Golden Yellow'. The names 'Dutch Yellow' (Maw, 1886), 'Large Yellow', 'Yellow Giant', 'Yellow Mammoth', and 'Grote Gele' (Scheepen, 1991) seem to be synonyms, and Bowles (1924, 1952) suggested that Rea's (1665) discussion of the 'greatest yellow *Crocus*' may be an early reference to this cultivar. In accordance with the the International Checklist for Hyacinths and Miscellaneous Bulbs (Scheepen, 1991), we adopt the preferred name 'Golden Yellow' here. Although it has been widely referred to as *C. flavus* 'Golden Yellow' (e.g. Scheepen, 1991), Mathew (in Brighton *et al.*, 1980) suggested that it was of hybrid origin. However, there are no reports of attempts to produce artificial hybrids between *C. flavus* and *C. angustifolius*, which are the species proposed as parents.

The chromosome numbers of many *Crocus* species and cultivars, including *C. flavus* ($2n = 8$) and *C. angustifolius* ($2n = 12$), were most recently established by Brighton and her colleagues (see e.g. Brighton, Mathew and Marchant, 1973; Brighton, 1976). With respect to the chromosome number of the two cultivars, Mather (1932) found $2n = 10$ in *C.* 'Stellaris'. In agreement with this Karasawa (1940) reports 10 univalents at meiosis but did not, on the basis of his findings, conclude on the hybrid nature of *C.* 'Stellaris' (Karasawa, 1943). Karasawa (1943) reported $2n = 14$ for 'Golden Yellow', and in meiotic configurations found univalents, bivalents and trivalents and he suggested that 'Golden Yellow' could be triploid. This was later supported by Brighton *et al.* (1980) and Heywood (1983) who showed that *C.* 'Golden Yellow' ($2n = 14$) has a bimodal karyotype. Based on their analysis, they proposed that it was triploid with eight large chromosomes originating from *C. flavus*

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FIG. 1. *Crocus* species and cultivated hybrids. Upper left: *C. flavus* (ex Macedonia). Upper right: *C. angustifolius*, plant and open flower (ex Taurica). Lower left: *C.* 'Stellaris' (ex Tubergen, NL). Lower right: *C.* 'Golden Yellow' (ex RVAU). Natural size.

($2n = 8$) and six smaller chromosomes from *C. angustifolius* ($2n = 12$).

The two yellow-flowered species and the two cultivars have been illustrated by various artists scattered in journals and books since the 18th century, in sources that today are very rare. Furthermore, the colours and morphological characters have not always been correctly reproduced, and the four *Crocus* have never been illustrated on the same plate. In Fig. 1 the four crocuses are shown in natural size and colours.

As yet, the methods of molecular cytogenetics have not been applied to members of the genus *Crocus*. *In situ* hybridization using probes of total genomic DNA is a valuable method to test for homology between genomes of plants and may identify the origin of whole parental genomes (e.g. Schwarzacher *et al.*, 1989; Bennett, Kenton and Bennett, 1992; Ørgaard and Heslop-Harrison, 1994) or chromosomes of uncertain origin in hybrid-derived plants (e.g. Friebe *et al.*, 1992; Schwarzacher *et al.*, 1992). Southern hybridization of genomic DNA to enzyme-digested DNA gives further information about relationships between species and hybrids (Anamthawat-Jónsson *et al.*, 1990; Ørgaard and Heslop-Harrison, 1993). The data can be combined usefully with that from karyomorphological analysis.

In the present work, we use genomic DNA:DNA *in situ* and Southern hybridization to examine relationships and genome organization in various *Crocus* species, and the origin of *C.* 'Stellaris' and the triploid *C.* 'Golden Yellow'.

## MATERIALS AND METHODS

### *Plant material, cloned and genomic DNA*

*C.* 'Golden Yellow' [C 7, cult. The Royal Veterinary and Agricultural University (RVAU), Copenhagen, Denmark] and *C.* 'Stellaris' (C 204, ex Potterton & Martin, UK) from commercial sources were used for root-tip fixations. *C. flavus* (1248/26) and *C. angustifolius* (1248/17) were obtained from long-standing displays in the Botanical Garden in Copenhagen, although their exact origin is unknown (*C. flavus* seems to have been obtained via RVAU, Copenhagen, from a commercial source in The Netherlands around 1925, while *C. angustifolius* seems to have been growing in the Botanical Garden at least since 1842; (F. Arnklit, pers. comm.). Genomic DNA from *C. chrysanthus* (Herb.) Herb. 'Goldilocks' (C 167, ex P. B. van Eeden, The Netherlands) was used as blocking DNA to reduce cross-hybridization in some experiments. DNA from *C. ancyrensis* (Herb.) Moore (C 32, commercial source, cult. RVAU, Copenhagen) and *C. biflorus* Miller ssp. *alexandri* (Velen.) B. Mathew (C 40, commercial source, cult. RVAU, Copenhagen) was used for restriction digestions.

The ribosomal rDNA sequence pTa71 used as a probe in both *in situ* and Southern hybridizations, contains a 9 kb *Eco*RI fragment of rDNA isolated from wheat, *Triticum aestivum* (L.) Thell. (Gerlach and Bedbrook, 1979; recloned and provided by R. B. Flavell and M. O'Dell) with the coding sequence for the 18S, 5·8S and 26S rRNA genes and the non-transcribed spacer sequences. Genomic DNA was isolated from young flowers using a CTAB protocol (modified after Howland, Oliver and Davy, 1991). When used as a probe, genomic DNA was sonicated to a length of 1–5 kb before labelling. Blocking DNA was prepared by sonicating total genomic DNA for 45 s or autoclaving for 5 min at 103·5 kPa, resulting in short fragments of DNA (100–300 bp long).

### *Southern hybridization*

Standard methods following Anamthawat-Jónsson *et al.* (1990) were used with the non-radioactive ECL (Amersham, UK) system for probe labelling and detection of hybridization sites. Genomic DNA was digested to completion using *Eco*RI, *Dra*I, *Hae*III or *Bam*HI restriction endonucleases. DNA fragments, with a *Hind*III-digest of lambda as a size marker, were separated by gel electrophoresis in 1·2% or 1·5% agarose gels stained with ethidium bromide. Both cloned (pTa71) and genomic DNA was used as probes with a hybridization stringency of 80%. Blots were reprobed several times after removal of probe by washing in ECL detection reagents before rehybridization.

### In situ *hybridization*

Plants for root tip preparation were grown in a growth cabinet at 5 °C. Root tips were pretreated in ice-water for 22–24 h, fixed in freshly made, cold 3:1 (v/v) ethanol–glacial acetic acid for 2–4 h at room temperature before transfer to 4 °C for at least 6 h and partially digested in a mixture of 2% cellulase (Onazuka R10, Serva) and 20% liquid pectinase (from *Aspergillus niger* Van Tiegh., Sigma) in enzyme buffer for 75–90 min at 37 °C. The soft meristematic tissue was squashed in 45% acetic acid as described by Schwarzacher *et al.* (1989). Before hybridization, chromosome preparations were treated with pepsin solution (5 $\mu$g ml$^{-1}$ in 0·01 M HCl; 200 $\mu$l per slide), covered with a plastic cover slip and incubated for 5 min at 60 °C. To reduce background hybridization due to cellular RNA, chromosome preparations were treated with 200 $\mu$l per slide of 1:10 (w/v) RNase in 2 × SSC (0·3 M sodium citrate and 0·3 M sodium chloride), covered with a plastic coverslip, and incubated for 1 h at 37 °C. Slides were then washed twice in 2 × SSC for 10 min, post-fixed in freshly depolymerized 4% (w/v) paraformaldehyde in water for 10 min, washed in 2 × SSC for 10 min, dehydrated in a graded ethanol series (70, 85 and 96%), 2 min each and finally air dried.

Genomic DNA probes were labelled with digoxigenin-11-dUTP (Boehringer Mannheim) or with biotin-11-dUTP (GIBCO-BRL) by nick translation (Schwarzacher *et al.*, 1989). The probe pTa71 was labelled directly with Rhodamine-4-dUTP (FluoroRed, Amersham).

Immediately prior to *in situ* hybridization, probes were mixed to a final concentration of 5 $\mu$g ml$^{-1}$ in a solution of 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0·1% (w/v) SDS (sodium dodecyl sulphate), and 2 × SSC (see Heslop-Harrison *et al.*, 1991). Hybridization stringency and the amount of probe(s) and blocking DNA in the

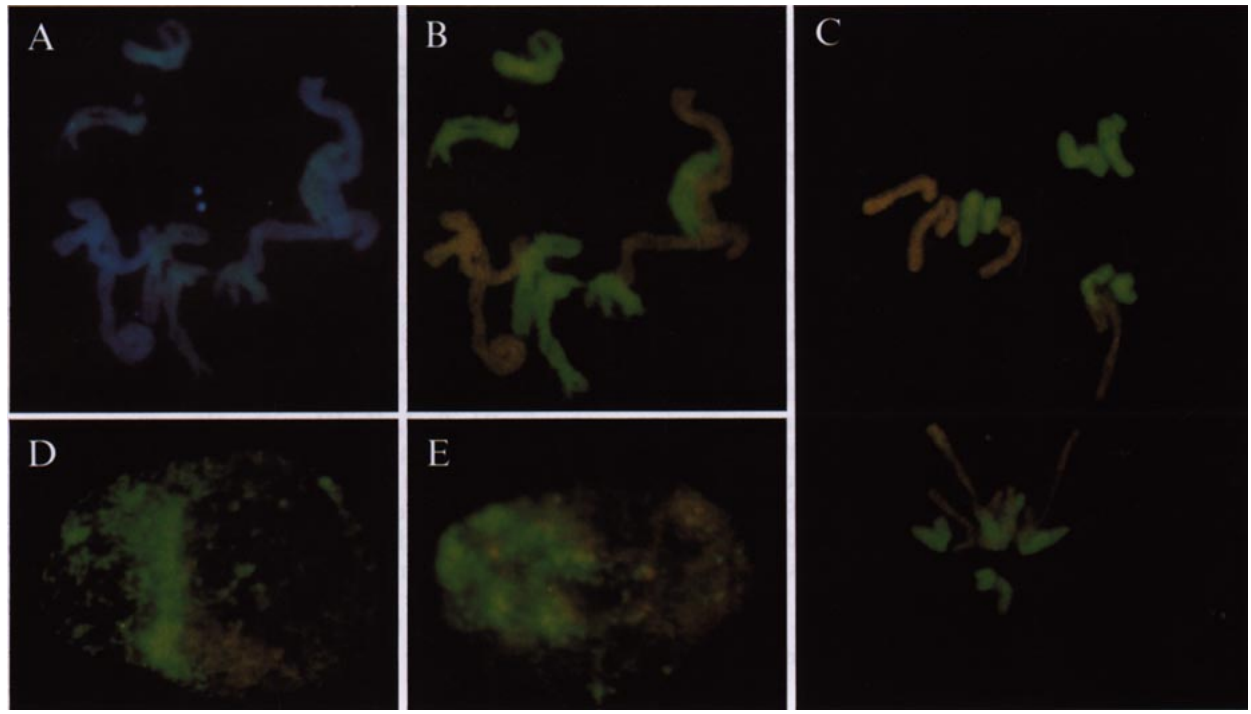


FIG. 2. Double target *in situ* hybridization to a chromosome preparation from a root-tip of *Crocus* 'Stellaris' ($2n = 2x = 10$). Genomic DNA from *C. flavus* ($2n = 8$) was labelled with Cy3 (detected yellow) while DNA from *C. angustifolius* was labelled with digoxigenin (detected green). Unlabelled genomic DNA from *C. chrysanthus* 'Goldilocks' was applied as blocking DNA. A, DAPI-staining for DNA showing chromosome morphology. At early metaphase (B) and anaphase (C) chromosomes originating from each parent can be easily distinguished as four large chromosomes from *C. flavus* and six smaller chromosomes from *C. angustifolius*. At interphase (D and E) there is a polarity in the nuclei in which groups of chromosomes from both genomes occupy discrete, non-intermixed, domains in the nuclei (seen as non-overlapping yellow and green domains in D and E). All micrographs × 1100.

hybridization mixture were modified in each experiment. The probe mixture for genomic *in situ* hybridization included unlabelled blocking DNA from a related species which was not used as probe. The amount of blocking DNA per slide was 30–80 times that of the probe concentration.

The probe mixture was denatured at 70 °C for 10 min, then 30–40 $\mu$l were loaded onto the slide preparation and covered with a plastic coverslip. Combined denaturation of slides and probes was conducted using a programmable temperature controller (Hybaid Omnislide, London). The preparations were denatured for 5 min at 80 °C. Then hybridization was carried out overnight at 37 °C. After hybridization, slides were washed in 2 × SSC for 5 min at 40 °C and then given a stringent wash in 20% formamide in 0·1 × SSC for 10 min at 42 °C to remove unhybridized or weakly hybridized probe DNA. The wash allows DNA sequences with more than 85% homology to the probe to remain hybridized. The formamide wash was followed by two washes in 2 × SSC for 5 min at 40 °C, and two washes in 2 × SSC at room temperature and then in 4 × SSC, 0·2% (v/v) Tween-20.

The digoxigenin at the hybridization sites on the chromosomes was detected using the fluorochrome FITC (fluorescein isothiocyanate) conjugated to sheep anti-digoxigenin antibody (Boehringer Mannheim). Detection of biotin-labelled DNA using streptavidin-Cy3 (Sigma) was carried out simultaneously (Leitch, Leitch and Heslop-Harrison, 1991). Slides were treated with 5% (w/v) BSA (bovine serum albumin) in 4 × SSC-Tween-20, 200 $\mu$l per slide for 5 min at room temperature, and then with a 1:400 dilution of streptavidin-Cy3 and digoxigenin-FITC in 5% (w/v) BSA buffer for 1 h, 30 $\mu$l per slide, covered with plastic coverslips and incubated for 1 h at 37 °C in a humid chamber. After incubation, slides were washed three times in 4 × SSC-Tween-20, 5 min each, at 37 °C.

After a brief wash in distilled water and air drying, the preparations were counterstained for 10 min at room temperature with the fluorochrome DAPI (4′, 6-diamidino-2-phenylindole; 2 $\mu$g ml$^{-1}$) in McIlvaine's citrate buffer (0·01 M citric acid, and 0·08 M sodium hydrogen phosphate, pH 7·0), 100 $\mu$l per slide, and washed briefly in 4 × SSC-Tween-20. Each preparation was mounted in about 100 $\mu$l antifade solution (AF1, Citifluor) to reduce fading of fluorescence.

The fluorescence signal was examined with a Leitz epifluorescence microscope with filter sets A, I2/3, N2 and an Omega Optical (Brattleboro, Vermont) triple bandpass filter set. Photographs were taken on Fujicolor Super HG 400 colour print film. The DAPI fluorescence was always photographed, but is not shown in all figures.

## RESULTS

### *Morphology of plants*

C. flavus (Fig. 1, upper left). The tunic is membranous, splitting from the base into parallel fibres, upwards continuing into a brown, persisting tube. The flower is pale to orange yellow, outside mostly without any lines or suffusion at the base, the segments are 20–35 mm long, oblanceolate, obtuse to subacute, giving the flower a somewhat pointed appearance; the flower tube is yellow to pale yellow.

C. angustifolius (Fig. 1, upper right). The tunic consists of coarsly reticulate fibres, splitting or united at the base, upwards with irregular sharp points. The flower is yellow to orange, outside mostly deeply brown feathered to heavily suffused, segments 20–35 mm long, elliptic to oblanceolate, subacute, reflexed shortly after anthesis (Fig. 1); flower tube brownish.

C. '*Stellaris*' (Fig. 1, lower left). The tunic is coriaceous with parallel veins and no splitting at the base, reticulated upwards, uniting into a few teeth. The flowers are yellow to orange, lighter yellow than in *C. angustifolius*, outside with three to five feathered, brown lines, segments 20–35 mm long, elliptic, subacute, giving the flower an elongate appearance; flower tube brownish. The morphology of, e.g. the tunic of the corm and the colours of the flowers in *C.* 'Stellaris' is intermediate between *C. flavus* and *C. angustifolius*.

C. '*Golden Yellow*' (Fig. 1, lower right). The tunic is membranous in the upper part, upwards continuing into a shorter or longer tube or a few points, and almost parallel fibres towards the base. The flowers are yellow, outside with 1–3(–5) short, greyish lines at the base, segments 35–45 mm long, obovate, obtuse, giving the flower a rounded appearance; flower tube whitish. *C.* 'Golden Yellow' is larger in all parts, e.g. corm and flower, than *C. flavus*, *C. angustifolius*, and *C.* 'Stellaris'. The morphology of, e.g. the tunic of the corm and the colours of the flowers in *C.* 'Golden Yellow' is more like *C. flavus* than *C. angustifolius*.

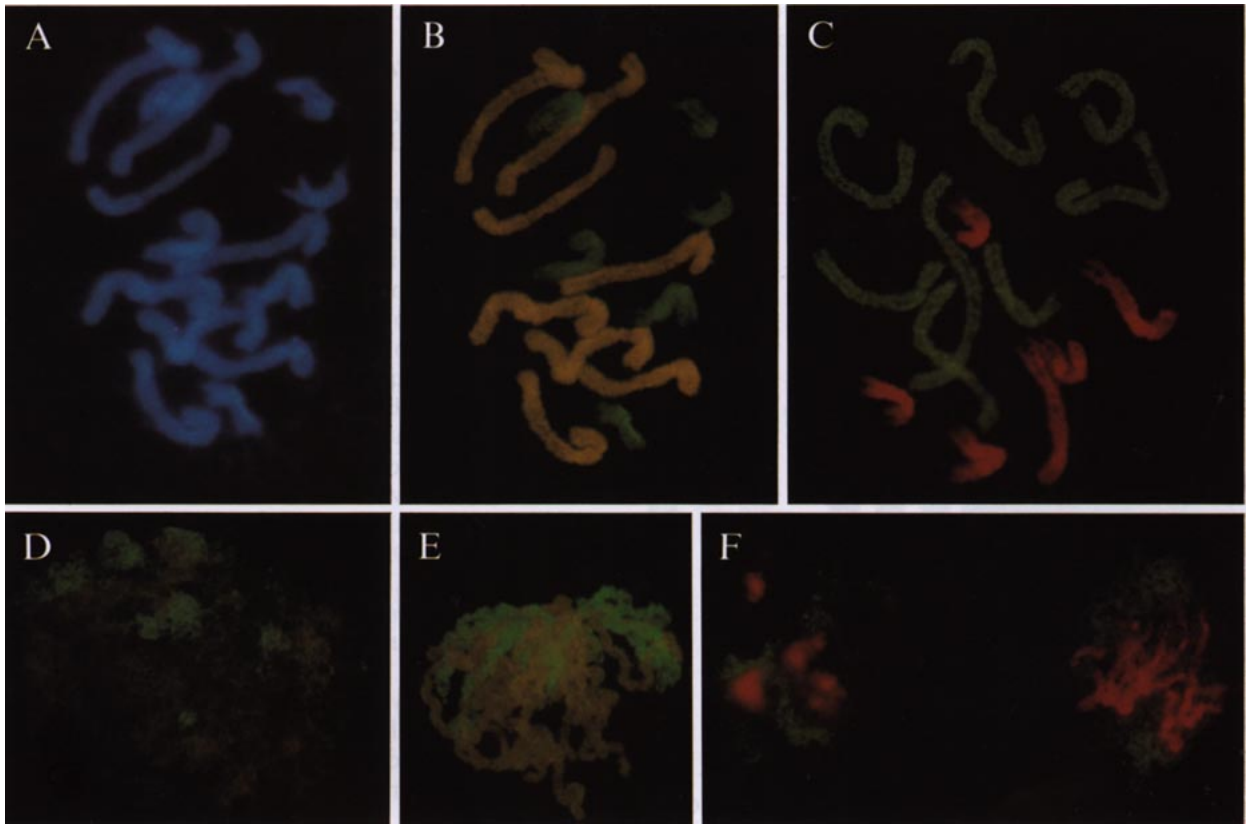


FIG 3. Double target *in situ* hybridization to root-tip chromosomes of *Crocus* 'Golden Yellow' ($2n = 3x = 14$). A, DAPI staining showing eight large and six smaller chromosomes. B, *In situ* hybridization of genomic Cy3-labelled DNA from *C. flavus* ($2n = 8$) and digoxigenin labelled DNA from *C. angustifolius* ($2n = 6$) with cross-hybridization reduced by inclusion of excess unlabelled total genomic DNA from *C. chrysanthus* 'Goldilocks' (40 times the total amount of probe DNA). The *C. flavus* probe DNA hybridize uniformly to eight of the chromosomes (yellow labelling) while the *C. angustifolius* probe hybridize to six, generally smaller chromosomes (green labelling). C, *In situ* hybridization of digoxigenin-labelled total genomic DNA from *C. flavus* and Cy3 labelled DNA from *C. angustifolius* and unlabelled blocking DNA from *C. chrysanthus* 'Goldilocks' (20 times the total amount of probe DNA). Eight of the chromosomes fluoresce green as they hybridize to the *C. flavus* probe while six of the chromosomes fluoresce red hybridizing to the *C. angustifolius* probe. Interphase nuclei (D), and prophase (E) from the *in situ* hybridization shown in (B), and (F) is an interphase nuclei after the *in situ* hybridization shown in (C). The chromosomal DNA occupies defined non-intermixed domains. All micrographs × 1100.

In situ *hybridization*

C. '*Stellaris*'. *In situ* hybridization of labelled genomic DNA from the species *C. flavus* and/or *C. angustifolius*, in the presence of excess unlabelled genomic DNA from *C. chrysanthus*, gave a clear discrimination of two sets of chromosomes (Figs 2 and 6) in root tip chromosome preparations from *C.* 'Stellaris'. At metaphase, the four larger chromosomes labelled with *C. flavus* DNA, while the six smaller chromosomes labelled with *C. angustifolius* DNA. The discrimination was particularly clear when probes from the two genomes were differentially labelled (Fig. 2). Probe hybridization was uniform throughout the length of the chromosomes. At interphase, separation of the differentially probed chromosomes was evident and the two sets of chromosomes, although decondensed, were apparently not intermixed (Fig. 2D–E). In general, the chromatin probed with *C. flavus* DNA tended to associate with the nucleolus.

The ribosomal probe pTa71 revealed three major rDNA sites in *C.* 'Stellaris', one intercalary on a chromosome which probed with *C. flavus* DNA and two sites on distal regions of non-labelled chromosomes (Fig. 6).

C. '*Golden Yellow*'. After *in situ* hybridization of genomic DNA to the cultivar *C.* 'Golden Yellow', two chromosome sets could be clearly distinguished at metaphase (Fig. 3A–C), interphase (Fig. 3D, F), and prophase (Fig. 3E, F) by simultaneous probing with differently labelled total genomic DNA from *C. flavus* and *C. angustifolius* after competitive blocking with unlabelled total genomic DNA from *C. chrysanthus*. Genomic DNA from *C. flavus* hybridized to the eight long chromosomes, and genomic DNA from *C. angustifolius* to the six shorter chromosomes. In different experiments, reciprocal probe/label combinations were used (c.f. Fig. 3B and C; 3D, E and F); both combinations gave clear discriminations.

As in *C.* 'Stellaris', the chromosomes showed uniform probe hybridization. The chromosomes of *C. angustifolius* were in more defined domains in this hybrid and chromosomes originating from the two genomes were not intermixed at interphase.

The rDNA probe pTa71 hybridized to four chromosomes showing two strong and two minor signals (Fig. 7). The two strong pTa71 probe sites were localized at intercalary positions on two of the *C. flavus* chromosomes (long yellow chromosomes). The two minor sites were localized terminally on chromosomes of *C. angustifolius* origin (short green chromosomes).

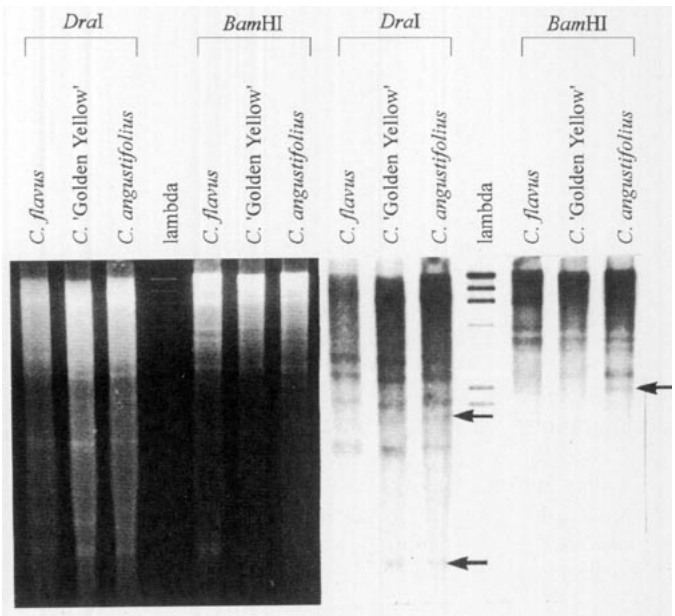


FIG. 4. *Southern hybridization of total genomic DNA from Crocus angustifolius to restriction enzyme digests of C. flavus, C.* 'Golden Yellow' *and C. angustifolius.* A, Ethidium bromide-stained gel showing equal loading of DNA on all *Crocus* lanes and the large number of repetitive DNA families giving restriction fragment bands on the gel. Most bands are apparently present in both species, although there are some differences in presence or strength of minor bands in both *DraI* and *Bam*HI digests. B, The DNA shown in (A) after transfer and probing. Again, there are no conspicuous differences between the lanes, although the strength of hybridization to the *C. angustifolius* lanes is the greater and hybridization sites are not visible in the *C. flavus* lanes (arrows). Centre: lambda *Hind*III size markers from top: 23·1, 9·4, 6·6, 4·4, 2·3, 2·0 kb.

*Hybridization to Southern membranes*

In ethidium bromide-stained gels of size-fractionated DNA digested with restriction enzymes *Dra*I and *Bam*HI (3 $\mu$g per lane), many repetitive DNA families gave restriction fragment bands. Most bands were present in both species and in *C.* 'Golden Yellow', but some bands were only present in one of the species and in *C.* 'Golden Yellow', other bands varied in strength between the lanes (Fig. 4A). After Southern hybridization using labelled total genomic DNA from *C. angustifolius* (5 ng cm$^{-2}$) all lanes showed almost equally strong hybridization (Fig. 4B). Strongly probed bands of major satellite families were visible overlying the smear of DNA hybridization. Some of the fragments in both digests were specific to *C. flavus* and *C.* 'Golden Yellow'.

Figure 5 shows the hybridization of total genomic DNA from *C. flavus* to *Dra*I and *Hae*III digests of genomic DNA from *C. flavus*, *C.* 'Golden Yellow', *C. angustifolius*, *C. biflorus* ssp. *alexandrii*, *C. chrysanthus*, and *C. ancyrensis*. The banding pattern in *C. flavus*, *C.* 'Golden Yellow' and *C. angustifolius* is essentially the same, but this pattern differs considerably from the patterns found in the reference species. In the *Hae*III digests, a 5 kb band is present only in *C. flavus* and *C.* 'Golden Yellow', but not in *C. angustifolius*. The banding pattern of *C. ancyrensis* differs strongly from that of the other species examined showing restriction fragments of different sizes.

## DISCUSSION

The cultivars are characterized morphologically as being more or less intermediate between the parental species. Although the cultivars have the same parental origin their appearance is different, presumably because of the con-

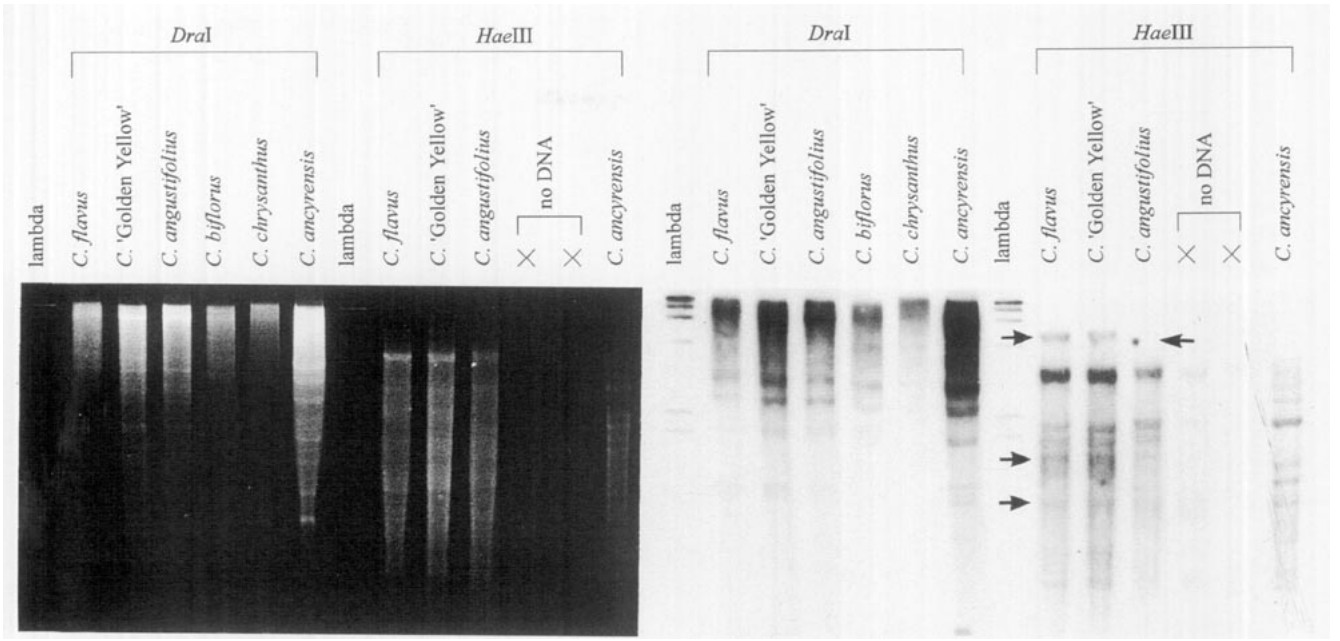


FIG. 5. Southern hybridization of total genomic DNA from *Crocus flavus* to restriction enzyme digests of *C. flavus*, *C.* 'Golden Yellow', *C. angustifolius*, *C. biflorus*, *C. chrysanthus* and *C. ancyrensis*. A, Ethidium bromide-stained gel showing unequal loading of DNA on lanes. Although all lanes have many restriction fragment bands, the patterns from *C. flavus*, *C.* 'Golden Yellow' and *C. angustifolius* (which are similar) differ from the other species. B, The DNA shown in (A) after transfer and probing. The differences between the species groups noted in (A) are emphasized. A 5 kb fragment differing between *C. flavus* and *C. angustifolius* is arrowed. Centre: lambda *Hin*dIII size markers as Fig. 4.

tribution of two sets of chromosomes from *C. flavus* in *C.* 'Golden Yellow' producing a hybrid morphologically closer to *C. flavus* than to *C. angustifolius*.

*In situ* hybridization using labelled total genomic DNA from *C. flavus* and *C. angustifolius* showed that the four large chromosomes of *C.* 'Stellaris' and the eight large chromosomes of *C.* 'Golden Yellow' share a common origin from *C. flavus*, while the six small chromosomes of both *C.* 'Stellaris' and *C.* 'Golden Yellow' apparently originate from *C. angustifolius*. The uniformity of labelling would not be expected if other species were involved. Repetitive sequences which give a hybridization signal using genomic *in situ* hybridization may evolve rapidly and hence use of a probe from a related species onto chromosomes often reveals both negative and positive bands along chromosomes (Anamthawat-Jónsson, Schwarzacher and Heslop-Harrison, 1993; Ørgaard and Heslop-Harrison, 1994). Thus, the present work confirms the hybrid origin of *C.* 'Stellaris' and *C.* 'Golden Yellow' and supports the hypothesis that the donors of the parental genomes are *C. flavus* and *C. angustifolius*.

The genome size of species of *Crocus* is large [the C value is 11·6 pg in *C. vernus* Hill (Bennett and Smith, 1976; Bennett, Smith and Heslop-Harrison, 1982)] and the present work is on the largest genome and chromosome size for which genomic *in situ* hybridization has been reported. As found in species of both Poaceae (Schwarzacher *et al.*, 1989) and Solanaceae (Parokonny *et al.*, 1992; Kenton *et al.*, 1993), the chromosomes of both *Crocus* genomes show remarkably uniform labelling with their own DNA without major unlabelled or strongly labelled sites. No translocations could be found between chromosomes of the two genomes. The hybrids are entirely propagated vegetatively so trans locations could be stably maintained.

When probing total genomic DNA to Southern blots, hybridization between highly repeated DNA restriction fragments on the blot and repeated sequences in the probe show bands corresponding to repetitive sequences in common between the probe and the target DNA. Analysis of the bands shows that the DNA restriction fragments differ among the limited number of species investigated here. Figures 4 and 5 show that the genomic DNA sequence composition of *C. flavus* and *C. angustifolius* is quite similar and differs only in a few bands. These may represent species-specific sequences or conserved regions of repetitive sequences, the substructure of which differs between species by alteration of particular restriction sites. The overall strength of hybridization is almost equal in all species investigated, indicating that major parts of the genome are conserved among a number of *Crocus* species. However, application of blocking DNA would probably have demonstrated some differentiation between the species investigated (Ørgaard and Heslop-Harrison, 1994).

Restriction maps of the intergenic spacer in the ribosomal genes have been used to examine relationships between species of the tribe Triticeae (Poaceae) (Molnar *et al.*, 1989). Although there are some discrepancies compared with generally accepted relationships (for example, the separation of *Hordeum bulbosum* L. from other species of the genus *Hordeum*), the restriction patterns generally correlate with presumed taxonomic relationships. The similar patterns of *C. flavus* and *C. angustifolius*, and the considerable difference between their patterns and that of *C. ancyrensis* is likely to indicate the closer relationship of the former species. In

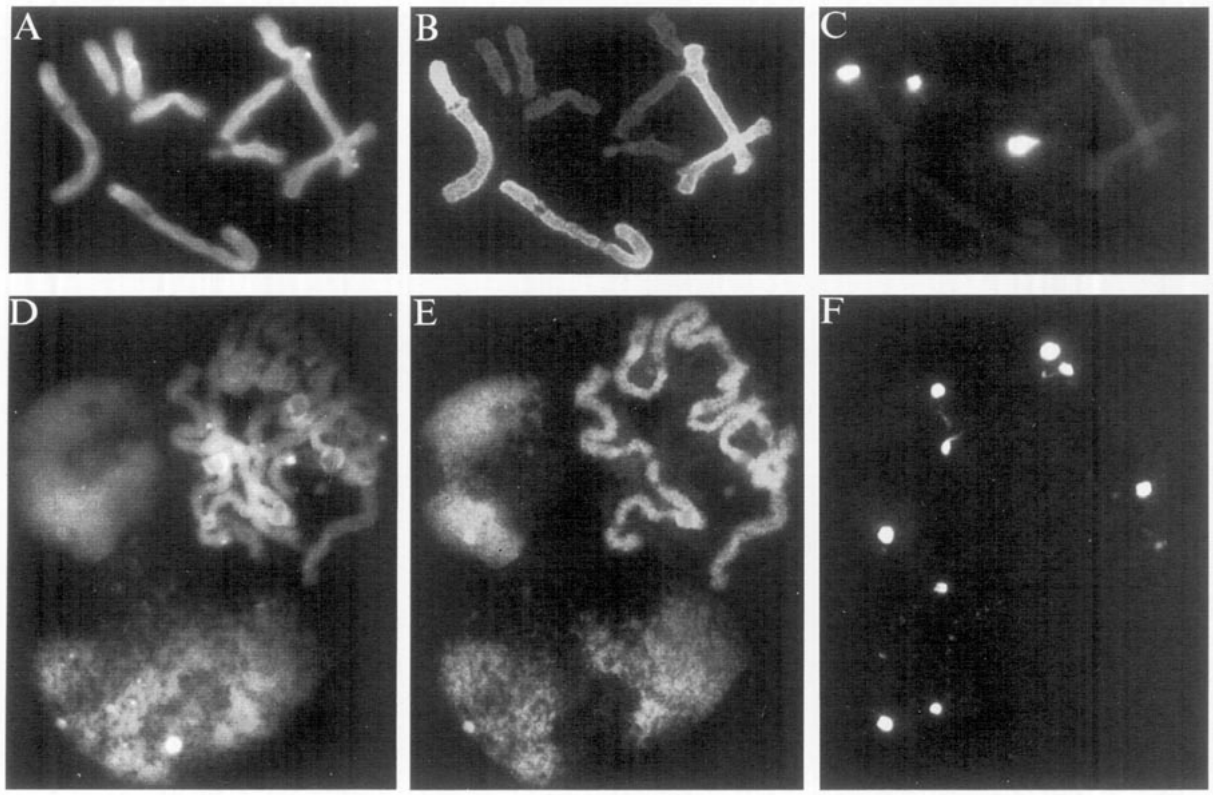


FIG. 6. *In situ* hybridization to a metaphase of *Crocus* 'Stellaris' probed with genomic DNA of *C. flavus* and a ribosomal DNA probe. A, D, DAPI stained nuclei and (A) metaphase showing all 10 chromosomes. B, FITC labelled metaphase showing brightly labelled chromosomes originating from *C. flavus*. C, rDNA hybridization showing one site on a labelled *C. flavus* origin chromosome and two sites (of different sizes) on unlabelled *C. angustifolius* origin chromosomes. E, Prophase and interphase nuclei showing parental genomic domains when probed with *C. flavus* probe. F, Three rDNA sites are seen in each interphase or prophase nucleus. In all three nuclei, the parts of the two rDNA loci on *C. angustifolius*-origin chromosomes are more dispersed, indicating they are preferentially expressed.

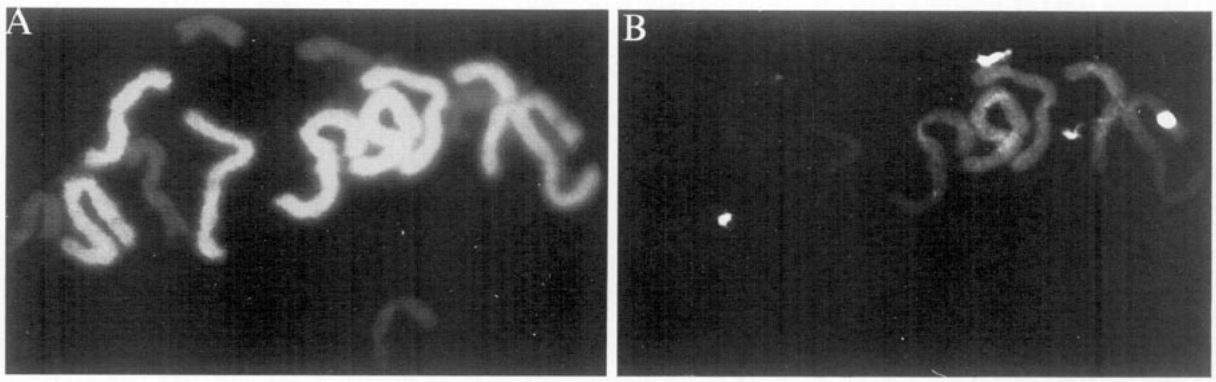


FIG. 7. *In situ* hybridization to a metaphase of *Crocus* 'Golden Yellow' probed with genomic DNA of *C. flavus* and a ribosomal DNA probe. A, After hybridization with FITC-detected *C. flavus* probe, eight chromosomes originating from *C. flavus* are brightly labelled while the other six chromosomes are weakly labelled. B, rDNA hybridization shows two sites on *C. flavus* origin chromosomes and two sites on *C. angustifolius* origin chromosomes.

contrast, Mathew (1982) regards *C. ancyrensis* as closely related to *C. angustifolius*. The two species have similar reticulate tunicea, but this character may have arisen independently. The number of suitable morphological characters within the genus is limited making molecular data valuable.

The origin of the triploidy of *C.* 'Golden Yellow' is unknown. A cross between a tetraploid *C. flavus* and a diploid *C. angustifolius* could explain the triploid hybrid, but tetraploid *C. flavus* is not known [however, Propach (1939) and Karasawa (1943) do mention $2n = 16$ for *C. flavus* as an unpublished result without further docu-

mentation]. Therefore, the most probable explanation is that *C. flavus* provided an unreduced, 2*n*, gamete. Although it is well known that maternal inheritance of plastids is not universal (Zhu, Mogensen and Smith, 1991), one could look at chloroplast DNA to see which parent contributed the plastids in order to elucidate whether a *C. flavus* gamete was female or male.

Spontaneously occurring triploids as the result of unreduced gametes have been recorded in a number of plants, and seem to be more frequent than usually assumed, e.g. *Cryptocoryne* (Jacobsen, 1977), *Hordeum* (Sandfaer, 1975), and *Lilium* (Noda, 1986; Noda and Schmitzer, 1990). Stefani (1986) investigated the $F_1$ hybrid between *Triticum durum* Desf. and *Haynaldia villosa* Schur. and found unreduced male as well as female gametes. In *Lilium* hybrids a number of cases of spontaneously occurring unreduced gametes have been recorded (e.g. Tuyl and Kwakkenbos, 1989; Tuyl, 1990).

The hybrid origin of the two yellow flowered spring crocuses, means that the orthographic writing of their names should be *C.* 'Stellaris' for the diploid hybrid, if regarded as a cultivar of garden origin, or *C.* × *stellaris* Haw. if of natural origin. Because the two parental species do not occur together in nature, natural hybrids are unlikely to exist, but spontaneous hybridization has probably occurred in cultivation. We therefore recommend the designation *C.* 'Stellaris', implying that it is of garden origin. Similarly, we recommend the name *C.* 'Golden Yellow' for the triploid hybrid.

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