Repetitive DNA sequences in *Crocus vernus* Hill (Iridaceae): The genomic organization and distribution of dispersed elements in the genus *Crocus* and its allies

S. Frello and J.S. Heslop-Harrison

Abstract: Eight clones of repetitive DNA were isolated from *Crocus vernus* Hill. The genomic organization of the clones was analyzed by in situ hybridization to *C. vernus* and Southern hybridization to a range of *Crocus* and other species. Seven clones were used for in situ hybridization. Sequence analysis showed that all eight clones were nonhomologous, and thus represented eight different sequence-families. In situ hybridization showed that six were dispersed in high copy numbers on all chromosomes of the *C. vernus* genome, whereas one was localized proximal to the secondary constriction, at the NOR (nucleolar organizer region) and was not further analyzed, as it was considered part of the 18S–25S rDNA repeat. Except for short palindromes, none of the sequences showed notable internal structures. Clone pCvKB4 showed homology to the reverse transcriptase gene of Ty1-*copia*-like retrotransposons; the others showed no homology to known sequences. When used as probes for Southern hybridization, four showed a ladder of 3–4 bands superimposed by irregular patterns, indicating organization in short tandem arrays. Each clone had a unique distribution among *Crocus* species (12–16 species analyzed with each clone) and six species of Iridaceae, Liliaceae, and Amaryllidaceae; all seven investigated sequences were Iridaceae specific and four were *Crocus* specific. The species distribution of these seven clones showed notable discrepancies with the taxonomic subdivision of the genus at the subgenus, section, and series levels. The results suggest that the phylogeny and taxonomic structure of the genus *Crocus* might need reconsideration. The analysis of repetitive DNA as a major and rapidly evolving part of the genome could contribute to the study of species relationships and evolution.

Key words: phylogeny, evolution, in situ hybridization, sequence analysis, dispersed elements.

Résumé : Huit clones d'ADN répétitif ont été isolés du *Crocus vernus* Hill. L'organisation génomique de ces clones a été analysée par hybridation in situ chez le *C. vernus* et par hybridation Southern chez une gamme d'espèces du genre *Crocus* et d'autres encore. Sept clones ont été employés en hybridation in situ. Les analyses de séquences ont montré que les huit clones étaient nonhomologues et représentaient ainsi des familles de séquences distinctes. L'hybridation in situ a montré que six des séquences étaient dispersées en grand nombre sur tous les chromosomes du *C. vernus* tandis qu'une séquence était située du côté proximal de la constriction secondaire, à l'organisateur nucléolaire (NOR), et n'a donc pas été analysé davantage étant considérée comme faisant partie de la répétition 18S–25S. À l'exception de courts palindromes, aucune des séquences ne montrait de structure interne notable. Le clone pCvKB4 montrait de l'homologie au gène codant pour la transcriptase inverse des rétrotransposons de type Ty1-*copia*. Les autres clones ne montraient de l'homologie à aucune séquence connue. Lorsque ces clones ont été employés comme sondes lors d'hybridations Southern, quatre clones ont produit des échelles de trois à quatre bandes superposées de motifs irréguliers, cela étant indicatif d'une organisation en courtes suites de répétitions en tandem. Chaque clone avait une distribution unique parmi les espèces de *Crocus* (12 à 16 espèces examinées selon le clone) et parmi six espèces d'iridacées, de liliacées et d'amaryllidacées. Les sept clones étaient spécifiques aux iridacées et quatre d'entre eux étaient spécifiques au genre *Crocus*. La distribution des ces clones au sein des espèces a montré des différences par rapport aux subdivisions taxonomiques du genre *Crocus* aux niveaux du sous-genre, de la section et de la série. Les résultats suggèrent que la phylogénie et la structure taxonomique du genre *Crocus* pourraient nécessiter certaines remises en question. L'étude de l'ADN répétitif, en tant que composante du génome à la fois importante et en rapide évolution, pourrait contribuer à l'étude des relations interspécifiques et de l'évolution.

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Mots clés : phylogénie, évolution, hybridation in situ, analyse de séquence, éléments dispersés.

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Introduction

Crocus vernus Hill, with the two subspecies *C. vernus* subsp. *vernus* and *C. vernus* subsp. *albiflorus*, is a well-defined taxon distributed throughout most mountainous regions of Southern Europe. The species is morphologically variable and presents a range of karyotypes at the diploid and polyploid levels; all accessions have large genomes and very large chromosomes. *Crocus* taxonomy has until now been based primarily on morphology, taking chromosome numbers into consideration (Mathew 1982). Little is known about the genetics and genome structure of the genus and, based on data from other species groups, such information is likely to help understand the evolutionary processes, relationships, and diversity within the genus.

The major fraction of most plant genomes is made up of repetitive DNA sequences; short sequence motifs repeated thousands of times, which may be present as tandem arrays at a discrete number of genomic locations, or dispersed over much of the genome (Flavell 1986; Kubis et al. 1998). The genomic function of most repetitive sequences is largely unknown, but some are genes (e.g., the rDNA), and others are involved in chromosome segregation at the centromere, or stabilization of chromosome ends (Vershinin et al. 1995). It has been suggested that repetitive sequence accumulation might be greater in slow-growing, long-lived species than in species with faster development (Charlesworth et al. 1994). Although some repetitive sequences are highly conserved (the rRNA genes, for example), many repetitive DNA motifs are restricted in their distribution to single species, species groups, or genera.

A major class of dispersed repetitive elements, the retrotransposons, amplify through an RNA intermediate, are abundant in all living organisms (Flavell et al. 1992), and are well studied in plants (e.g., Kumar et al. 1997; Flavell et al. 1997). Retroelements alone may represent 50% of the total DNA in a plant genome (Pearce et al. 1996), although other abundant dispersed repeats with no known homology to retroelements have also been found in species such as barley (Busch and Hermann 1997) and sugar beet (Kubis et al. 1997). Avramova et al. (1995) suggest that some dispersed sequences function as matrix attachment regions (MARs) and influence gene transcription. The abundance of dispersed repeats makes investigation of their evolution, occurrence, organization, and structure important to our understanding of the plant genome. Because of the diversity and rapid evolution of repetitive sequences, they are potentially valuable markers in phylogenetic and biodiversity studies (de Bustos et al. 1996; Svitashev et al. 1994) and for the investigation of hybrids (Itoh et al. 1990).

As part of a project in *Crocus* cytology, phylogeny, and evolution (Ørgaard et al. 1995) we present here the investigation of multiple clones of repetitive DNA from *C. vernus*. The clones were sequenced and investigated with respect to genomic organization and physical distribution on the *C. vernus* genome, and their presence in various *Crocus* species as well as more distantly related genera.

Materials and methods

Plant material

Table 1 provides the details of the origins of the plant accessions used in this study. All accessions are grown at the institution indicated by the accession number.

DNA extraction and cloning

DNA was extracted from flowers (preferably just before opening) by standard methods (Sambrook et al. 1989). A recombinant DNA library was made from a *Sau*3AI partial digest of genomic DNA from *C. vernus* in the *Bam*HI site of pUC18. Highly repetitive cloned sequences were identified by probing a dot-blot of the clones with labelled genomic DNA. Selected clones were named (p for plasmid, Cv to designate the species, and KB for the karyobiology laboratory), labelled, and hybridized to Southern transfers of *Sau*3AI digests of DNA from *C. vernus*, and clones showing strong hybridization signals were used in further work. Selected clones were sequenced commercially and analyzed by in situ and Southern hybridization.

In situ hybridization

Preparation of young roots from corms and in situ hybridization followed the methods described by Schwarzacher and Heslop-Harrison (2000). Fresh root tips were incubated 24 h in ice water, fixed in alcohol : acetic acid (3:1) and digested for 40–50 min in a mixture of pectinase and cellulase, sometimes with the addition of 0.5% pectolyase. In situ hybridization implemented 40 ng of probe DNA labelled with biotin or digoxigenin in 40 µL hybridization buffer per slide. Preparations with denatured probe were denatured at 70°C for 8 min and allowed to hybridize overnight at 37°C. The most stringent wash following hybridization was either at 42°C in 0.1× SSC (20× SSC is 3 M NaCl, 0.3 M sodium citrate) and 20% formamide, allowing target sequences of more than 85% homology to remain hybridized (low stringency) or at 42°C in 0.032× SSC and 25% formamide, allowing target sequences of more than 95% homology to remain hybridized (high stringency).

Streptavidin-Cy3 and antidigoxigenin-FITC (fluorescein isothiocyanate) were used to detect probe hybridization sites, and preparations were counterstained with DAPI (4′,6-diamidino-2-phenylindole). Micrographs were made on 400 ASA Fuji Superia or SuperHG colour film with an epifluorescence microscope, digitized to Kodak PHOTOCD and processed with Adobe PHOTOSHOP, using only functions affecting the whole image equally.

Molecular analysis of DNA

Sequence homologies with other sequences were investigated by searching the GenBank and EMBL databases (release 103). Five micrograms DNA from the Iridaceae species and 15 µg DNA from each of the other species were digested with *Sau*3AI and size-separated on a 1% agarose gel. DNA was visualized with ethidium bromide and photographed to confirm equal loading before Southern blotting on Hybond N^+ membranes. Probe labelling and hybridization was performed with the ECL Random Prime method (Amersham) according to the manufacturer's instructions. X-ray film was used to visualize the signal. Each blot was used only once.

Results

In situ hybridization

When used for in situ hybridization, clones pCvKB3,

Table 1. Accession numbers and origin of plants used in this study.

Species	Acc. No.	Origin
Crocus vernus Hill	KVL, 95-7	Italy, Passo del Futta
	KVL, 95-70	Italy, Toscana, Passo Cereto
	KVL, 95-91	Switzerland, Col de Mosses
	KVL, 95-93	Switzerland, Rigi
	KVL, 95-114	Austrian Alps, Passo mille Croci
	KVL, 95-116	Germany, Imstal
	KVL, C 371	Yugoslavia, Ljuboten
C. kosaninii Pulevi	HBH, P1992/5284	Cultivated material; no specified origin
C. malyi Vis.	HBH, P1992/5296	Cultivated material; no specified origin
C. imperati Ten.	KVL, 95-44	Italy, Campania, Valico delle Croci
C. medius Balbis	KVL, s.n.	Italy, Mt. Bignone
C. kotchyanus Koch	KVL, C 78	Cultivated material; no specified origin
C. vallicola Herbert	KVL, 90-95	Turkey, Zigana Pass
C. cartwrightianus Herbert	KVL, C 336	CB, cultivated material; no specified origin
C. veluchensis Herbert	HBH, s.n.	Cultivated material; no specified origin
C. dalmaticus Vis.	HBH, s.n.	Cultivated material; no specified origin
C. chrysanthus Herbert	KVL, 93-39	Greece, Mt. Hortiatis
C. korolkowii Maw	KVL, C 170	Eeden, cultivated material; no specified origin
C. antalyensis Mathew	KVL, s.n.	Eeden, cultivated material; no specified origin
C. flavus Weston	KVL, C 49	Cultivated material; no specified origin
C. speciosus M. Beib.	KVL, C 79	Cultivated material; no specified origin
C. laevigatus Bory & Chaub.	KVL, 94-3	Greece, Crete, Lefka Ori
C. banaticus Gay	HBH, P1992/5240	Zw, cultivated material; no specified origin
Romulea bulbocodium (L.) Sebast. & Mauri	Kew, s.n.	Cultivated material; no specified origin
Iris magnifica Vved.	KVL, 86-0024	Cultivated material; no specified origin
Erythronium 'Pagoda'	KVL, 84-1838	Cultivated material; no specified origin
Tulipa turkestanica Regel	KVL, 86-0030	Cultivated material; no specified origin
Fritillaria imperalis L.	KVL, 84-1645	Cultivated material; no specified origin
Narcissus pseudo-narcissus L.	KVL, 91-1641	Cultivated material; no specified origin

Note: Different *C. vernus* accessions have been used both for Southern and in situ experiments with the different clones. Abbreviations: s.n., Without number; HBH, The Botanical Garden of the University of Copenhagen, Denmark; KVL, Royal Veterinary and Agricultural University, Copenhagen; Eeden, provided by P.W. van Eeden, The Netherlands; CB, Provided by Cambridge Bulbs, U.K.; Zw, provided by Zwanenburg Nurseries, Haarlem, The Netherlands.

pCvKB4, pCvKB5, pCvKB6, pCvKB9, and pCvKB10 showed a rather similar dispersed organization over the lengths of the chromosomes, with areas of slightly weaker or stronger hybridization (Figs. 1–3); these minor differences, seen on multiple metaphases, different accessions, and in both *C. vernus* subspecies, are described below. pCvKB2 hybridized to part of the 18S–25S rRNA locus, labelling much of the satellite and often part of the chromosome arm proximal to the satellite (Fig. 1*c*). pCvKB5 was used for in situ hybridization at two different stringencies: at the lower stringency (80–85%, Fig. 1*b*), it showed a dispersed pattern along the whole length of all chromosomes with small gaps at the centromeres and larger gaps (arrows) at the sites of the rDNA (pCvKB2, Fig. 1*b*, 1*c*) whereas a slightly less uniform signal was obtained at the higher stringency (95%, not shown). pCvKB3 showed strong hybridization to intercalary regions with less signal in telomeric and centromeric regions (results not shown). pCvKB4 (not shown) showed a dispersed distribution with gaps at the centromeres, stronger hybridization signals on both sides of the centromere of most chromosomes, and gaps at the rDNA loci. pCvKB6 (Fig. 3*b*) showed a more uniform hybridization pattern than pCvKB5, whereas pCvKB9 (Fig. 2*b*) hybridized slightly more strongly to some intercalary regions, with weaker hybridization at centromeres and some distal regions. pCvKB10 (Fig. 2*a*) was less uniform than other sequences, with some chromosomal regions and satellites showing stronger hybridization.

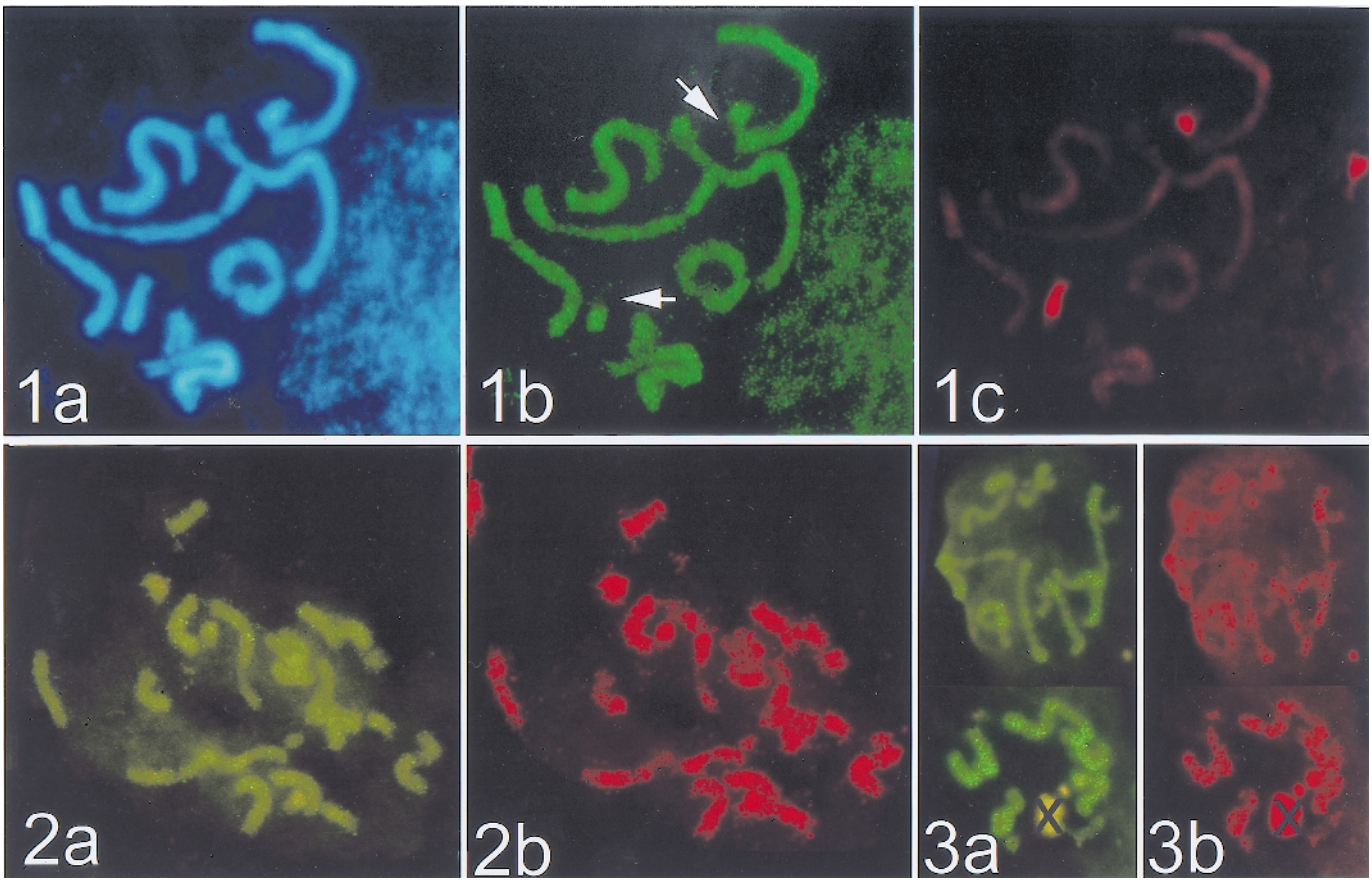
Sequence analysis

Table 2 summarizes information about eight nonhomologous sequences reported here, including their database accession numbers. pCvKB1 had an AT content of 45% (compared with an average of 57 ± 2.5% SD for the other sequences), and an unusual internal structure of 32 bp, including overlapping 8-bp (three) and 6-bp (two) palindromic sequences (Fig. 4). Only pCvKB4 showed homology to existing sequences: it included a fragment of a reverse transcriptase gene from a *copia*-like retroelement, revealed by >60% homology at the nucleotide level to genes isolated from *Platanus occidentalis*, *Hordeum vulgare*, and *Helianthus annuum*. No notable features were detected in the other repeats.

Southern hybridization

Table 3 summarizes the strength of hybridization of the seven clones to genomic DNA digests from *Crocus* (Table 3*a*) and other genera (Table 3*b*). No clones hybridized to species from the Liliaceae and Amaryllidaceae; only pCvKB4, pCvKB5, and pCvKB6 were present in the

Fig. 1. In situ hybridization (red and green signal) of repetitive DNA sequences from *C. vernus* to metaphase chromosomes from *C. vernus* subsp. *vernus* ×1200. (*a*) Staining of chromosomes with DAPI. (*b*, *c*) Dispersed hybridization of pCvKB5 (green, *b*) showing exclusion from the rDNA regions (arrows; pCvKB2, red, *c*). **Fig. 2.** In situ hybridization (red and green signal) of repetitive DNA sequences from *C. vernus* to metaphase chromosomes from *C. vernus* subsp. *albiflorus* ×1200. pCvKB10 (green, *a*) shows weaker hybridization than pCvKB9 (red, *b*), but relatively equal hybridization to most chromosome segments. pCvKB9 is dispersed but reduced in broad centromeric regions and some whole chromosome arms. **Fig. 3.** In situ hybridization (red and green signal) of repetitive DNA sequences from *C. vernus* to metaphase chromosomes from *C. vernus* subsp. *vernus* ×S1200. Both pCvKB5 (green, *a*) and pCvKB6 (red, *b*) show dispersed but unequal hybridization to most chromosome arms. 'x' indicates stain precipitate.



Iridaceae genera *Romulea*, and only pCvKB5 and pCvKB6 in *Iris*, the others being specific to *Crocus*. All clones showed different genomic arrangements by Southern hybridization to genomic DNA digests with *Sau*3AI (shown for pCvKB4 and 5 in Figs. 5 and 6). The *copia*-like sequence pCvKB4 hybridized with variable strength to all *Crocus* species except *C. korolcowii*. It revealed multiple restriction fragments shared by a number of species at 1000 bp and below. High-molecular-weight bands had an underlying hybridization smear (Fig. 5). Southern hybridization of pCvKB5 (971 bp, Fig. 6) revealed more than 15 abundant *Sau*3AI restriction fragments in *C. vernus* (between 250 and 2500 bp), and an exceptional species distribution pattern, being absent in half the *Crocus* species tested but present in *Romulea* and *Iris*. The sequence included *Sau*3AI sites, so some fragments probably represent internal domains. Some bands were parts of a ladder indicating that the sequence occurs in short tandem arrays, although in situ hybridization showed pCvKB5 to be dispersed over the chromosomes (Fig. 1*b*).

pCvKB1 gave two weak bands, shared by most species where the clone is detected. *C. vernus* had additional bands. pCvKB3 produced multiple bands, some of which corresponded to a ladder, with a similar banding pattern in all species varying in intensity. pCvKB6 gave a ladder with conserved low-molecular-weight bands, even in the remote genera *Romulea* and *Iris*. The high-molecular-weight bands showed a more complex pattern. pCvKB9 gave a ladder-like pattern in *C. vernus* with six bands between 200 and 800 bp. Two bands at 1300 bp and 1450 bp were present in several species. Except for this, the probe showed multiple polymorphic multiple bands with no detectable pattern, different in all *Crocus* species where it is detected. pCvKB10 produced a few bands over a smear in most species.

Discussion

We have investigated the chromosomal and species distribution of cloned nonhomologous repetitive DNA elements from *C. vernus*. The in situ hybridization experiments showed that all but one of the sequence families investigated have an essentially uniform dispersed distribution pattern on all chromosomes of *C. vernus*, but with small differences in the detailed pattern (Figs. 1–3). One sequence, pCvKB2,

Table 2. Data on eight cloned nucleotide sequences from *C. vernus*: genomic distribution, sequence length, AT content, homology to other known sequences, and internal structure.

Category	pCvKB1	pCvKB2	pCvKB3	pCvKB4	pCvKB5	pCvKB6	pCvKB9	pCvKB10
Figs. showing Southern hybridization	—	—	—	7	8	—	—	—
Figs. showing in situ hybridization	—	1*c*	—	—	1*b*, 3*a*	3*b*	2*b*	2*a*
EMBL accession number	AJ131445	AJ131446	AJ131447	AJ131448	AJ131449	AJ131450	AJ131453	AJ131454
Distribution in the *C. vernus* genome	unknown	at satellites	dispersed	dispersed	dispersed	dispersed	dispersed	dispersed
Sequence length (bp)	267	738	894	233	971	185	299	62
AT content	45%	57%	57%	58%	58%	52%	60%	58%
Homologies	none	none (45S rDNA associated)	none	Ty1-*copia*-like	none	none	none	none
Internal structure	pattern of palindromes	none	none	none	none	none	none	none

Note: —, data not shown.

was associated with the 18S–25S rDNA (Fig. 1). Two homologous repetitive sequences from *C. vernus*, pCvKB7 and pCvKB8, units of a family of tandem repeats with subterminal locations on many chromosomes, are under investigation. Our survey of repetitive elements only found one pair of homologous sequences, and no copies of presumably abundant 5S rDNA, LINE, or *gypsy*-like retroelements. We therefore suggest that many more families of repetitive DNA can be found in this species. However, the absence of visible restriction fragments in ethidium-bromide-stained agarose gels suggests the absence of very abundant repeats.

The sequence pCvKB4 was homologous to a fragment of a Ty1-*copia*-like retrotransposon. In many species with large chromosomes, such elements show a dispersed distribution over the chromosomes with gaps at the centromeres and rDNA loci (Brandes et al. 1997). This pattern was also seen in *C. vernus*. At the level of nucleotide sequence, variation is high enough that *copia*-like retrotransposons can be used to discriminate species (e.g., gymnosperms, Kamm et al. 1996; *Avena*, Katsiotis et al. 1996). With one exception (see Table 3*a* and 3*b*), the presence of pCvKB4 divides species of *Crocus* and *Romulea* from the more remote species tested.

The mechanism of amplification and dispersion of non-retroelement-related dispersed repetitive elements is not known, since known amplification mechanisms such as unequal crossing-over would not produce the dispersal seen. It is possible that the other sequences analyzed may be diverged or unidentified fragments of retroelements, or co-transcribed with them. When used as probes for Southern hybridization to DNA of other species of *Crocus* and related genera, each of the seven sequences analyzed here showed a unique distribution pattern among the species (Table 3), although differences between pCvKB3, pCvKB4, and pCvKB6 were small. This indicates that individual clones were not fragments of compound repeats. None of the dispersed sequences was conserved in all *Crocus* species; the pCvKB8 family also showed a unique species distribution (Frello and Heslop-Harrison 2000). The genomic organization of the pCvKB8 family revealed by Southern hybridization (Figs. 5, 6, and Results) indicated that some sequences have similar genomic organizations in the different species in which it is present; major restriction fragments were shared in all hybridizing species.

A model of evolution of repetitive elements involves the appearance of a particular sequence at a single point in a phylogenetic lineage, with subsequent amplification and homogenization of the new sequence across the whole genome. Essential refinements of this model take into account the fact that sequences can disappear during evolution, and that most sequences arise as variants of pre-existing sequences. This model fits the Iridaceae phylogeny, with two of the seven sequences being present in the distant genus *Iris*, and one more in *Romulea*. It is also supported by extensive data in the tribe Triticeae (e.g., Svitashev et al. 1994), the Chenopodiaceae (Kubis et al. 1997), and other species.

Within the genus *Crocus* as currently understood (Mathew 1982), such a sequential model of sequence evolution does not fit our data. *C. banaticus* is taxonomically isolated as the sole member of subgenus *Crociris* based on unique morphological features. However, the subset of four of the seven repetitive sequences included in its genome does not separate

Fig. 4. The DNA sequence of pCvKB1. Between bp 119 and 150, the sequence shows a pattern of palindromes. Underlined: 6-bp palindromes. Bold: 8-bp palindromes. Bolding and underlining indicates overlap.

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GATCTGAGATGTGTCTTCGTTTTGGTTAGACCGATTCTGCGAACGGCCTA   50
GCCCCAAGCGGGCCAAGCACGTAGCGATAAGAAAACTTAACACCAGGTTC  100
ATTGGTGCTTTGGAGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTG  150
GCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTAC  200
CCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATA  250
GCGAAGAGGCCCGCACCGATC
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Table 3. Presence of seven clones from *C. vernus* on genomic DNA from *Crocus* and allied species, revealed by Southern hybridization (as shown in Figs. 7 and 8). Numbers indicate strength of hybridization signal.

(3*a*.) Results from the genus *Crocus*, subdivided by subgenus/section/series, according to Mathew (1982).

Iridaceae: subgenus/section	Series	Species	pCvKB1	pCvKB3	pCvKB4	pCvKB5	pCvKB6	pCvKB9	pCvKB10
Crocus/crocus	*verni*	*vernus*	2	2	2	2	2	2	2
Crocus/crocus	*verni*	*kosaninii*	1	2	2	1	—	—	—
Crocus/crocus	*versicolores*	*malyi*	—	2	—	—	—	—	2
Crocus/crocus	*versicolores*	*imperati*	1	1	1	0	2	2	—
Crocus/crocus	*longiflori*	*medius*	1	1	1	0	2	1	0
Crocus/crocus	*kotchyani*	*kotchyanus*	—	1	1	0	—	—	—
Crocus/crocus	*kotchyani*	*vallicola*	—	1	—	—	1	0	0
Crocus/crocus	*crocus*	*cartwrightianus*	0	2	—	—	2	1	0
Crocus/nudiscapus	*reticulati*	*veluchensis*	0	2	—	—	1	1	1
Crocus/nudiscapus	*reticulati*	*dalmaticus*	—	2	2	1	—	—	—
Crocus/nudiscapus	*biflori*	*chrysanthus*	1	2	2	1	2	1	2
Crocus/nudiscapus	*orientalis*	*korolkowii*	0	0	0	0	0	1	0
Crocus/nudiscapus	*flavi*	*antalyensis*	—	—	—	1	—	—	—
Crocus/nudiscapus	*flavi*	*flavus*	1	2	2	—	2	1	2
Crocus/nudiscapus	*speciosi*	*speciosus*	1	1	2	0	2	0	0
Crocus/nudiscapus	*laevigati*	*laevigatus*	1	2	1	0	2	1	2
Crociris/ —		*banaticus*	0	1	1	1	2	0	0

(3*b*.) Results from other genera than *Crocus*.

Family	Genus	Species	pCvKB1	pCvKB3	pCvKB4	PCvKB5	pCvKB6	pCvKB9	pCvKB10
Iridaceae	*Romulea*	*bulbocodium*	—	0	1	1	1	—	—
	Iris	*magnifica*	0	0	0	1	1	—	0
Liliaceae	*Erythronium*	cv. 'Pagoda'	—	0	0	0	—	—	—
	Tulipa	*turkestanica*	0	0	0	0	0	—	0
	Fritillaria	*imperalis*	0	0	0	0	0	—	0
Amaryllidaceae	*Narcissus*	*pseudo-narcissus*	0	0	0	0	0	—	0

Note: —, Not investigated; 0, hybridization signal not detectable or extremely weak; 1, signal weaker than *C. vernus*; 2, signal strength as strong as *C. vernus* or stronger.

it from species in the other subgenus *Crocus*. In contrast, six of the seven repetitive sequences are not detected in *C. korolkowii*, placed in the subgenus *Crocus*. Furthermore, the subdivision of the subgenus *Crocus* into two sections (see Table 3) is not reflected in the distribution of the sequences; no sequence was largely absent from section *nudiscapus* and present in section *crocus*.

Is the sequential model of sequence evolution wrong? Or is the valuable taxonomic classification of *Crocus* unrelated to evolution in the genus? If evolution has been explosive, with rapid radiation of new species, then the slow processes of repetitive sequence divergence and homogenization could not be expected to be informative. In such cases, taxonomy may be based on informative but fundamentally autapomorphic characters based on a small number of genetic differences. More recent inter-taxon hybridization, with continuing homogenization of repetitive sequences, would make it extremely difficult to reconstruct the phylogeny, even using combinations of molecular, genetic, chromosomal, and morphological analyses. We think that such a pattern for the evolution of the genus *Crocus* is more likely than the occurrence of many parallel and repeated events in sequence evolution.

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Fig. 5. *Sau*3AI digests of genomic DNA from *Crocus* and allies after agarose gel electrophoresis and ethidium bromide staining showing smears of cut DNA. No major bands are visible in the digests. Southern transfer of the gel probed with the repetitive DNA clone pCvKB4 shows presence of the sequence in all *Crocus* species, except *C. korolkowii*, and *Romulea*. Multiple restriction fragments are shared between most lanes showing hybridization, although the signal strength varies. Lanes: a, *C. vernus*; b, *C. kosaninii*; c, *C. imperati*; d, *C. medius*; e, *C. kotchyanus*; f, *C. dalmaticus*; g, *C. chrysanthus*; h, *C. korolkowii*; i, *C. flavus*; j, *C. speciosus*; k, *C. laevigatus*; l, *C. banaticus*; m, *Romulea bulbocodium*; n, *Iris magnifica*; o, *Erythronium* 'Pagoda'; p, *Tulipa turkestanica*; q, *Fritillaria imperalis*; r, *Narcissus pseudonarcissus*; s, 100-bp ladder with 100 bp just below the illustration, double band at 800 bp.

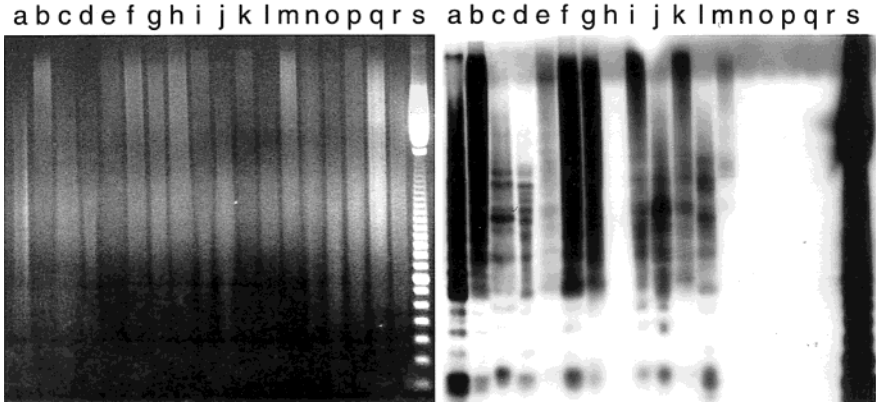
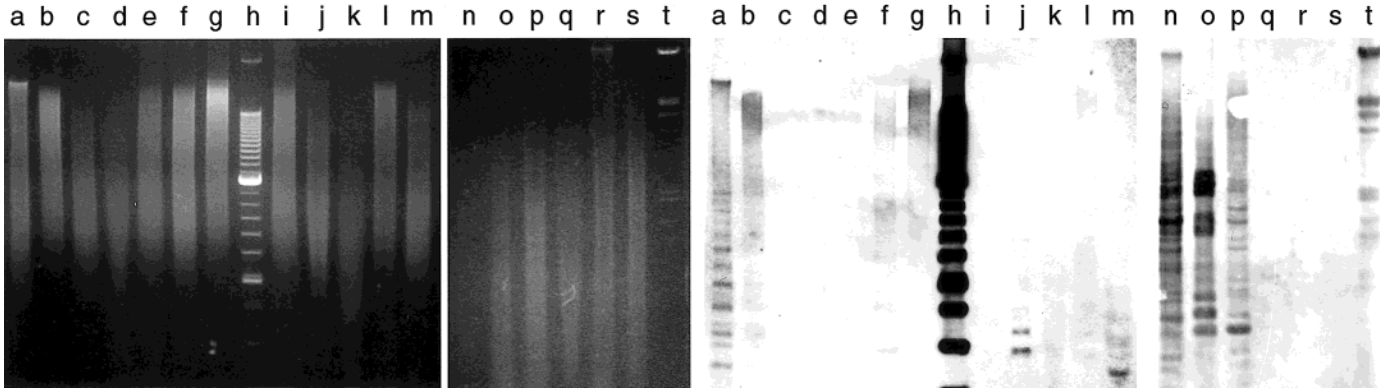


Fig. 6. *Sau*3AI digests of genomic DNA from *Crocus* and allies after agarose gel electrophoresis and ethidium bromide staining showing smears of cut DNA. Southern transfer of the gel probed with the repetitive DNA clone pCvKB5 showing presence of the sequence in 6 of the 12 *Crocus* species tested. More than 15 restriction fragments are present in *C. vernus*, but other species have mostly different and a much smaller number of hybridizing fragments. Lanes: a, *C. vernus*; b, *C. kosaninii*; c, *C. imperati*; d, *C. medius*; e, *C. kotchyanus*; f, *C. dalmaticus*; g, *C. chrysanthus*; h, 250-bp ladder from lowest band, double band at 1 kb; i, *C. korolkowii*; j, *C. antalyensis*; k, *C. speciosus*; l, *C. laevigatus*; m, *C. banaticus*. Second gel (longer autoradiograph exposure than lanes a to m; lane n loaded with less DNA than o to s; cf. lanes a and n): n, *C. vernus*; o, *Romulea bulbocodium*; p, *Iris magnifica*; q, *Tulipa turkestanica*; r, *Fritillaria imperalis*; s, *Narcissus pseudonarcissus*; t, lambda-*Eco*RI–*Hin*dIII double digest.



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