Quantitative delineation of karyotype variation in *Papaver* as a measure of phylogenetic differentiation and origin

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Karyomorphology of 30 species of *Papaver* L. (Papaveraceae) and *in situ* localization of rDNA sites on chromosomes of *P. somniferum* was used to establish phylogenetic affinities, ancestry and chromosomal variation during speciation. A simple biometrical parameter, the 'Dispersion Index', was employed to facilitate quantitative evolutionary gradation between the closely related karyotypes that fall under the same class of karyotypic asymmetry. The karyoevolutionary data thus obtained is combined with the morphotaxonomic information to project species interrelationships and probable phylogenetic order. Over 2-fold diminution in relative chromosome size is associated with speciation in this genus. Diminution is almost uniform except the smallest pair. The degree of reduction in the short arm is proportionately more than that in the long arm. The opium poppy, *Papaver somniferum* ($2n = 22$), a deviation from $x = 7$, appears to have originated via chromosomal introgression through aneuallopolyploidy involving addition of nucleolar chromosomes from two or more species. Possibly, a progenitor triploid hybrid between species like *P. glaucum* ($2n = 14$) and *P. gracile* ($2n = 28$) may have preceded the speciation of *P. setigerum* ($2n = 22$) and *P. somniferum* ($2n = 22$) in concurrent succession.

PAPAVER is the largest and most advanced genus in the family Papaveraceae with more than 80 species, almost all of which are laticiferous and are source of one or more alkaloids spread over 170 alkaloids from 13 alkaloid groups[1]. Five alkaloids, namely morphine, codeine, thebaine, papaverine and narcotine owe major pharmacological and clinical significance[2]. Since this taxon has become differentiated karyologically, morphologically, physiologically and biosynthetically[3], an integrated account of interspecific affinities is needed for an understanding of introgression of biosynthetic and ecological features.

Using morphological and floral characters, the genus divided into 9 sections[4], was subsequently revised[5]. Following a 'geographical–morphological method' of determining affinities, four cladistic groups of sections in the genus have since been recognized[6]. The genus has three basic chromosome numbers, $x = 6, 7, 11$, together with intra- and inter-specific polyploidy. This complicates the issue of genome evolution. In an attempt to determine species relationships, efforts have been made by various workers to produce interspecific hybrids[7–10]. Although meiosis in interspecific hybrids provides useful information about genomic affinities, the relevant karyomorphological data have not been applied to supplement it. Therefore, the present study was undertaken (i) to obtain karyomorphological details and karyotypic affinities, (ii) to use karyological data in projecting species relationship, and (iii) to delineate the mode of chromosome evolution and the direction of phylogenetic change.

Materials and methods

Thirty species of *Papaver* L. were studied. The names of the species, section within the genus, source and their growth habit are listed in Table 1.

For analysis of somatic chromosomes, 1–3 cm long roots from rapidly growing germinating seeds were pretreated for 3–3½ h at 12–14°C in an equal mixture of saturated aqueous solution of *para*-dichlorobenzene and 8-hydroxyquinoline. Roots were subsequently fixed in Carnoy's fixative after thorough washing, stained in 2% aceto-orcein-N. HCl (9 : 1) for 2–4 h and squashes were prepared. At least 3–5 well-spread metaphase plates with similar degree of chromatin condensation were used to make chromosomal measurements.

For constructing the karyotype, the chromosomes were arranged in order of decreasing size and increasing asymmetry and numbered. They were grouped into 8 types (A–H) based on their length in decreasing order. The karyotype formula was determined from chromosome morphology based on centromere position in accordance with the classification of Levan *et al.*[11] (Table 1, Footnote a) and karyotype symmetry according to Stebbins[12] (Table 1, Footnote b). The karyotype symmetry classes of Stebbins were further quantitatively differentiated into finer karyoevolutionary gradations following the parameter of

This paper is dedicated to Prof. A. K. Sharma in honour of his 75th birth anniversary.

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Table 1. The examined species of *Papaver*, their sectional classification and karyotype characteristics

Sl. no.	Species	Source of material	Growth habit	Section within genus	2*n* no.	Chromosome length shortest : longest	Karyotype symmetry	Dispersion Index (DI)**	Haploid chromatin length (μm)	Karyotype formula
Group I										
1.	*P. nudicalue* L.	Mus Nat Hist, Paris	P	Meconella	14	1 : 1.34	2A	2.58	32.57	1stsC + 1mD + 3smD + 1stD 1smE
2.	*P. kerneri* Hayek	Bot Gard, Vienna	P	Meconella	14	1 : 1.40	2A	3.27	16.63	7smF
3.	*P. rhaeticum* Leresche	Hort Bot, Amsterdam	P	Meconella	14	1 : 1.63	2A	4.76	22.47	1smsD + 2smE + 1stE + 3sm
4.	*P. alpinum* L.	RBG, Edinburgh	P	Meconella	14	1 : 1.73	2A	5.14	17.93	1smE + 1smsF + 3smF + 1 F + 1smG
5.	*P. alboroseum* Hult*.	Acad Sci, Leningrad	P	Meconella	28	1 : 1.85	3A		35.42	1msE + 1smE + 7mF + 2smF 3mG
6.	*P. nordhagenianum* ssp. *islandicum* Love	Bot Gard, Montreal	P	Meconella	14	1 : 3.44	2B	12.85	19.38	1stsD + 1stsE + 2smF + 2stF + 1smG
7.	*P. fugax* Poir.	Hor Bot, Amsterdam	B	Meconidium	14	1 : 1.34	1A	2.62	22.55	3smE + 3stE + 1stF
8.	*P. tauricolum* Boiss.	RBG, Kew	B	Meconidium	14	1 : 1.53	1A	3.39	24.33	1smsD + 4smE + 1stE + 1sm
9.	*P triniifolium* Bois.	Mus Nat Hist, Paris	B	Meconidium	14	1 : 1.51	1A	3.77	26.33	1smsD + 6smE
10.	*P. persicum* Lindl.	Acad Sci, Leningrad	B	Meconidium	14	1 : 1.71	2A	4.90	14.69	1mF + 3smF + 3smG
Group II										
11.	*P. apokrinomenon* Fed.	RBG, Kew	P	Pilosa	14	1 : 1.34	2A	2.96	20.12	3smE + 4smF
12.	*P. spicatum Bois.* & Bal	Mus Nat Hist, Paris	P	Pilosa	14	1 : 1.47	3A	5.07	23.04	1smsE + 3smE + 3smF
13.	*P. heldreichii Bois.**	RBG, Edinburgh	P	Pilosa	28	1 : 1.71	3A		31.69	3mF + 6smF + 5mG
14.	*P. lateritium* Koch	RBG, Edinburgh	P	Pseudopilo.	14	1 : 2.84	2B	5.37	18.09	2stsE + 1smF + 2stF + 1tF + 1smG
15.	*P. ruifragum* Bois Reut	RBG, Edinburgh	P	Pseudopilo.	14	1 : 3.53	2B	7.19	16.66	1stsE + 1smsF + 1smF + 3stF + 1smH
16.	*P. atlanticum* Bal Cos	Bot Gard, Berlin	P	Pseudopilo.	14	1 : 2.49	2B	7.96	17.78	1stsE + 1mF + 3smF + 1stF - mG
Group III										
17.	*P. orientale* L*	Inst Hmelijarstvo, Pivovarstvo	P	Macrantha	42	1 : 2.32	2B		97.69	1mB + 4smC + 1stC + 2ml + 2smsD + 8smD + 1sm + 1stE + 1mF
18.	*P. bracteatum* Lindl.	Inst Hmel Pivovast.	P	Macrantha	14	1 : 2.01	3B	8.87	39.18	1smsA + 1smB + 1mC + 2smC + 1smD + 1mE
19.	*P. rhoeas* L.	Hort Bot, Antwerp	A	Rhoeadium	14	1 : 1.37	2A	3.46	28.84	1msD + 1mD + 1smD + 1mE + 3smE
20.	*P. commutatum* FisMey	Acad Sci. Leningrad	A	Rhoeadium	14	1 : 1.39	2A	3.75	23.81	1smsD + 5smE + 1smF
21.	*P. dubium* L. *dubium*	Hort Bot, Antwerp	A	Rhoeadium	14	1 : 1.46	3A	5.24	33.04	1smsC + 1smC + 4mD + 1smD
22.	*P. dubium-lavigatum* Kad*	Acad Sci, Leningrad	A	Rhoeadium	28	1 : 1.71	2A		34.13	1smsE + 8smF + 3stF + 2smG
23.	*P. gracille* Boiss.	Dr JW Kadereit	A	Papaver	14	1 : 1.22	1A	1.98	21.49	1smsE + 3smE + 3smF
24.	*P. glaucum* Bois & Haus	RBG, Kew	A	Papaver	14	1 : 1.51	3A	4.19	22.15	1smsE + 3smE + 2mF + 1smF
25.	*P. setigerum* DC*	Mus Nat Hist, Paris	A	Papaver	22	1 : 1.94	2A		27.55	1mD + 1smsD + 1smD + 1mE + 2smE + 3stE + 1mF + 1smF
26.	*P. somniferum* L*	CIMAP, Lucknow	A	Papaver	22	1 : 2.06	2B		45.19	1smsB + 1smC + 1smD + 1stD + 1mE + 5smE + 1sn
27.	*P. setigerum* DC*	Orto Bot, Napoli	A	Papaver	44	1 : 2.15	3B		50.23	1smsE + 1smE + 9mF + 10smF + 1stF
Group IV										
28.	*P. hybridum* L.	Univ Gard, Cambridge	A	Argemonid.	14	1 : 1.28	2A	2.80	21.47	2mE + 3smE + 2smF
29.	*P. ocellatum* Waronow	Acad Sci, Leningrad	A	Argemonid.	14	1 : 1.28	2A	3.21	17.99	2mF + 1sms F + 3smF + 1 stsF
30.	*P. pavoninum* Schrenk*	RBG, Kew	A	Argemonid.	12	1 : 1.79	2B		19.93	1msD + 1smD + 1smsE + 3smF
31.	*P. argemone* L*	Bot Gard, Hamburg	A	Argemonid.	42	1 : 2.13	2B		62.20	3mE + 1smsE + 4smE + 1stE + 2mF + 8smF + 1stF + 1smG

Notes and abbreviations:

(a) Chromosome form based on centromere position indicating long : short arm ratio; m = medium region (4.01 : 3.99–5 : 3), sm = submedian region (5 : 3–6 : 2 st = subterminal region (6 : 2–7 : 1), t = terminal region (7 : 1–7.99 : 0.01), s = SAT chromosome.

(b) Classification of karyotype symmetry is based on the difference in length between the largest and smallest chromosome within the complement < 2 : 1 = A, 2 : 1 4 : 1 = B; and further within class categorization is indicated by numerical prefix 1, 2, 3. . . in increasing order of proportion of chromosomes with arm ratio < 2 : 1 on 0–scale (0–0 = 1, 0.01–0.5 = 2, 0.51–0.99 = 3, 1.0 = 4).

(c) Chromosome class based on length of chromosome: A = more than 7 μm–up to 8 μm, B = more than 6 μm–up to 7 μm, C = more than 5 μm–up to 6 μm, D = more tha 4 μm–up to 5 μm, E = more than 3 μm–up to 4 μm, F = more than 2 μm–up to 3 μm, G = more than 1 μm–up to 2 μm and H = up to 1 μm.

(d) Growth habit: P = Perennial, B = Biennial and A = Annual.

**DI values are given only for the species that have basic diploid set (2*n* = 14) so as to allow for the uniformity in comparison.

*These species are considered more evolved on account of numerical change.

Chromosome–Dispersion Index[13]. The values of Dispersion Index (DI) for a given karyotype are estimated from the following equations. This is explained by taking the example of *P. bracteatum* using the raw karyotypic data provided in Table 2 (serial no. 1).

$$\text{Centromeric gradient (CG)} = \frac{\text{Length of median short arm}}{\text{Length of median chromosome}} \times 100 = \frac{2.25}{5.74} \times 100 = 39.2$$

$$\text{Coefficient of variation (CV) for chromosome length} = \frac{\text{S.D.}}{\text{Mean}} \times 100 = \frac{1.267}{5.6} \times 100 = 22.62$$

Dispersion index (DI) =

Proportionate measure of CG with respect to CV = 22.62% of 39.2 = 8.87.

The DNA probe *p*Ta*71*, containing a 9 kb *Eco*R1 fragment of the 18S–26S rDNA isolated from *Triticum aestivum*[14] and cloned in *pUC*19 was used for physical mapping of rDNA sites on the somatic chromosomes of *Papaver somniferum* by fluorescence *in situ* hybridization technique (FISH) following the standard protocol[15–17].

Results

Karyomorphology

The karyotypic features are summarized in Table 1. The structural characteristics of the various karyotypes are denoted by the symbol A and B along with a numerical prefix depicting particular class of karyotype symmetry[12]. Measurements of the individual chromosomes within the complement for the species that have similar number of somatic chromosomes ($2n = 14$) are given in Table 2; along with the values of 2C nuclear DNA amounts adopted from our earlier work[18]. The values of DI calculated from this data are included in Table 1. The average values for the total length of haploid complement at metaphase on sectional basis are given in Table 3. The somatic chromosomes of various species representing varying chromosome numbers are shown in Figure 1.

Localization of rDNA sites on somatic chromosomes of P. somniferum

Three rDNA sites (one major and two minor) homologous to 18S–26S–rRNA gene families were detected in the NOR region of chromosome 1, in the telomeric region of short arm of chromosome 2 and the subterminal region of short arm of chromosome 4, respectively (Figure 2).

Chromosome variation and evolution

Alterations in chromosome number, size and structure are common in the genus. There is over two-fold variation in

Table 2. Length of short and long arm of individual chromosomes in microns in the 22 species of *Papaver* ($2n = 14$) and 2C DNA content

Sl. no.	Species	Chromosome 1		Chromosome 2		Chromosome 3		Chromosome 4		Chromosome 5		Chromosome 6		Chromosome 7		2C DNA (pg)
		short	long	short	long	short	long	short	long	short	long	short	long	short	long	
1.	*P. bracteatum*	2.70	5.00	2.25	4.00	2.00	4.00	2.70	3.04	2.25	2.96	1.25	3.20	1.51	2.32	9.79
2.	*P. dubium*	2.08	3.80	1.86	3.50	2.00	2.81	1.29	3.30	1.74	2.55	1.69	2.40	1.52	2.50	7.43
3.	*P. nudicaule*	1.53	3.80	1.42	3.49	1.26	3.45	1.47	3.20	1.00	3.67	1.60	2.69	1.20	2.79	8.23
4.	*P. rhoeas*	1.53	3.40	1.79	2.80	1.80	2.33	1.20	2.73	1.13	2.80	1.27	2.46	1.00	2.60	7.14
5.	*P. triniifolium*	1.50	3.10	1.26	2.74	1.15	2.76	1.27	2.50	1.10	2.60	1.00	2.31	0.74	2.30	6.04
6.	*P. tauricolum*	1.67	2.70	0.89	2.70	1.04	2.50	0.80	2.69	1.10	2.35	0.92	2.12	0.90	1.95	6.73
7.	*P. commutatum*	1.86	2.20	1.30	2.49	1.02	2.62	1.04	2.23	0.92	2.20	0.92	2.10	1.11	1.80	5.82
8.	*P. spicatum*	1.29	2.70	1.70	2.24	1.20	2.17	1.14	2.09	1.05	1.90	0.85	2.00	0.80	1.91	5.82
9.	*P. fugax*	0.91	2.90	0.90	2.60	1.02	2.10	0.89	2.23	0.88	2.20	0.80	2.28	0.65	2.20	5.34
10.	*P. rheaticum*	1.40	2.68	1.13	2.50	1.00	2.50	0.73	2.60	0.94	1.76	0.70	2.00	0.80	1.71	–
11.	*P. glaucum*	1.60	2.32	1.15	2.20	0.89	2.40	1.03	2.20	1.10	1.80	1.08	1.77	0.90	1.70	6.20
12.	*P. gracile*	0.92	2.43	0.92	2.33	1.10	2.10	0.95	2.20	0.85	2.10	0.85	2.00	0.84	1.90	5.55
13.	*P. hybridum*	1.18	2.20	1.04	2.29	0.92	2.30	1.30	1.87	1.16	1.80	0.80	1.96	0.90	1.75	4.94
14.	*P. apokrinomenon*	1.12	2.12	1.00	2.17	0.82	2.29	1.00	1.73	0.80	1.93	0.73	2.00	0.80	1.61	–
15.	*P. nordhagenianum*	1.20	2.96	1.24	2.76	0.93	1.96	0.93	1.56	0.60	1.83	0.50	1.70	0.40	0.81	4.78
16.	*P. lateritium*	0.96	2.33	0.78	2.40	0.58	2.25	0.32	2.40	1.14	1.40	0.57	1.80	0.40	0.76	5.58
17.	*P. ocellatum*	1.07	1.80	0.94	1.87	0.94	1.75	0.82	1.80	1.10	1.40	0.90	1.35	0.75	1.50	4.65
18.	*P. alpinum*	1.10	2.20	0.81	2.08	1.15	1.40	0.60	1.88	0.80	1.63	0.87	1.50	0.60	1.31	–
19.	*P. atlanticum*	1.30	2.49	0.55	2.30	0.76	1.90	0.90	1.51	0.64	1.76	1.00	1.15	0.56	0.96	4.68
20.	*P. rupifragum*	0.80	2.67	0.70	2.08	0.52	2.26	0.58	1.85	0.40	1.74	0.70	1.38	0.40	0.58	4.71
21.	*P. kerneri*	0.88	2.00	0.70	1.86	0.80	1.64	0.69	1.69	0.55	1.70	0.76	1.30	0.60	1.46	5.03
22.	*P. persicum*	0.80	1.98	0.80	1.46	0.64	1.50	0.68	1.40	0.60	1.36	0.60	1.25	0.43	1.20	4.64
	C.V.	34.33	27.57	37.72	23.29	37.38	25.86	46.08	24.43	44.44	27.54	32.26	26.29	39.51	35.67	23.36

average chromosome length even in the species that have similar chromosome number, i.e. $2n = 14$. The chromosome size ranges from 1.0 to 7.7 μm in perennials, 1.6 to 4.6 μm in biennials, 1.6 to 5.9 μm in annuals, and $2n$ number from $2n = 12$ (with one pair of large metacentrics) to $2n = 44$; majority having $2n = 14$. Curiously, despite differences in habit, ranging from perennial to annual, the size of the shortest chromosome is rather short in perennials, although accretion of chromatin material generally lead to increase in size of other chromosomes within the complement. Such a situation is most pronounced in the perennial section *Pseudopilosa* wherein the smallest chromosome pair is distinctly smaller and characteristic of the group. The application of the DI has been found useful to differentiate quantitatively the closely related karyotypes belonging to the same class of asymmetry. The higher values of DI are considered to indicate higher levels of karyotypic specialization.

Table 3. The haploid metaphase chromosome length in *Papaver* spread over taxonomic sections and cladistic groups

Cladistic group	Section	Haploid chromatin length* (μm)
Group I	Meconella	21.12
	Meconidium	21.97
Group II	Pilosa	19.67
	Pseudopilosa	17.51
Group III	Macrantha	35.87
	Rhoeadium	25.69
	Papaver	21.18
Group IV	Argemonidium	20.86

*The values are calculated assuming $n = 7$.

Species affinities

To complement Kadereit's delimitation of sectional affinities in *Papaver*[6], the species affinities were delineated on the basis of karyoevolutionary parameters. The species in the sections were first arranged in an advancing order of specialization *pari passu* increased order of karyotype asymmetry. Wherever the differentiation between the closely related taxa was not possible by karyotype asymmetry, the new parameter of DI was used as an adjunct to further determine the evolutionary course. Any haploid number deviating from 7 is considered to be derived. The phylogenetic sequence thus obtained is depicted in Figure 3.

Discussion

Based on morphotaxonomic parameters and geographical distribution, Kadereit[6] (and pers. commun.) recognizes four groups among the sections studied here. The first comprises sections *Meconella* and *Meconidium* which appear to be ancestral by virtue of their valvate capsules and yellow filaments. The second consists of sections *Pilosa* and *Pseudopilosa* which still have yellow filaments, but poricidal capsules, and in that respect are somewhat more advanced than the preceding group. The

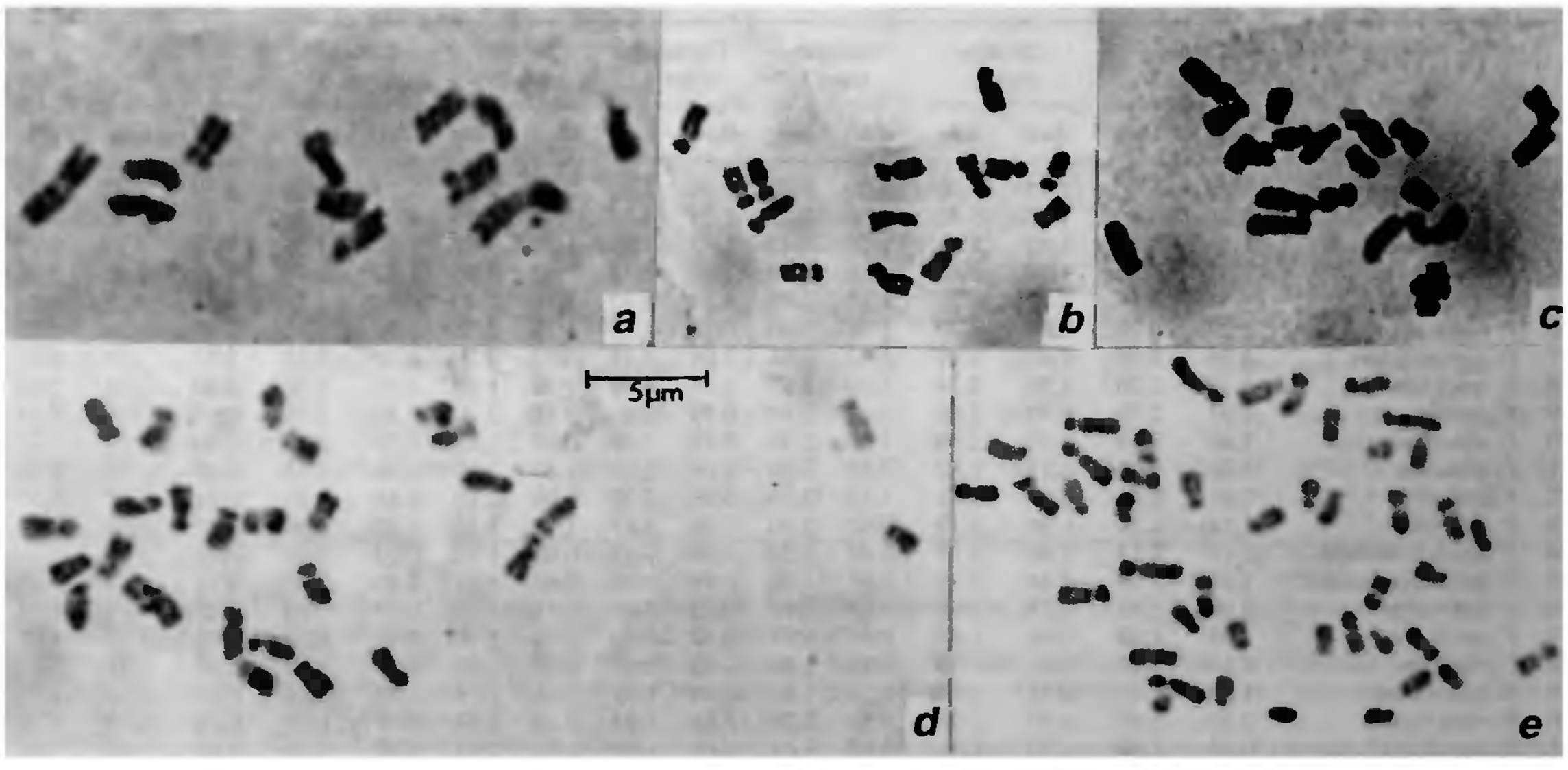

Figure 1. Somatic chromosomes of the representative species of *Papaver* showing varying chromosome number. *a*, *P. pavoninum* ($2n = 12$); *b*, *P. gracile* ($2n = 14$); *c*, *P. somniferum* ($2n = 22$); *d*, *P. dubium* subsp. *laevigatum* ($2n = 28$); *e*, *P. setigerum* ($2n = 44$).

third comprises sections *Macrantha*, *Rhoeadium* and *Papaver*, which all have poricidal capsules and black filaments. Within this group section, *Macrantha* is least advanced. The fourth and last group consists only of section *Argemonidium*, which stands apart from the rest of the genus on account of a number of anatomical and morphological characters; so much so that the removal of this section along with *Meconella* from the genus *Papaver* has been suggested on the basis of *cp* DNA analysis[19]. Groups 2 and 3 appear most closely related to each other.

An attempt has been made to deduce the probable phylogenetic sequence by first illustrating the evolutionary order among the sectional groups followed by the within-section evolutionary arrangement of the species (Figure 3). Such an arrangement was derived by associating karyotypic characteristics (chromosome number and symmetry, relative chromosome length, DI) with other taxonomic characters such as growth habit, morphological similarities, geographical distribution, and genomic affinities. The species arrangement thus obtained has been further used to determine the evolutionary pattern in chromosome variation and direction of change.

Pattern of chromosome variation within the genus and origin of opium poppy

Although three different basic numbers $x = 6, 7, 11$ occur in the genus *Papaver*, $x = 7$ is by far the most frequent. The two series in the Papaveraceae: Coryodalo–Hypecoideae and Chelidoniodeae, have the basic numbers $x = 4$ and $x = 3$, respectively[7]. These series are independent but may have undergone convergent evolution to give the $x = 7$ of Papaveroideae (including *Papaver*). It is most likely that $x = 7$ is ancestral and $x = 6$ might have occurred by Robertsonian fusion of two acrocentrics giving a metacentric. This is seen in *P. pavoninum* ($x = 6$) which has one pair of large metacentrics (Figure 1 *a*). However, the origin of $x = 11$ from $x = 7$ or 6 needs special consideration. It might have developed by aneu-allopolyploidy. A case study of *Papaver somniferum* may serve as a possible indicator.

The ancestors of opium poppy

Based on morphotaxonomic affinities, it was suggested[20] that *P. setigerum* ($2n = 44$) is a close relative and probably the ancestor of the opium poppy, *P. somniferum* ($2n = 22$). Proximity between the two species is substantiated from the meiotic behaviour of their interspecific hybrids, where all the 11 chromosomes of *P. somniferum* show homoeologous pairing with a group 11 chromosomes of *P. setigerum*, although the remaining 11 chromosomes of the latter maintain separate identity as a group of 3 and 8 chromosomes, indicating that *P. setigerum* is endowed with three genomes, i.e. A, B and

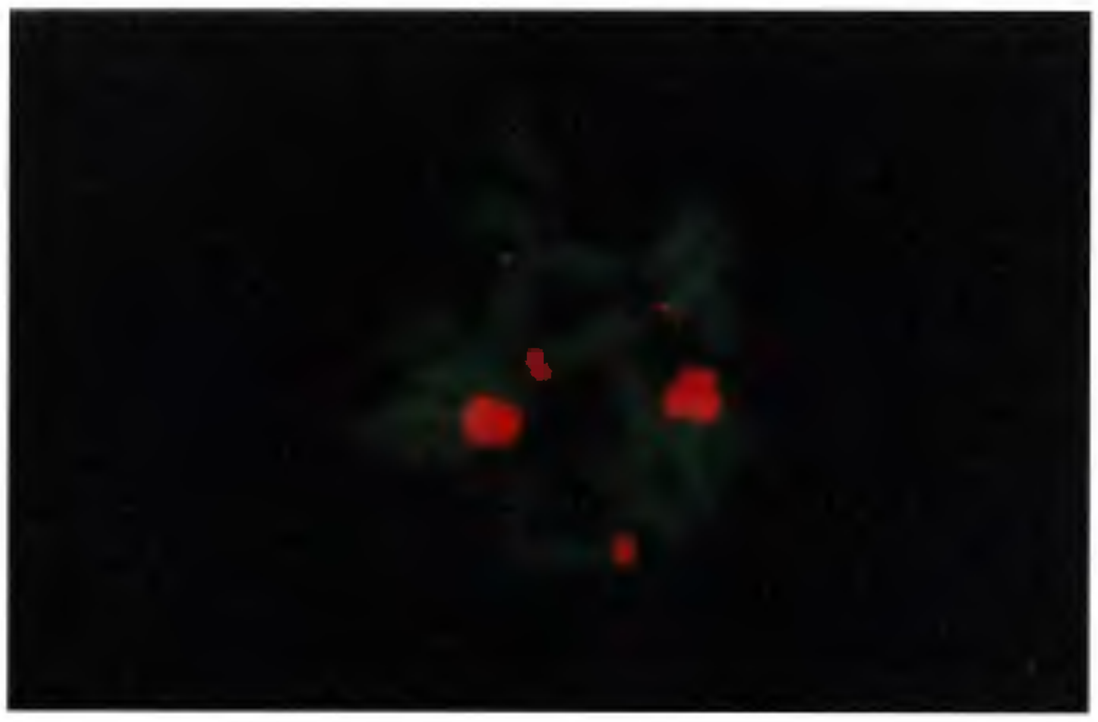

Figure 2. Fluorescent detection of 18S–26S rDNA site on somatic chromosomes of *P. somniferum*. Biotin labelled rDNA probe *p*Ta71 detected by fluorescein conjugated to avidine–red fluorescence. Note, one pair of large site along the NOR region of chromosome 1, and two other pairs of minor sites.

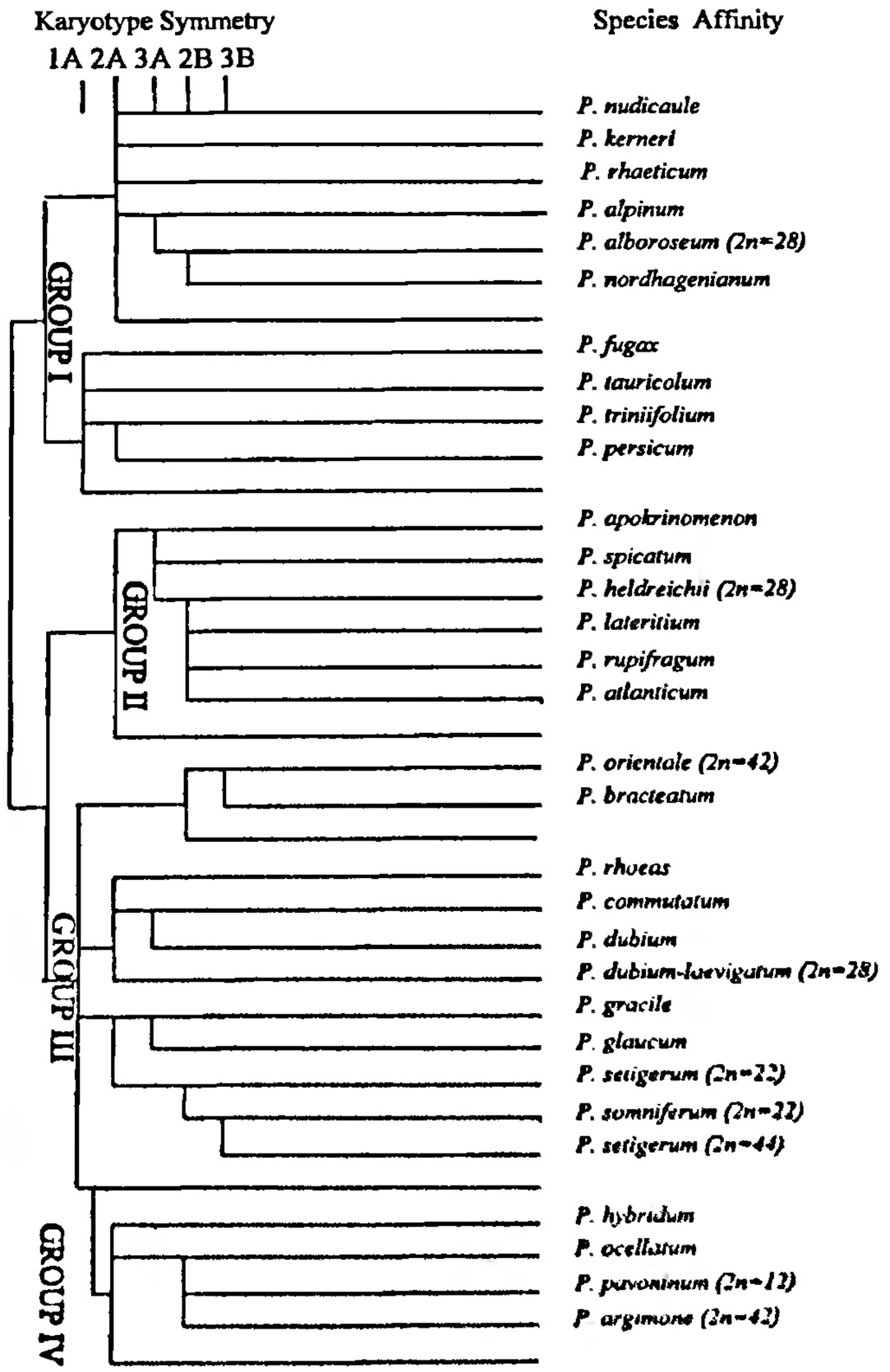

Figure 3. Probable phylogenetic affinities between the species of *Papaver*.

C, respectively[21]. But, the DNA dot blot analysis of the 9 gene families encoding for major latex proteins (MLPs) of opium poppy show that they belong to two distinct subfamilies[22]. Thus, it seems likely that $x = 11$ of opium poppy, the so-called A genome, may owe its origin to at least two different genomes. Our present observations on the occurrence of rDNA sites on three different linkage groups in *P. somniferum* further add towards a distinct possibility of origin of this species through genomic introgression of 2 or 3 species.

In quest for ancestors of opium poppy, it may be important to examine the integrated proposal[23] that *P. somniferum* ($2n = 22$) and *P. setigerum* ($2n = 22, 44$) are intermediate between *P. glaucum* ($2n = 14$) and *P. gracile* ($2n = 28$). The morphological differences between the two species *P. sominiferum* and *P. setigerum* could be interpreted as segregation of parental characters with *P. somniferum* showing more similarity to *P. glaucum* and *P. setigerum* more to *P. gracile*[24]. The affinity between these species is also reflected in the degree of karyotype symmetry as recorded from the present study. Further, the occurrence of a modal number of $2n = 28$ in the segregating F_2 progenies of the interspecific hybrids between *P. somniferum* ($2n = 22$) and *P. setigerum* ($2n = 22$) has been encountered[10]. Such unexpected chromosome behaviour may be considered to suggest that a multiple of 7 chromosomes is probably more conducive to genomic stability in this genus. Our karyomorphological data, more particularly the symmetry of the karyotype, however show that *P. setigerum* ($2n = 22$) with 2A class of karyotype symmetry might have preceded the speciation of *P. somniferum* with 2B karyotype. The tetraploid *P. setigerum* ($2n = 44$) could be assumed to have originated much later, as it has a more specialized 3B karyotype, possibly as a consequence of interspecific hybridization between *P. somniferum* and *P. setigerum* ($2n = 22$) followed by polyploidization and genomic adjustments. Nevertheless, genomic *in situ* hybridization (GISH)[16,25,26] with putative donors/close relatives of *P. setigerum* and *P. somniferum* may help judicious identification of diploid progenitors of this species, as also to resolve the derivation of $2n = 22$ chromosomes.

Chromosome evolution, direction of change and species affinities

The divergence and evolution of angiosperms is often accompanied by large-scale changes in amounts of chromosomal DNA[27] and both increases and decreases in genome size have been postulated to occur[28,29]. In some cases the changes are direct consequences of polyploidy, whereas in others they may involve chromosome repatterning coupled with addition or deletion of certain specific chromosomal segments[30,31]. An exhaustive analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants[32], and a correlation between the degree of evolutionary 'advancement' and an increase in genome size *vis-à-vis* increase in chromosome number has been identified[31]. However, when the issue of chromosome variation is examined at the inter-species level, the situation is intriguing. Whereas obvious result of polyploidy is an increase in genome size, it does not necessarily increase the chromosome size. Nevertheless, numerical variation emanating from Robertsonian changes may involve addition or deletion of certain specific chromosomal segments, and the other structural changes that bring about change in karyotype symmetry are consummated by tandem amplification or deletion. Such tandem changes may generally involve increase or decrease in the proportion of noncoding, repetitive DNA sequences[33], more so the retroelements[34]; an equal change at individual chromosome level would add to karyotype asymmetry and hence the evolutionary advancement.

In the divergence and evolution, have the chromosomes of *Papaver* become longer or shorter with phylogenetic advancement? All the species investigated are arranged in a phylogenetic sequence in increasing order of specialization taking into account their perennial/biennial or annual habit, morphological similarities and systematic position in conjunction with karyotaxonomic criteria. When the karyotypic characteristics are fitted into this arrangement (Tables 1, 2 and Figure 3), it is clear that chromosome evolution in the genus has been associated with diminution in chromosome size, haploid chromatin length, 2C DNA content *vis-à-vis* an enhanced order of karyotype asymmetry and DI concurrent with evolutionary specialization of the species. Such an evolutionary trend of reduction in chromatin material is also reflected even for the average haploid chromatin length for a given taxonomic section in a particular cladistic group (Table 3).

The evolutionary reduction in chromatin material/nuclear DNA content is of the magnitude of over two-fold even in the species that all have similar chromosome number of $2n = 14$ (Tables 1 and 2). This prompted us to examine the pattern of diminution of individual chromosomes within the complements. The CV estimated for the homoeologous chromosomes/chromosome arms, spread over species, is considered to serve as a general guiding parameter to detect the extent of gradual variation. These values are given in Table 2. The revelations are that the diminution in individual chromosome size is shared almost equally by all the chromosomes (short : long arm) within the complement except the smallest pair, irrespective of the size of the chromosome. Supporting evidence for an equal change in DNA amounts at individual chromosome level has been provided in *Vicia*[35], *Festuca* and *Lolium*[36]. However, the extent of diminution is proportionately higher for short arms than long ones in *Papaver*.

The occurrence of chromosome diminution poses the question as to why such a loss of chromosomal material during evolutionary advancement does not affect the very survival of the descendant species, particularly at the diploid level?. Two complementary possibilities are worth mentioning in this context. To make the deleterious effect less serious with such an evolutionary change, the chromatin loss is shared by the whole complement instead of constraining particular linkage groups, or the loss may chiefly involve the less important chromosomal segments comprised of repetitive DNA elements, both tandem and dispersed ones[37]. In fact, a progressive reduction in intercalary C-banding pattern has earlier been depicted in 9 species of *Papaver*[38]. It is, therefore, surmised that repeat elements, more particularly the retroelements[34] have contributed in generating genetic and species diversity in *Papaver*.

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