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Karyotype, DNA Content and Meiotic Behaviour in Five South American Species of *Vicia* (Fabaceae)

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This paper presents the karyotype, DNA content and meiotic behaviour of five species of *Vicia* from Argentina (*V. macrograminea* Burk., *V. graminea* SM., *V. epetiolaris* Burk., *V. pampicola* Burk. and *V. nana* Vog.). All the species have the same chromosome number and karyotype formula ($2n = 14$; 6 **m** + 4 **st** + 4 **t**). Each species, however, displays a characteristic number and position of the nucleolar organizer region (NOR) and different sizes of the respective satellites, confirmed by Ag-NOR banding. Moreover, significant differences were found in the total chromosome volume (TCV) and DNA content of the species. Positive correlations between DNA content and TCV, and between DNA content and type of life cycle were also found. TCV and DNA content are lower in *V. nana* (annual) and higher in *V. macrograminea* (biennial–perennial). The material displayed marked karyotypic orthoselection, with similar karyotypes in all studied species, even when the overall chromosome size varied. Evolutionary changes in DNA amount are proportional to the relative length of each chromosome arm, maintaining karyotypic uniformity. Significant differences were found between the meiotic behaviour of *V. graminea* and that of the other species. *V. graminea* has a lower frequency of ring bivalents and chiasmata per cell, and also has a lower interstitial chiasma frequency. In general, the results are congruent with the morphological data reported for these species.

Key words: *Vicia* species, karyotype, orthoselection, nuclear DNA content, NOR banding, meiotic behaviour.

INTRODUCTION

The genus *Vicia* (Fabaceae) includes about 150 species distributed throughout the temperate zones of both hemispheres. The Mediterranean area is its principal centre of diversification. A smaller centre of diversification in temperate South America is based solely upon the endemic Section Australes Kupicha (Subg. *Vicilla*) (Kupicha, 1976; Hanelt and Mettin, 1989; Maxted, Callimassia and Bennett, 1991). About 20 species are native to Argentina and the majority of them belong to Section Australes (Burkart, 1967, 1987; Giangualani, 1982, 1984).

Speciation and evolution within this genus have involved a variation in chromosome number and ploidy level ($2n =$ 10, 12, 14, 24, 28; $x = 5$, 6, 7) (Raina and Rees, 1983; Hanelt and Mettin, 1989; Maxted *et al*., 1991), in chromosome size and in 2C nuclear DNA amount at the diploid level (3·66–27·07 picograms) (Raina and Rees, 1983; Maxted *et al*., 1991; Bennett and Leitch, 1995; Li and Liu, 1996).

The amount of DNA contained in a nucleus is positively correlated with several cellular parameters, such as the total length and/or volume of chromosomes at metaphase of mitosis and/or meiosis (Bennett, 1987; Grant, 1987). Moreover, the DNA C-value in plants is positively correlated with characteristics which interact to determine the growth rate, and also limit the minimum generation time and type of life-cycle (Bennett, 1987). Many other characters are correlated with the C-value (Bennett, 1987; Grant, 1987). Cytological and chemosystematic studies were formerly carried out in *Vicia* species of Argentina. These include chromosome numbers (Palacios, 1971) and phenolic compound patterns (Ferrari, Palermo and Naranjo, 1986).

In the present work, the karyotype formula, Ag-NOR banding, chromosome volume and DNA content of five species from the mesopotamic-pampean area are compared and their meiotic behaviour analysed. The correlation between these characteristics is discussed.

MATERIALS AND METHODS

Plant material

Vicia macrograminea Burk.: Argentina, Prov. Chaco, Dep. 1° de Mayo, Col. Benitez, CAN 668, 509; *V. graminea* Sm.: Argentina, Chaco, Dep. 1° de Mayo, Cpo. Antequera, CAN 632, 626; Argentina, Buenos Aires, Pdo. de San Isidro, Pya. Anchorena, CAN 625; *V. pampicola* Burk.: Argentina, Entre Ríos, Dep. La Paz, La Paz, CAN 667; Argentina, La Pampa, Dep. Ultracán Chacharramendi, El Carancho, CAN 1070; *V. epetiolaris* Burk.: Argentina, Entre Ríos, Dep. La Paz, La Paz. CAN 669; Argentina, Chaco, Dep. 1° de Mayo, Col Benítez, CAN 1082; Holland, CAN 615; Argentina, Buenos Aires, Pdo. San Isidro, CAN 630; *V.*

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TABLE 1. *Nuclear DNA content, total chromosome volume (TCV) and seed cycle*

Species	Collection number Naranjo (SI)	Asymmetry indexes A_1	Asymmetry indexes A_2	DNA (2C)pg Mean ± s.e.* (samples)	TCV (μm^3) Mean ± s.e.	Seed weight (mg) Mean ± s.e.	Seed cycle†
V. macrograminea	668	0·50	0·17	11·88 ± 0·08[a] (114)	37·89 ± 0·20	7·90 ± 0·12	B-P
V. graminea	632	0·48	0·19	10·14 ± 0·06[b] (80)	34·97 ± 0·88	5·93 ± 0·10	A-B-T
	625			9·90 ± 0·06[b] (40)			
V. pampicola	667	0·53	0·09	10·66 ± 0·07[c] (80)	30·37 ± 1·14	3·31 ± 0·10	A
	1070			9·47 ± 0·08[b] (20)			
V. epetiolaris	669	0·55	0·21	8·30 ± 0·06[d] (30)	21·85 ± 0·68	4·81 ± 0·16	A
	1082			9·25 ± 0·06[e] (60)			
	615			9·20 ± 0·06[e] (20)			
V. nana	1073	0·55	0·13	8·93 ± 0·06[d] (60)	24·52 ± 0·50	1·66 ± 0·02	A

* Different superscripts represent significant differences.
† A, Annual; B-P, biennial to perennial; A-B-T, annual to biennial or triennial (from Burkart, 1967, 1987).

nana Vog.: Argentina, Chaco, Dep. Bermejo, Pto Alcaraz, CAN 1073. Voucher specimens are deposited in the 'Instituto de Botánica Darwinion' Herbarium (SI).

Cytological analysis

Root tips were pretreated for 2·5 h in 0·002 M 8 hydroxyquinoline at 20 °C, fixed in 3:1 (absolute ethanol:acetic acid) and stained in Feulgen solution after 50 min of hydrolysis in 5 N HCl at 20 °C. The total chromosome volume (TCV) was estimated using the formula:

$$\mathrm{TCV} = 2\ (\Pi \times r^2 \times \mathrm{TCL})$$

where r is the average radius of the chromatid and TCL the total chromosome length. The nomenclature used for the description of chromosome morphology is that proposed by Levan, Fredga and Sandbert (1964). To estimate karyotype asymmetry, two numerical parameters were used according to Romero Zarco (1986):

$$A_1 = \frac{\left(\begin{matrix}\text{intrachromosomal}\\ \text{asymmetry index}\end{matrix}\right) 1 - \left[\Sigma_1^n \left(\frac{\text{short arm}}{\text{long arm}}\right)\right]}{n}$$

and

$$A_2 = \frac{(\text{interchromosomal index})\ \text{standard division}\ (S)}{\text{mean length}\ (X)}$$

Both indexes are independent of chromosome number and size. Determination of karyotype parameters was carried out using a Mini-Mop (Kontron) Image Analyser and working with photomicrographs. Mean descriptive values for karyotypes were calculated from a minimum of five scattered metaphase plates measured in each accession. The Ag-NOR banding technique was carried out according to Hizume, Sato and Tanaka (1980) with modifications proposed by Lacadena *et al.* (1984). For meiotic studies, immature flowers were fixed in 3:1 (absolute ethanol:acetic acid) and the anthers squashed in 2% acetic haematoxylin. Slides were made permanent by freezing with liquid CO_2, removing the coverslip, dehydrating in absolute alcohol and mounting in Euparal.

Feulgen staining and cytophotometry

DNA content was measured in 20 telophase nuclei (2C) of the root apex of germinating seedlings. Roots (0·5–1 cm long) were fixed in 3:1 (ethanol:acetic acid) for 1–4 d. The staining method was performed as described in Tito, Poggio and Naranjo (1991). After fixation, root tips were rinsed for 50 min in distilled water. Hydrolysis was carried out with 5N HCl at 20 °C for 50 min (preliminary studies had shown this to be the optimum time for *Vicia* and *Allium cepa*). Root tips were then washed in three changes of distilled water for 10 or 15 min each and stained in Feulgen solution at pH 2·2 for 2 h (Teoh and Rees, 1976). The material was then rinsed three times in SO_2 water for 10 min each, kept in distilled water and squashed in 45% acetic acid. The coverslip was removed after freezing with CO_2 and the slide dehydrated in absolute alcohol and mounted in Euparal. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm using the scanning method in a Zeiss Universal Microspectrophotometer (UMSP 30). The DNA content per basic genome expressed in picograms was calculated using *Allium cepa* 'Ailsa Craig' as a standard (2C = 33·55 pg; Bennett and Smith, 1976). The differences in DNA content were tested by analysis of variance and comparisons between means using Scheffe's method.

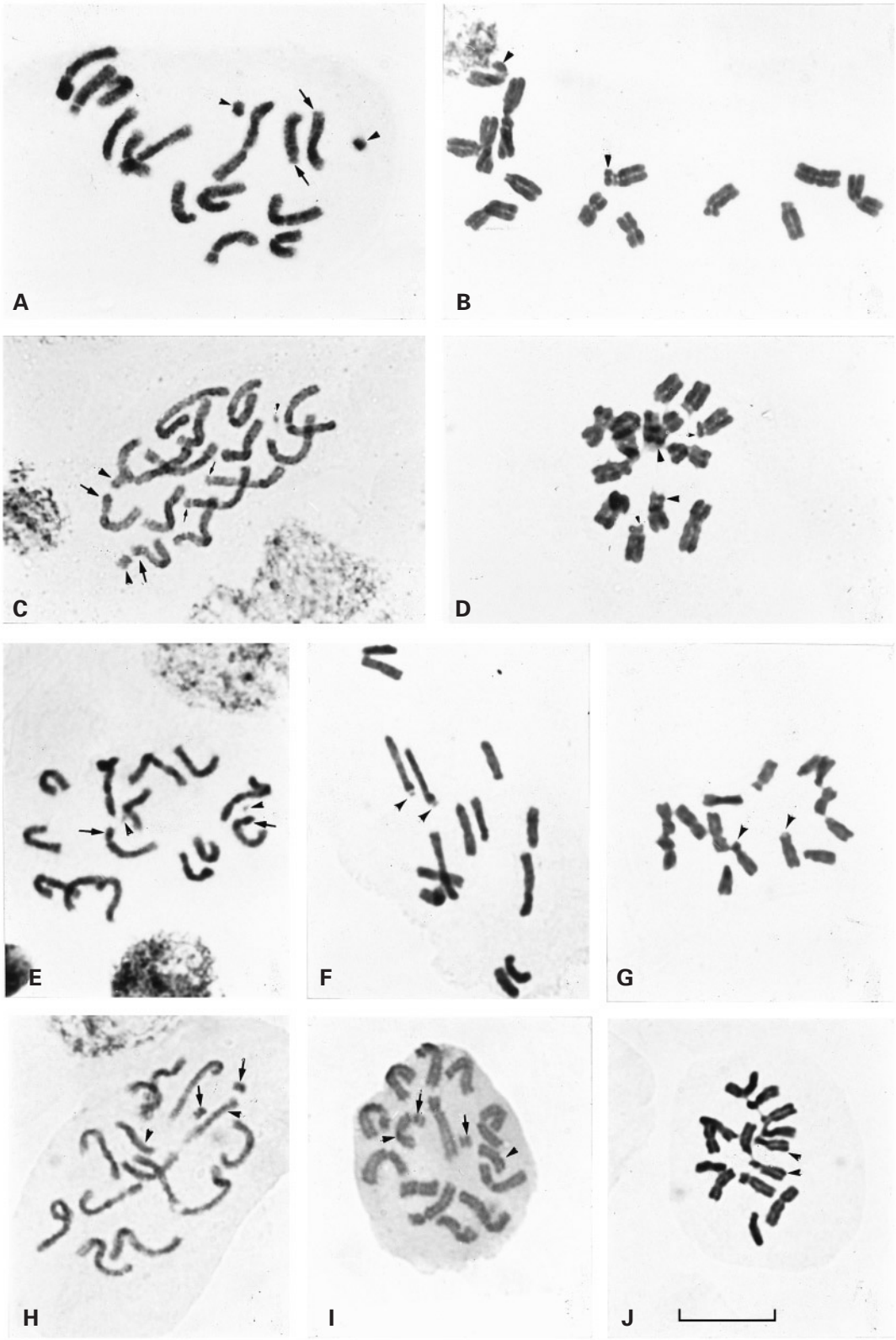

FIG. 1. Somatic chromosomes of *Vicia* spp. A and B, *V. macrograminea*; C and D, *V. graminea*; E, F and G, *V. pampicola*; H, I and J, *V. nana*; see text for explanation. Arrowheads show satellites and arrows show the corresponding chromosome. Bar = 10 μm. The mitotic metaphases B, D, G, and J were used to make the corresponding karyograms of Fig. 3.

Seeds were dried to a constant weight (five replicates of 20 seeds per species) and their dry weight was recorded.

RESULTS AND DISCUSSION

Karyotype

The chromosome number for the five species is $2n = 14$ ($x = 7$), which confirms previous reports (Palacios, 1971; Veerasethakul and Lassetter, 1981; Raina and Rees, 1983). The karyotype characteristics are given here for the first time (Table 1, Figs 1–3), except for *V. graminea*, the karyogram of which was shown previously by Veerasethakul and Lassetter (1981). The karyotype formula is similar (6 **m** + 4 **st** + 4 **t**) for all five species. The karyotype asymmetry of the five species is very similar, particularly regarding values of the A_1 parameter. There are differences, however, in the number and location of nucleolar organizer regions (NOR), in the size of the corresponding satellites, and in the total chromosome volume (TCV) and DNA content (Table 1). In Fig. 3, the type and location of satellites for each species are represented in the corresponding idiograms.

The Ag-NOR banding confirms that the secondary constriction stained positively in all species. The maximum number of nucleoli is two, except in *V. graminea* where there are four. Figure 2 B–D shows Ag-NOR banding in prophase and metaphase of *V. epetiolaris*. In all species, extended secondary constrictions (NOR) were observed in mitotic prophase (Figs 1 A, C, E, H and 2 A). This extension makes the satellites appear detached from the corresponding chromosomes. The same phenomenon has been observed in several plants and has been interpreted differently; for instance, Markarian and Schulz-Schaeffer (1958) considered that the filament connecting the satellite to the chromosome is fragile during prophase and breaks easily. Our observations suggest that the filament of the nuclear organizer undergoes a condensation cycle during prophase. As the nucleolus disintegrates as a result of HCl-hydrolysis, the filament stretches and the satellite consequently moves

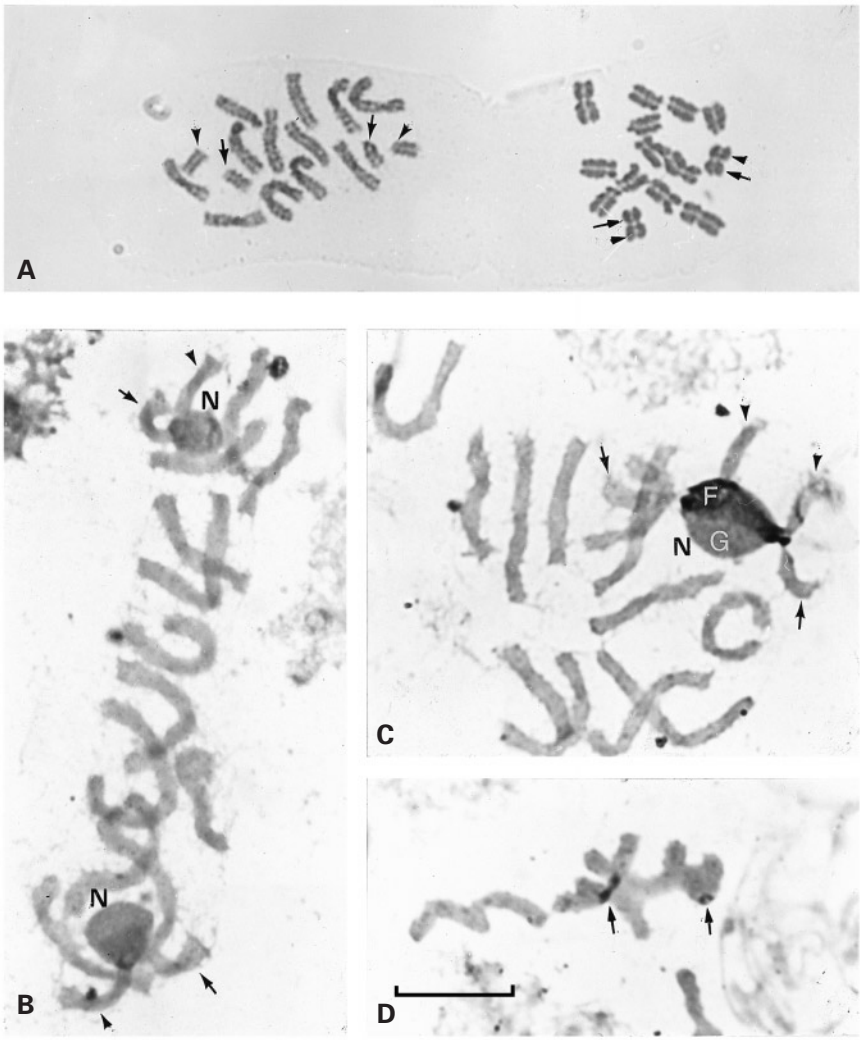

FIG. 2. *Vicia epetiolaris*. A, Adjacent prometaphase (L) and metaphase (R) stained with Feulgen; arrows show the chromosome and arrowheads the linear satellite; the metaphase was used to make the karyogram of Fig. 3. B–D, Ag-NOR banding. B, Two nucleoli (N); arrows show the chromosome and arrowheads show the linear satellite. C, One segregated nucleolus (N); F, fibrilar zone; G, granular zone. D, Arrows show NOR regions. Bar = 10 μm.

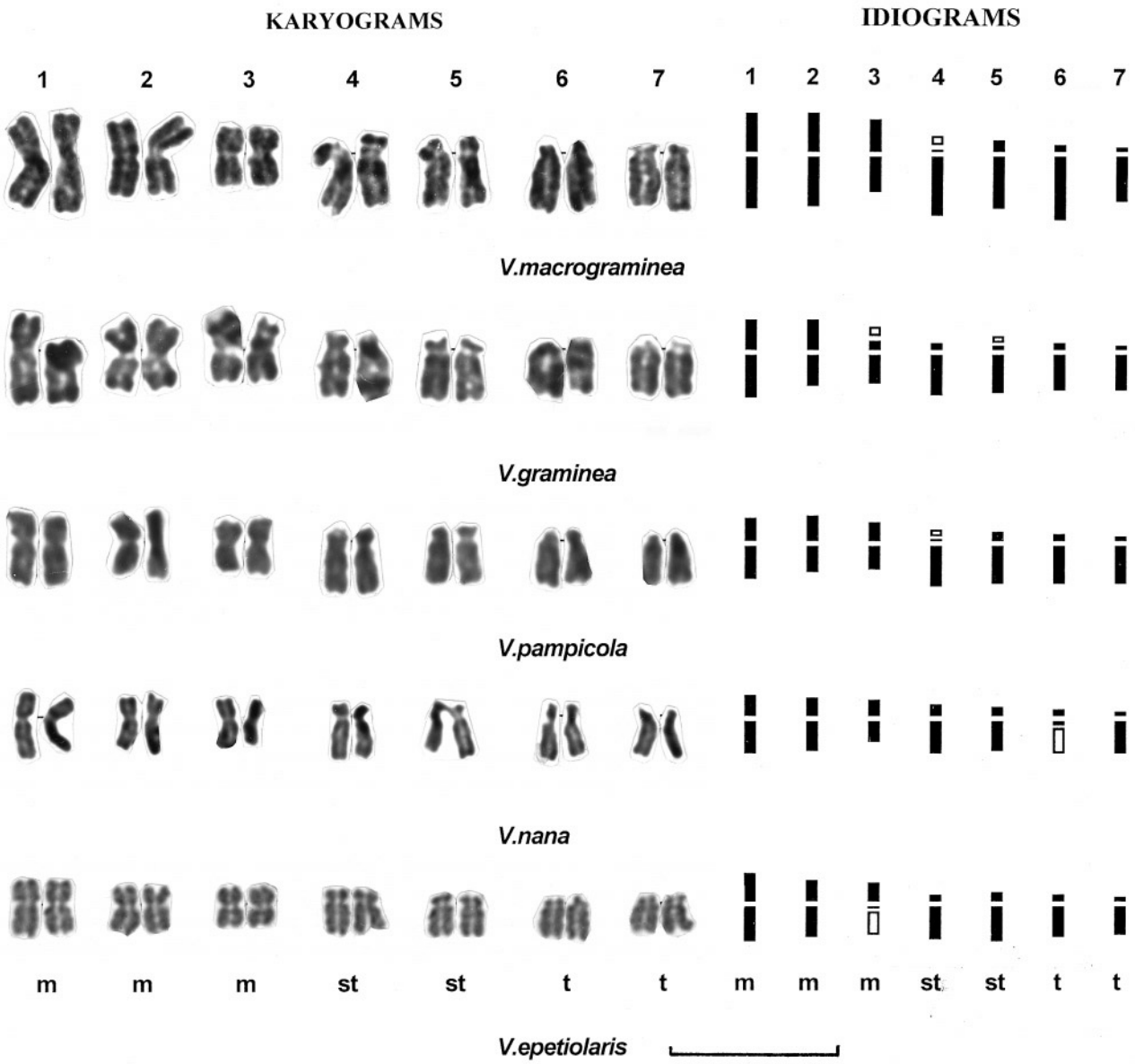

FIG. 3. Karyograms and idiograms of the five species in Figs 1 B, D, G, J and 2 A (right), respectively; in the idiograms the satellites are represented in white. Bar = 10 μm.

away. While metaphase is approaching the filament begins to contract until the satellite returns to its normal position in the chromosome arm, as seen at mid-metaphase. In Fig. 2 B (Ag-NOR banding) a nucleolus can be seen that remains attached to the chromosome and satellite in *V. epetiolaris*; this figure should be compared with the same stage in Fig. 2 A (left) stained with Feulgen, where the nucleolus disintegrated because of the acid hydrolysis.

DNA content

The chromosome length at mitotic metaphase varies between 2·49 and 6·22 μm (Fig. 3). The TCV (total chromosome volume) shows differences among the studied species, being lower in *V. epetiolaris* (21·8 μm^3) and *V. nana* (24·5 μm^3) and higher in *V. macrograminea* (37·9 μm^3) (Table 1). A similar relation in DNA content was found among these species. Analysis of variance and the Scheffe contrasts showed significant differences in DNA content ($F = 21{\cdot}79$, $P < 0{\cdot}001$) (Table 1). Significant positive correlation was found between TCV and DNA content ($r = 0{\cdot}92$, $P < 0{\cdot}0001$). In the subgenus *Vicia*, Maxted *et al.* (1991) and Raina (1990) observed that the distribution of DNA content among species seemed to be independent of the taxonomic position. However, in the taxa studied here the DNA content variation among species is in agreement with the morphological variation. Analysis of the morphological characteristics (Burkart, 1967, 1987) indicates that *V. macrograminea* and *V. nana* are the species with the greatest differences among those studied in this paper.

Chooi (1971) studied the DNA content and karyotype of 45 species of *Vicia* and concluded that the increase or

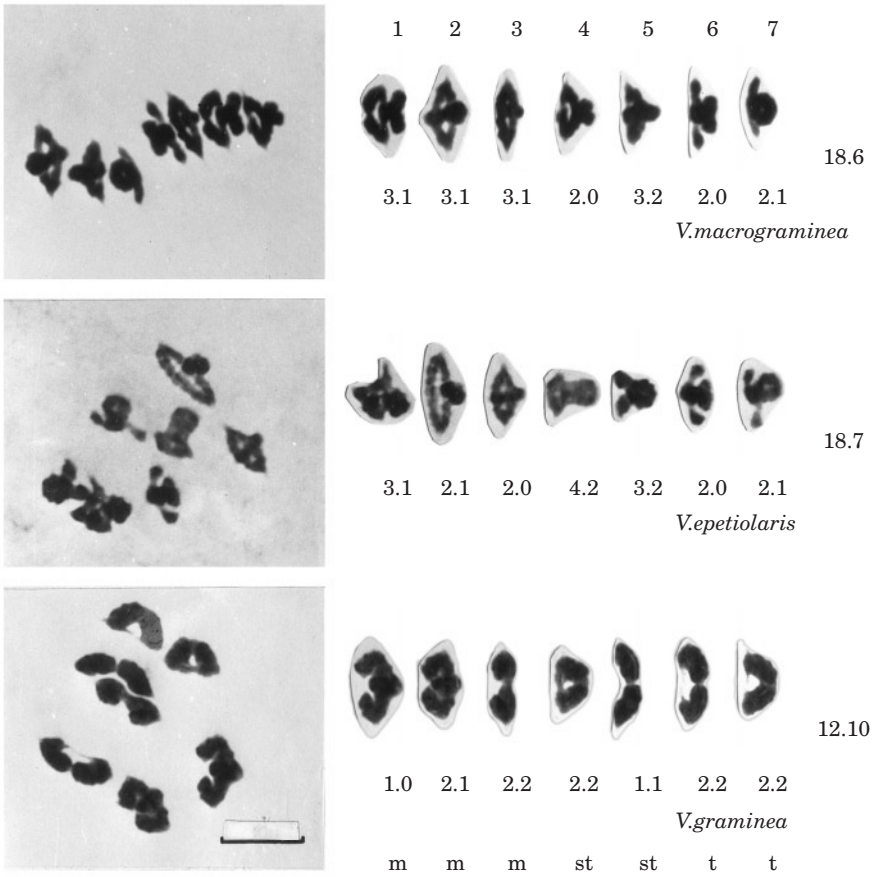

FIG. 4. Meiotic metaphase 1 of *V. macrograminea*, *V. epetiolaris* and *V. graminea* and their corresponding meiotic karyograms. The numbers under each bivalent represent both the total chiasmata and the terminal ones (e.g. 3.1); those on the right represent the total and terminal chiasmata per cell (e.g. 18.6). Bar = 10 μm.

decrease in DNA content per cell could have been a way of genome diversification. He found that in the Section to which *V. graminea* belongs, perennial species generally possess higher DNA content than annual ones and pointed out that the evolution from perennial to annual habit would proceed with DNA losses. Among the species studied here, *V. macrograminea*, which has the highest DNA content, is biennial or perennial, whereas the remaining species are annual, except *V. graminea* which can be annual or biennial (Table 1). Shortening of the cell cycle with the accompanying diminution of DNA content has also been found in other groups. Bennett (1972, 1987) found DNA content was correlated with the minimum generation time. Tito *et al.* (1991) found that DNA content is positively correlated with the length of the vegetative period in lines of maize. Several cases of species where a high TCL and TCV is accompanied by a high amount of DNA have been mentioned in the literature (Poggio and Hunziker, 1986; Poggio, Wulf and Hunziker, 1986; Poggio and Naranjo, 1990).

Rees (1984) proposed two strategies to explain the non-random distribution of DNA changes: proportional and equal addition of DNA content to each chromosome. Present results show that in the five species of *Vicia* studied here, the distribution of DNA changes among chromosome complements is not random. The addition of a proportional amount of DNA relative to the length of each chromosome arm has maintained a constant karyotype formula and the degree of chromosome asymmetry. In species from another Section of *Vicia*, increase in DNA content is distributed equally among all the chromosomes of the complement, leading to a more symmetrical karyotype (Raina and Rees, 1983). Both types of strategy (proportional and equal addition of DNA content to each chromosome) are present in the genus *Vicia*.

In many plants including *Allium* and *Vicia*, a linear relation was found between DNA content and seed weight (Bennett, 1987). A similar relation was found in some species and cultivars of *Phaseolus* (Castagnaro, Poggio and Naranjo, 1990). Among the species of *Vicia* analysed here, *V. macrograminea*, the species with the highest DNA content, has the highest seed weight (7·9 mg) (Table 1). *V. nana*, with a low DNA content, has very light seeds (1·66 mg). *V. epetiolaris*, with a DNA content equivalent to that of *V. nana*, has heavier seeds (4·81 mg) even though this weight does not reach that of *V. graminea* seeds (5·93 mg). *V. pampicola*, however, does not fit this scheme as it has a high DNA content but light seeds (3·33 mg) (Table 1). This indicates that the relationship between DNA content and seed weight is not straightforward and requires further study.

TABLE 2. *Meiotic configuration at Metaphase I*

Species	Number of cells studied	Ring bivalents Mean ± s.e.* (range)	Total chiasmata Mean ± s.e. (range)	Terminal chiasmata Mean ± s.e. (range)	Ri†
V. epetiolaris (CAN 630)	56	$5{\cdot}39 \pm 0{\cdot}11^{a}$ (4–7)	$17{\cdot}55 \pm 0{\cdot}27^{a}$ (13–21)	$6{\cdot}57 \pm 0{\cdot}20^{a}$ (4–10)	24·55
V. macrograminea (CAN 509)	121	$4{\cdot}95 \pm 0{\cdot}07^{a}$ (4–7)	$17{\cdot}88 \pm 0{\cdot}14^{a}$ (15–22)	$5{\cdot}87 \pm 0{\cdot}14^{a}$ (3–9)	24·88
V. graminea (CAN 626)	61	$4{\cdot}82 \pm 0{\cdot}11^{a}$ (3–7)	$13{\cdot}44 \pm 0{\cdot}13^{b}$ (10–17)	$9{\cdot}57 \pm 0{\cdot}21^{b}$ (6–14)	20·44

* Different superscripts represent significant differences in each column.
† Recombination index (Darlington, 1958) = $n(7)$ + total chiasmata.

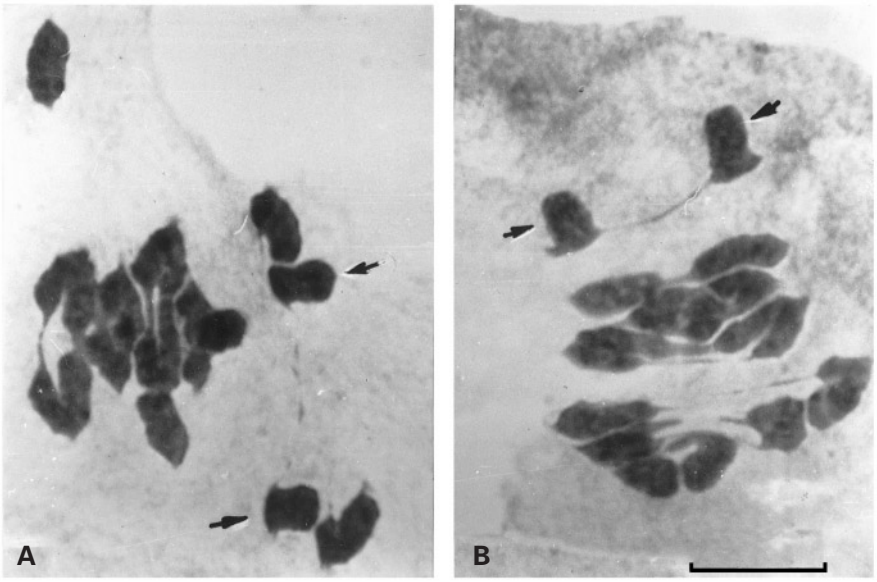

FIG. 5. *V. graminea*. Metaphase 1 showing 'ditactic' bivalents (with one chiasma in the short arms). Bar = 10 μm.

Meiotic behaviour

All the species showed regular meiosis, forming seven bivalents at Metaphase I (Fig. 4). Significant differences in the frequency of ring bivalents and the frequency and localization of chiasmata were found between three representative species (Table 2). Consequently, there are differences in the recombination index among species (Table 2). *V. macrograminea*, *V. epetiolaris*, *V. pampicola* and *V. nana* have similar meiotic behaviour. *V. graminea* differs significantly from the other species (Table 2, Fig. 4), because of the reduction of chiasma frequency and the tendency towards localization of chiasmata in the distal region. Usually, **t** chromosome number 7 (Fig. 3) of these species does not form chiasmata in its short arm (Fig. 4, see bivalents no. 7 of *V. macrograminea* and *V. epetiolaris*), but in *V. graminea* the short arm showed a chiasma frequency much higher than expected on the basis of its minute size. In extreme cases, a large proportion of the chiasmata is confined to short arms, whereas long arms have no chiasmata. The bivalent then appears strikingly different from the usual type at Metaphase I, since the long arms are orientated parallel to the equator of the spindle (Fig. 5A, B). McClung (1928) first recognized bivalents of this type in three species of the grasshopper *Stethophyma* and called them 'ditactic' bivalents. This type of bivalent has also been found in other genera of grasshoppers (see review in White, 1973).

CONCLUSIONS

(1) In general, the chromosome differences between the *Vicia* species studied here are congruent with the relationship supported on morphological data reported for these species. (2) Despite the constancy of the karyotype formula and asymmetry, each species displays a characteristic number and position of the nucleolar organizer region (NOR), different sizes of the respective satellites, and different total chromosome volume (TCV) and DNA content. Moreover, significant differences were found between the meiotic behaviour of *V. graminea* compared to that of the other species. (3) The constancy of the karyotype formula and asymmetry, and the change in chromosome size and DNA

content suggest that karyotype orthoselection has occurred in the group of *Vicia* species studied here, by means of the addition an amount of DNA proportional to the relative length of each chromosome arm. In another group of species of *Vicia*, the increase in DNA content is effected through an equal addition of DNA content to each chromosome, leading to a more symmetrical karyotype (Raina and Rees, 1983) as discussed above. Therefore, in *Vicia* there are at least two strategies by which DNA changes are distributed (proportional and equal addition of DNA content to each chromosome); both are probably adaptive.

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