VARIATION IN NUCLEAR DNA CONTENT IN THE GENUS VICIA

W. Y. CHOOI

Department of Botany, The University of Adelaide, Adelaide, South Australia 5001

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IN recent years much evidence has been gathered to support the idea that many organisms contain far more DNA than would be required to specify the proteins required for structural and metabolic functions if each DNA message were carried only once. In addition, large changes in DNA content per genome can evolve quite rapidly (see review by HOLLIDAY 1970). In view of these findings, three main hypotheses have been proposed:

1. *The segmental duplication hypothesis*: Rearrangement of a chromosomal segment, consequent upon breakage and rejoining, may lead to the formation of gametes bearing the segment in duplicate (REES and JONES 1967; JONES and REES 1968).

2. *The local multiplicity hypothesis*: Short chromosomal segments, comparable in size to that of a gene, are replicated and joined end to end within a single-stranded chromosome. When referring to this hypothesis, the chief mechanism implied will be that proposed by KEYL (1965). However, for the purpose of this paper, duplications which are the result of unequal crossing over at meiosis, as at the Bar locus in *Drosophila melanogaster* (STURTEVANT 1925), are indistinguishable.

3. *The lateral multiplicity hypothesis*: The total basic genetic information in the chromosome is multiplied to produce a multistranded chromosome.

Evidence distinguishing between these hypotheses must also be relevant to the problem of how DNA is organized into chromosomes. Various other approaches have been made toward solving this problem; these include electron microscopy of chromosomes (KAUFMANN, GAY and MCDONALD 1960; DUPRAW 1965; SOLARI 1967; LAFONTAINE and LORD 1969; WOLFE and MARTIN 1968; ABUELO and MOORE 1969), kinetics of chromosome breakage (GALL 1963), the study of meiosis in hybrids formed between species with different DNA contents (KEYL 1965; JONES and REES 1968), studies on the mechanisms of chromosome replication (TAYLOR 1958; PEACOCK 1963; HEDDLE 1969), and radiosensitivity of chromosomes (SPARROW and EVANS 1961). In general, these attempts have achieved only limited success. More definite results have, however, been achieved in studies using the methods of DNA-DNA hybridization; these have shown that many organisms contain a fraction of DNA with nucleotide sequence of varying degrees of repetition (BRITTEN and KOHNE 1968; WALKER, MCLAREN and FLAMM 1969).

The following is an investigation of the nuclear DNA variation and karyotype variation in 45 species of the genus Vicia. It reveals that widespread changes in nuclear DNA content and karyotype accompanied the divergence and evolution

of species within the genus. In addition, it provides information about the nature and distribution of the chromosome structural changes which account for the variation in nuclear DNA.

MATERIALS AND METHODS

Measurement of DNA content per cell by Feulgen microspectrophotometry: Seeds were germinated in vermiculite in perspex boxes at room temperature and kept moist with tap water. Seedlings were selected in which radicles were approximately 2 cm long. The actively growing roots (laterals in the case of *V. faba*) were washed free of vermiculite and drained of surface water. Root tips of two species were fixed simultaneously in the same vial, viz., *V. faba* as the control and the species with which it was compared. These were subsequently treated identically for the entire procedure. Root tips were fixed in acetic–alcohol (1 part acetic acid : 3 parts ethyl alcohol) for 10 min and transferred through a descending alcohol series. After hydrolysis the root tips were squashed one at either end of a single slide and stained with leuco basic fuchsin. The rest of the procedure used is as described by MARTIN and HAYMAN (1965).

Several slides of each of the 45 species, including *V. faba*, were prepared by the method described above. Photometric measurements were made using a Barr and Stroud integrating microdensitometer GN2 (DEELEY 1955). Fifty cells were measured from at least five slides for each species. The DNA content per cell was calculated for each species relative to *V. faba* (*V. faba* was given the arbitrary value of 100). The results were expressed as the relative DNA content per cell (RDC/cell) ± standard error.

Karyotype analysis, measurement of area, and relative DNA content per chromosome arm: Actively growing seedlings with roots about 2 cm long were transferred to a solution of 8-hydroxyquinoline at 18°C for 3–3¾ hr depending on the size of the chromosomes. The seedlings were then fixed in acetic alcohol (1:3) for 10 min, transferred through a descending series of alcohols and hydrolyzed in 1N HCl at 60°C for about 10 min.

Roots tips (2 mm) were squashed in acetic orcein (1% orcein in 45% acetic acid) (DARLINGTON and LA COUR 1960). Metaphase cells with flat, well spread chromosomes were photographed using Ilford Micro-neg pan film and printed on Kodak F4 paper.

In previous studies (MARTIN and HAYMAN 1965), the DNA content of a chromosome arm has been calculated from the mean percentage length and relative DNA content. In this study, mean percent area, rather than mean percent length was measured, because this quantity proved to be more appropriate for chromosomes with small and, following 8-hydroxyquinoline treatment, highly contracted short arms. The area of each chromosome arm was measured by weighing a carefully cut-out photograph of the chromosomes on an E. Mettler balance, Type H6. The error involved in weighing was minimized by using the maximum possible enlargement without loss of resolution. The area of each chromosome arm was calculated as a percentage of the total area of all the chromosomes in the cell and the mean obtained from both homologues in all the cells measured. In some species, some of the chromosomes were of about the same size and shape, making it difficult or impossible to characterize the individual pairs. In these cases the chromosomes were grouped.

The amount of DNA in a chromosome arm was calculated by multiplying its mean percent area by the relative DNA content per cell. The assumptions involved were recognized but, considering the way the data were to be used, this was thought to be justified. These amounts are in arbitrary units but are directly comparable from species to species. The average relative DNA content per chromosome was calculated for each species by dividing the relative DNA content per cell by the haploid chromosome number.

Sometimes a test of significance was carried out between the areas of the chromosome arms of two species. The chromosome arms are considered to be the same (or shared) if they are not significantly different at the 5% level of significance.

RESULTS

Variation in DNA content per cell between Vicia species: The basic chromosome number, relative DNA content per cell, and average DNA content per chromosome of the 45 species studied are shown in Table 1.

REES *et al.* (1966) and MARTIN (1968) have independently reported the DNA contents per cell of seven and twelve species, respectively. Except for *V. sepium* (reported in both the above papers) and except for *V. narbonensis* and *V. hirsuta* (reported by REES *et al.* 1966), the DNA values reported here are generally in agreement with those in the above two reports.

From Table 1, it can be seen that although the diploid chromosome number varies from only 10 to 14 (except in the two polyploid species) throughout the genus Vicia, relative DNA content per cell varies approximately six-fold from 15.7 in *V. eriocarpa* to 100 in *V. faba.*

Karyotypes of Vicia species: Although the karyotypes of 45 species were analyzed, the karyotypes of only five pairs of species will be compared. These are shown in Figures 6A, B, C, D, and E.

DISCUSSION

If the genus Vicia is considered as a whole, the relative DNA content per cell of the 45 species throughout the range from 15.7 to 100 forms a continuous series without any apparent distributional pattern. However, if the genus is split into its four component sections (BALL 1968), the DNA contents per cell in the sections Vicia and Faba appear to have disjunct distributions while those in the sections Ervum and Cracca do not (Figure 1). There seem to be three disjunct groups in the two sections Vicia and Faba; this will be discussed shortly.

It has not been possible to make a detailed comparison between the direction of DNA change (i.e., gain or loss) and the phylogeny of the species since there has not been a taxonomic study placing species in a phylogenetic sequence within sections of the genus. The four sections have, however, been placed in order of morphological evolutionary advancement, Ervum being the most primitive, then Cracca, with Vicia and Faba the most advanced (CHOOI 1970). Since there is a larger range of DNA contents per cell in the more advanced sections (Figure 1), it can be said that evolutionary increase in DNA content per cell has probably accompanied morphological advancement of the species.

Figure 2 shows that as would be expected, there is a linear relationship between relative DNA content per cell and average relative DNA content per chromosome. The four most notable exceptions are of interest. *V. hajastana* and *V. melanops* seem to have larger chromosomes than expected from their DNA contents. This can be accounted for in *V. melanops* if its large metacentric chromosome (chromosome *2*) is postulated to have been derived from the fusion of two smaller acrocentrics (Figure 3). If the relative DNA content per cell is then divided by a haploid chromosome number of 6 rather than 5, its DNA content per cell would then bear the same relationship to the average DNA content per chromosome as the rest of the other species. The same explanation can be

TABLE 1

DNA content per cell, haploid chromosome number, average DNA content per chromosome, and life cycle of 45 species of Vicia

	DNA value (arbitrary units) ± Standard error	Haploid chromosome number	Average DNA per chromosome	Annual (a) or perennial (p)
Section Faba				
V. bithynica	34.3 ± 1.3	7	4.9	a
V. faba	100 (standard)	6	16.7	a
V. narbonensis	54.5 ± 1.8	7	7.8	a
Section Vicia				
V. galeata	32.2 ± 0.6	7	4.6	a
V. grandiflora	24.9 ± 0.8	7	3.6	a
V. hajastana	56.2 ± 1.7	5	11.2	a
V. hybrida	51.1 ± 1.3	6	8.5	a
V. hyrcanica	50.5 ± 0.3	6	8.4	a
V. incisaeformis	35.5 ± 0.3	7	5.1	a
V. lathyroides	19.7 ± 1.2	6	3.3	a
V. lutea	55.6 ± 1.3	7	8.0	a
V. melanops	86.1 ± 1.5	5	17.2	a
V. michauxii	62.3 ± 0.3	7	8.9	a
V. pannonica	50.9 ± 1.0	6	8.5	a
V. peregrina	71.1 ± 0.6	7	10.2	a
V. sativa	19.8 ± 1.0	6	3.0	a
subspecies cordata	17.2 ± 0.9	5	3.4	a
macrocarpa	19.3 ± 0.5	6	3.2	a
angustifolia	23.0 ± 1.0	6	3.8	a
pilosa	18.9 ± 0.9	7	2.7	a
V. sepium	35.4 ± 0.7	7	5.1	p
Section Cracca				
V. articulata	45.3 ± 1.5	7	6.5	a
V. atropurpurea	18.2 ± 0.9	7	2.6	a/p
V. benghalensis	26.2 ± 0.2	7	4.2	a/p
V. biennis	22.4 ± 0.4	7	3.2	a
V. cassubica	31.0 ± 0.2	6	5.2	p
V. cracca	39.8 ± 0.4	14	2.8	p
V. dumetorum	55.8 ± 0.5	7	8.0	p
V. neglecta	35.4 ± 0.3	6	5.9	a
V. orobus	40.3 ± 0.3	6	6.7	p
V. pisiformis	49.9 ± 0.3	6	8.3	p
V. ramuliflora	35.1 ± 0.1	6	5.9	p
V. sylvatica	64.6 ± 0.8	7	9.2	p
V. tenuifolia	33.5 ± 0.4	12	3.0	p
V. unijuga	36.3 ± 0.3	6	6.1	p
V. villosa	17.1 ± 0.5	7	2.5	a
subspecies dasycarpa	24.4 ± 1.4	7	3.5	a
eriocarpa	15.7 ± 0.3	7	2.3	a
Section Ervum				
V. disperma	25.3 ± 1.1	7	3.6	a
V. ervilia	38.7 ± 0.9	7	5.5	a
V. graminea	38.8 ± 0.3	7	5.5	a
V. hirsuta	30.0 ± 0.7	7	4.3	a
V. meyeri	47.0 ± 0.3	7	6.7	a
V. pubescens	19.8 ± 0.2	7	2.8	a
V. tetrasperma	27.80 ± 0.7	7	4.0	a

a/p ≡ annual, sometimes perennial.

given to *V. hajastana* if its large satellite chromosome (chromosome *1*) is postulated to have been derived from the fusion of two smaller chromosomes.

The two polyploid species *V. tenuifolia* and *V. cracca* differ from the rest of the diploid species in that they have smaller chromosomes than expected from their DNA contents. It has often been inferred that polyploidization results in a decrease in chromosome size (MANTON 1950; DARLINGTON 1958; SOUTHERN 1967; GRANT 1969). The alternative explanation is that only Vicia species with the smallest chromosomes (average of 2–3 units per chromosome) can form viable polyploids. Which of the two hypotheses is correct is uncertain.

Table 1 shows that the average DNA content per chromosome throughout the genus Vicia varies approximately over a 7-fold range from 2.3 (in *V. eriocarpa*)

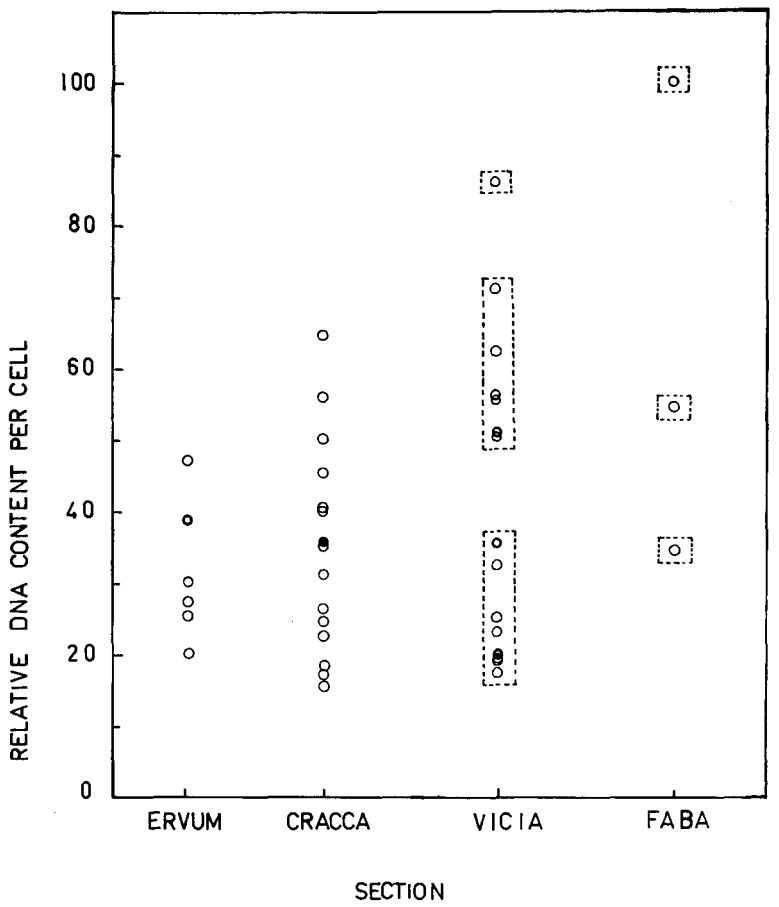


FIGURE 1.—Graph showing the distribution of the relative DNA content per cell of 45 species of Vicia in the four sections of the genus.

Open circle denotes the relative DNA content per cell of one species.

Thicker open circle denotes the relative DNA content per cell of two species.

Thickest open circle denotes the relative DNA content per cell of three species.

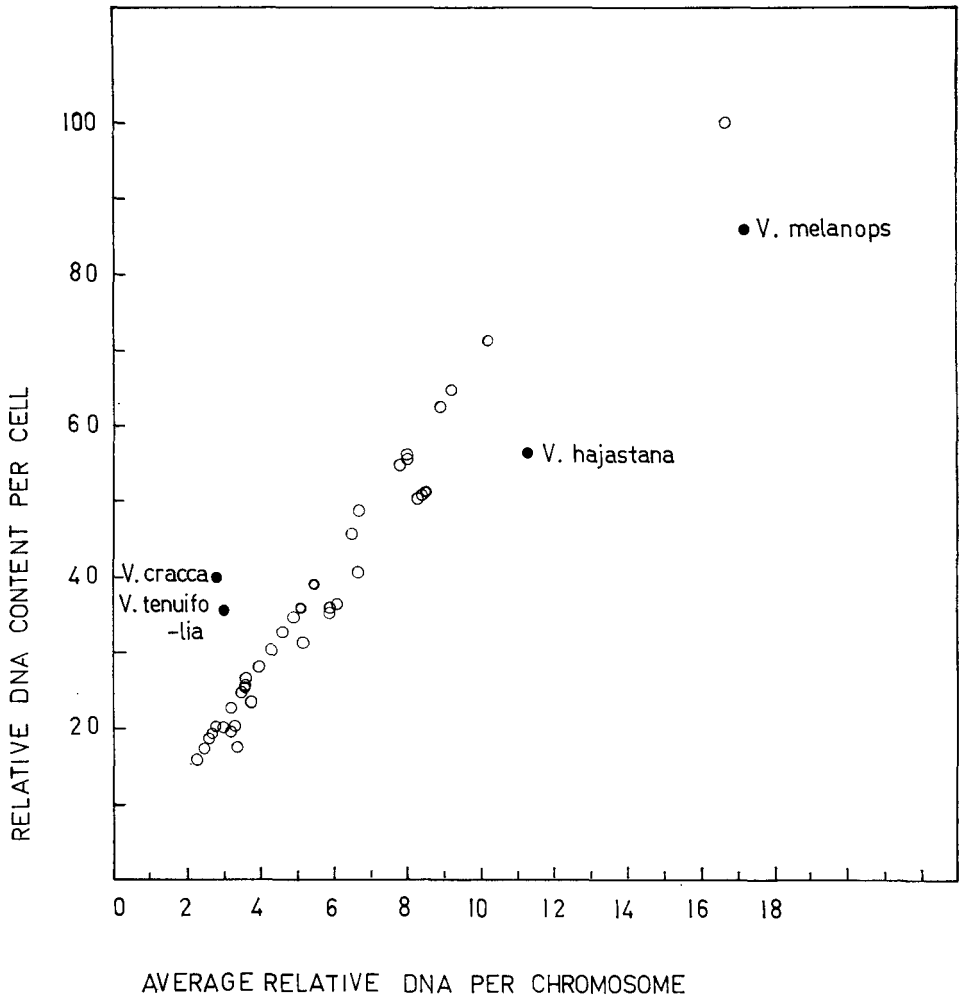


FIGURE 2.—Graph showing the relationship between the relative average DNA content per chromosome and the relative DNA content per cell for 46 species of Vicia.

Open circle denotes the relative DNA content per cell and the relative average DNA content per chromosome of one species.

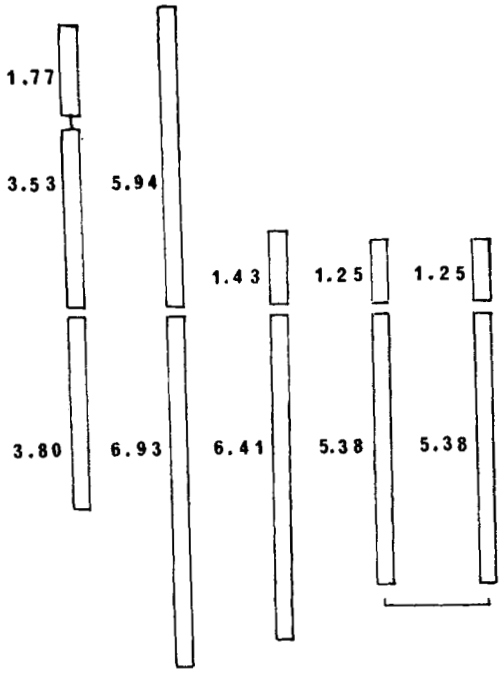
Thicker open circle denotes the relative DNA content per cell and the relative average DNA content per chromosome of two species.

FIGURE 3.—The karyotypes of *V. melanops* and *V. hajastana*. RDC/cell = relative DNA content per cell; aar = average arm ratio of the chromosome complement.

V. melanops

RDC / cell = 86.13 ± 1.48

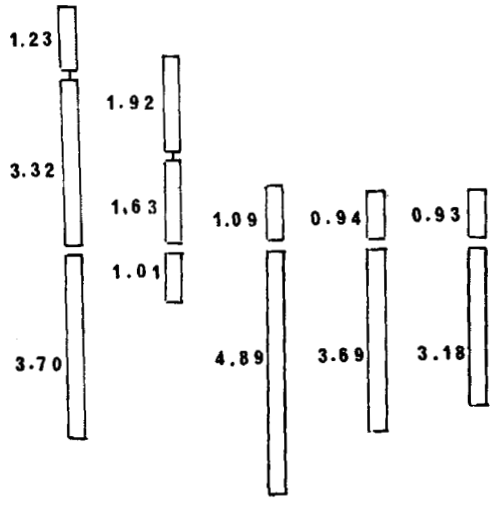
2n = 10 aar = 3.1



V. hajastana

RDC / cell = 56.22 ± 1.73

2n = 10 aar = 3.3



to 17.2 (in *V. melanops*). If the genus is considered as a whole, the values for chromosome sizes form a continuous series; however this is not so within sections. Continuous series occur in the sections Ervum and Cracca (Figure 4) but in the section Vicia there are three disjunct groups about means of 4.0, 9.5, and 17.2. In the section Faba the only three values are 4.9, 7.8, and 16.7. It is noteworthy that: (1) In all sections (except in the section Faba which, because of its small size, can be ignored) there are species with very small chromosomes. (2) There is little variation in size between chromosomes constituting a chromosome complement.

Table 2 shows that variation in chromosome sizes within species is very significantly smaller than variation in chromosome sizes between species; the ratio of between-species variance to the within-species variance is 130.

Variation in DNA content per cell between taxonomically synonymous species and taxonomic subspecies: According to Ball (1968), *V. dasycarpa* (RDC/cell = 24.4 ± 1.4) and *V. eriocarpa* (RDC/cell = 15.7 ± 0.3) are subspecies of *V. villosa* (RDC/cell = 17.1 ± 0.5). A direct comparison (instead of indirectly via *V. faba*)

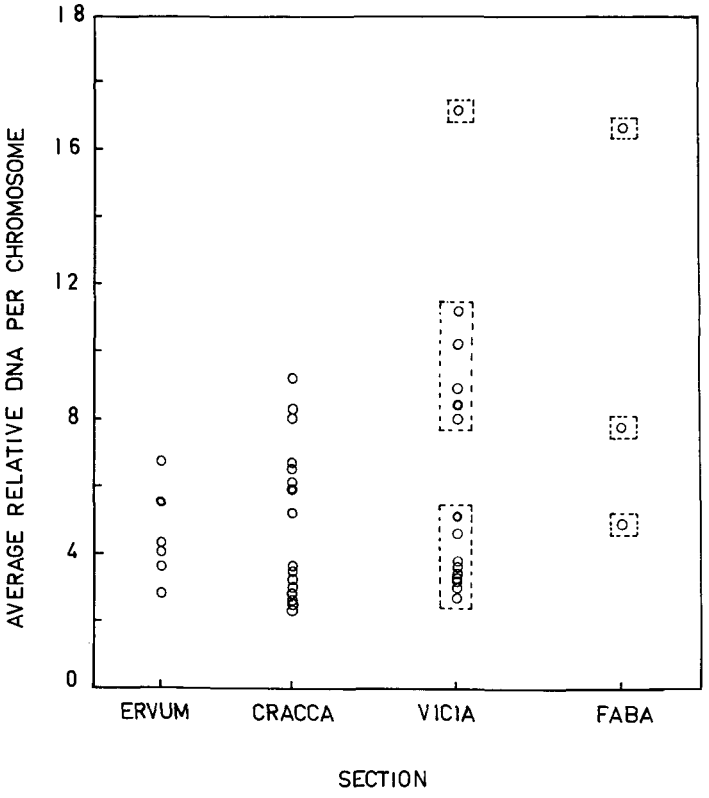


Figure 4.—Graph showing the distribution of average DNA per chromosome of 45 species of Vicia in the four sections of the genus.

Open circle denotes the average DNA per chromosome of one species.

Thicker open circle denotes the average DNA per chromosome of two species.

TABLE 2

Chromosome size (amount of DNA in arbitrary units) in 45 Vicia species

Source	Analysis of variance: Sums of squares	Degrees of freedom	Estimated variance
Variation between species	3035.78	44	70.60
Variation within species	141.34	263	0.537
Total	3177.12	306	
		Variance ratio = 130.7	
		$P < 0.001$	

TABLE 3

The relative DNA content per cell of V. dasycarpa *versus that of* V. villosa

	Analysis of variance: Degrees of freedom	Sums of squares	Mean square	F value
Between slides	1	39.5	39.5	7.6***
Between species	1	406.7	406.7	78.1***
Interaction	1	4.3	4.3	0.8 n.s.
Within samples	76	396.0	5.2	
Total	79	846.4		
	Mean of *V. dasycarpa* = 18.8			
	Mean of *V. villosa* = 14.2			

between *V. dasycarpa* and *V. villosa* shows that the difference is highly significant (Table 3).

Although *V. benghalensis* (RDC/cell = 26.2 ± 0.2) is regarded as taxonomically synonymous with *V. atropurpurea* (RDC/cell = 18.2 ± 0.9) (METTIN and HANELT 1968), their relative DNA contents per cell also appear to be significantly different. A similar result is obtained when a direct comparison (as in Table 3) is made between *V. benghalensis* and *V. atropurpurea*. *V. benghalensis* has 44% more DNA per cell than *V. atropurpurea*.

V. cordata, *V. macrocarpa*, *V. angustifolia*, and *V. pilosa* are regarded as subspecies of *V. sativa* (HANELT and METTIN 1966). *V. macrocarpa* (RDC/cell = 19.3 ± 0.5), *V. cordata* (RDC/cell = 17.2 ± 0.9), and *V. pilosa* (RDC/cell = 18.9 ± 0.9) have relative DNA contents per cell that are similar to that of *V. sativa* (RDC/cell = 19.8 ± 1.0). However, a direct comparison (as in Table 3) between *V. angustifolia* (RDC/cell = 23.0 ± 1.0) and *V. sativa* shows that their relative DNA contents per cell are significantly different.

These examples show that increase or decrease in DNA content per cell is probably one way of species diversification and this may or may not be manifested at the morphological level.

Correlation between reduction in DNA content per cell and reduction in life cycle: In the genus Vicia, the most primitive and the most advanced sections (Vicia and Faba) consist predominantly of annuals. The section Cracca, how-

ever, consists of both annuals and perennials. In the section Cracca, with two exceptions, perennials have more DNA per cell than annuals (Figure 5). Because HANELT and METTIN (1970) have indicated that evolution has proceeded from a perennial habit to an annual habit in the section Cracca and because perennials in the section Cracca have larger DNA contents per cell than annuals. evolution from a perennial habit to an annual habit in the section Cracca is probably accompanied by a loss in DNA per cell. The morphological data of HANELT and METTIN (1970) have also shown that *V. grandiflora* (annual) is very closely related to *V. sepium* (perennial). Their biochemical evidence appears to favor the suggestion that *V. grandiflora* is a derivative of *V. sepium.* If this is true, evolution has probably proceeded from a perennial habit to an annual habit. In this work it has been shown that *V. sepium* has 10.5 units more DNA per cell and larger chromosomes than *V. grandiflora* (Table 1). It appears, therefore, that a shortening of the life cycle is accompanied by a loss in DNA content per cell. The fact that the most primitive section (Ervum) of the genus consists only of annuals does not spoil the correlation since the section Ervum is believed to have broken away from the original group of species and to have undergone independent speciation (HANELT and METTIN 1970).

Karyotypes of Vicia species: One difficulty in interpreting the direction of karyotype change in the genus Vicia has been the fact that species within the genus have not been placed in a taxonomic phylogenetic sequence. However, because some species are known to be taxonomically more closely related than others, the karyotypes of closely related species which have significantly different DNA contents per cell can be examined in pairs to determine the relationship between evolutionary changes in DNA content per cell and changes in karyotype. Although the karyotypes of 45 species were analyzed, the karyotypes of only five pairs of species are compared here. The degree of taxonomic relatedness between Vicia species is based on the work of METTIN and HANELT (1968) and BALL (1968).

The karyotypes of *V. meyeri* and *V. hirsuta*, *V. tetrasperma* and *V. pubescens*, *V. benghalensis* and *V. atropurpurea*, *V. villosa* and *V. dasycarpa*, and of *V. narbonensis* and *V. faba* were compared. As is readily seen in Figures 6A. B, C, D, and E, the additional DNA found in one of each of the species pairs is: (1) located in both the long and short arms of all the chromosomes in the genome (except in *V. benghalensis* where the additional DNA is distributed to six out of seven pairs of chromosomes in its genome); and (2) an addition which is not evenly or proportionally distributed throughout all the chromosome arms. Usually both long and short arms increase, but this situation does not always hold. e.g., in the pair *V. narbonensis–V. faba.* This has resulted in different arm ratios and hence changes in karyotype.

The evidence presented above suggests that provided the number of sites involved is large, either segmental duplications or local multiplicity can best account for the change in average DNA content per chromosome in the species pairs considered and so in the whole genus. If lateral multiplicity were the whole explanation, the increase in DNA content per cell would be expected to be evenly

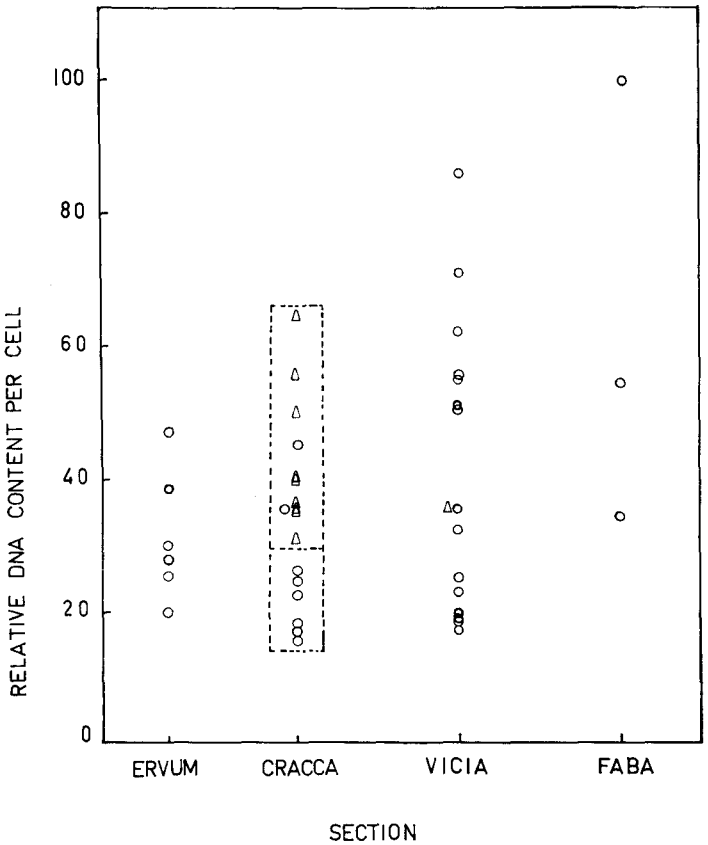
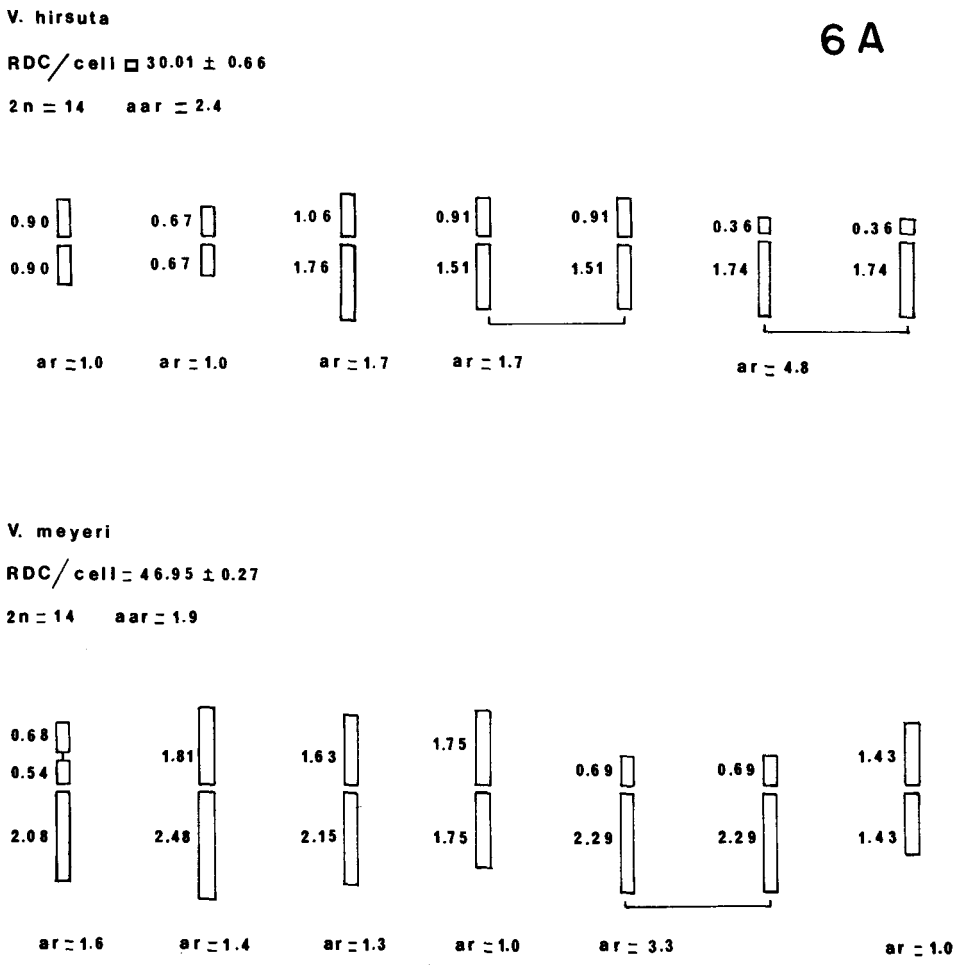


FIGURE 5.—Graph showing the relative DNA contents per cell of annual (○) and perennial (△) species of Vicia in the four sections of the genus.

distributed to all the chromosomes and chromosome arms. Further relevant evidence is derived from the distribution of average DNA content per chromosome in the sections Ervum and Cracca. A continuous distribution of average DNA content per chromosome is found in the two sections. Increase in DNA content per cell which takes place in small steps with no apparent distributional pattern is not consistent with lateral multiplicity according to which disjunct distributions forming a geometric series would be expected.

However, there are two observations that appear to be more consistent with a lateral multiplicity hypothesis. They are: (1) chromosomes constituting a genome are fairly uniform in size (Table 2); and (2) species averages of DNA content per chromosome fall into three disjunct groups in the two sections Vicia and Faba. In the section Vicia the disjunct groups form a 4 : 9.5 : 17.2 series, while a 4.9 : 7.8 : 16.7 series is formed in the small section Faba. If it is assumed that these two series approximate to 1 : 2 : 4 ratios, the disjunct grouping would be consistent with the lateral multiplicity hypothesis in which a geometric increase in the number of lateral strands should have taken place. An alternative



hypothesis is that the increase in DNA content per cell is due to segmental duplication and/or local multiplicity. For this to be so however, the number of sites would have to be large and evenly scattered throughout all the chromosome arms. In the sections Vicia and Faba, natural selection must act to produce adaptive peaks at or near multiples in a geometric series.

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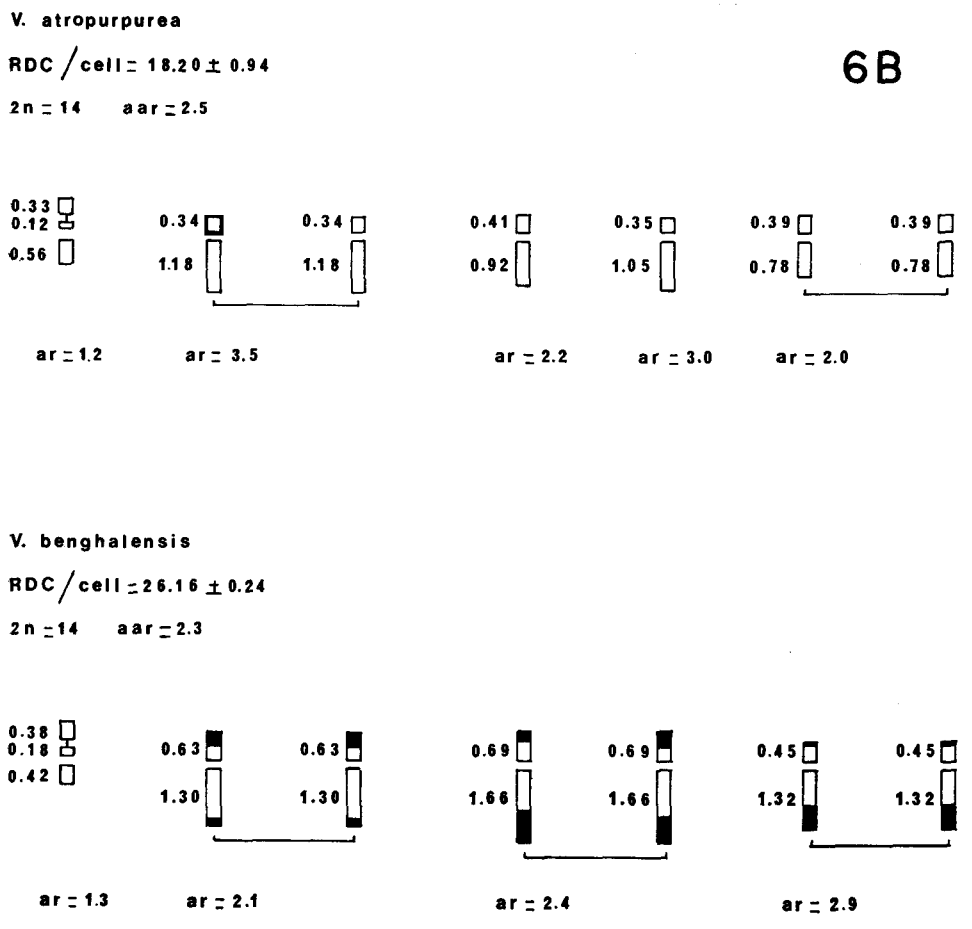
SUMMARY

Cytophotometric determinations of the DNA contents per cell made for 45 species of the genus Vicia show that there is a six-fold variation in DNA content



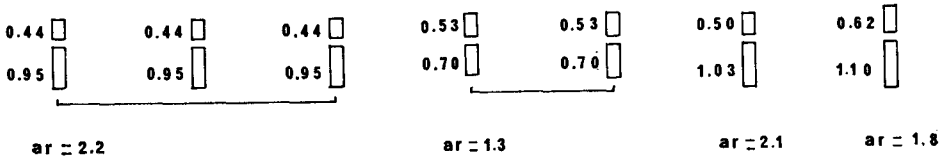
per cell throughout the genus. The DNA content per cell varies within all four sections of the genus. The variability increases in the taxonomically more advanced sections so that increasingly higher values occur. However, it is possible that evolution from a perennial habit to an annual habit in the section Cracca is accompanied by the loss of DNA. The distributions of DNA contents per cell of species in the more primitive sections (Ervum and Cracca) form continuous series while those in the more advanced sections (Vicia and Faba) are disjunct and approximate to geometric series 1 : 2 : 4. —Analyses of the karyotypes of five pairs of closely related species were also carried out. The change in DNA content per cell appears to affect all the chromosomes of a genome, but the two arms of a chromosome are not affected proportionally. This has resulted in a change of arm ratios. Most of the cytological data appear to favor increase by either local multiplicity or segmental duplications.

V. pubescens

RDC / cell = 19.77 ± 0.24

2n = 14 aar = 1.9

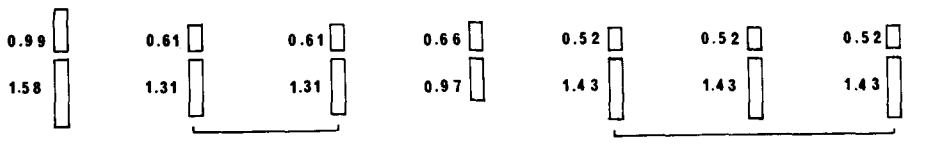
6C



V. tetrasperma

RDC / cell = 27.80 ± 0.75

2n = 14 aar = 2.2

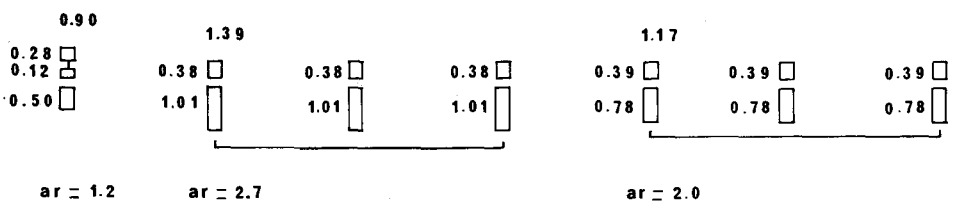


V. villosa

RDC / cell = 17.12 ± 0.48

2n = 14 aar = 2.2

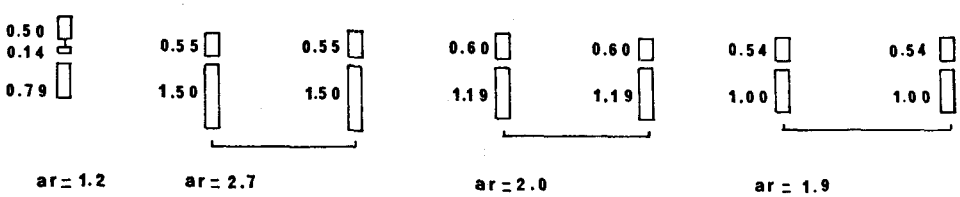
6D



V. dasycarpa

RDC / cell = 24.38 ± 1.39

2n = 14 aar = 2.1



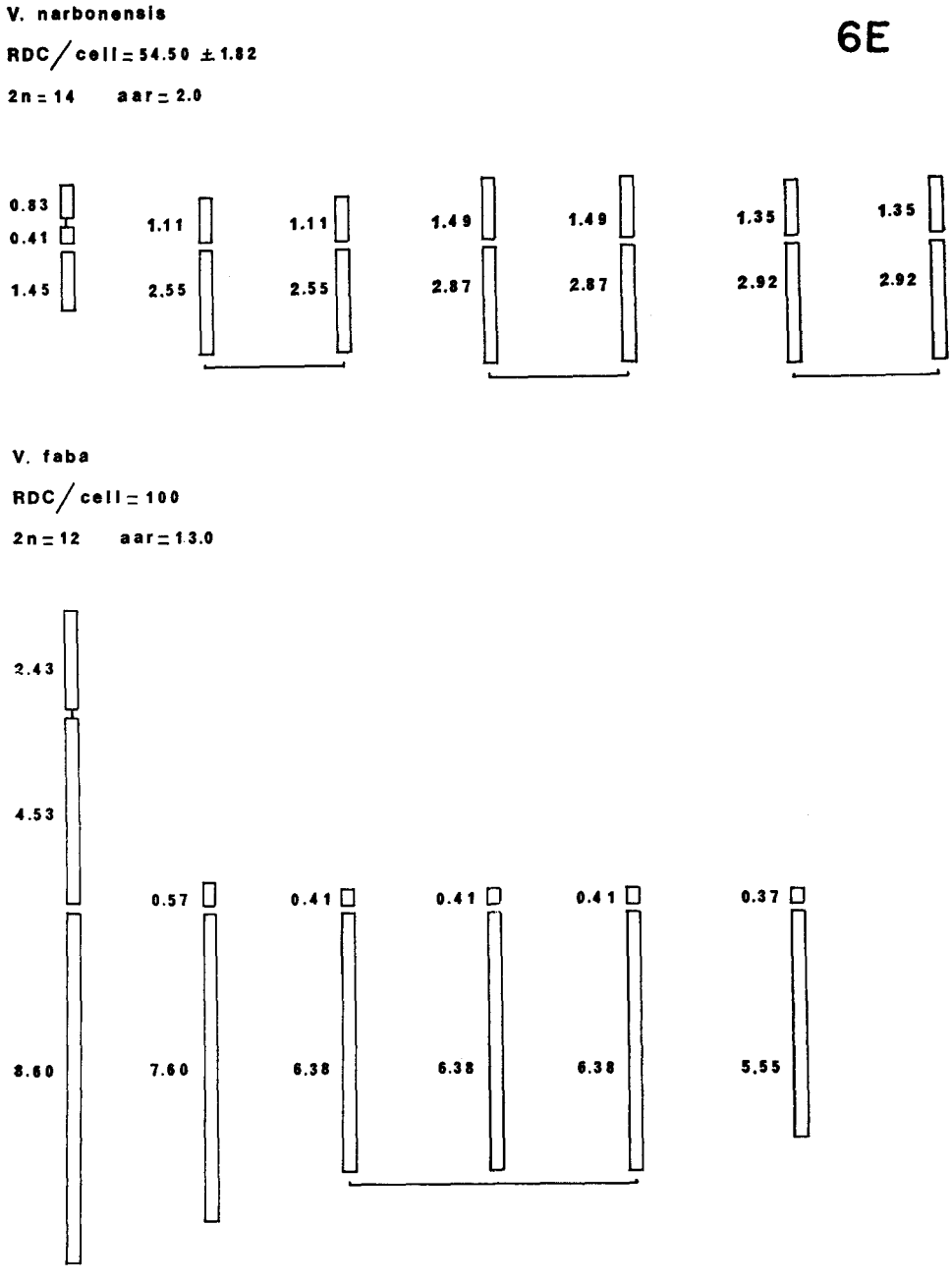


FIGURE 6.—Comparison of the karyotypes of
V. hirsuta and *V. meyeri* (A);
V. atropurpurea and *V. benghalensis* (B);
V. pubescens and *V. tetrasperma* (C);
V. villosa and *V. dasycarpa* (D);
V. narbonensis and *V. faba* (E).

RDC/cell = relative DNA content per cell; ar = arm ratio of the chromosome; aar = average arm ratio of the chromosomes of the complement; ⌣ denotes chromosomes are grouped; additional DNA is shaded.

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