VARIATION IN NUCLEAR DNA CONTENT BETWEEN

SPECIES IN THE GENUS *VICIA*.

A thesis submitted to the University of Adelaide

as a requirement for the degree of Doctor of

Philosophy

by

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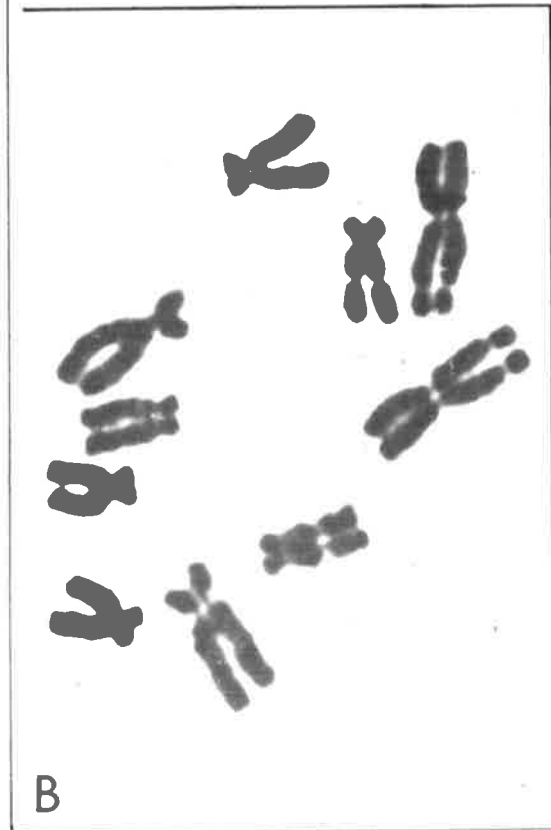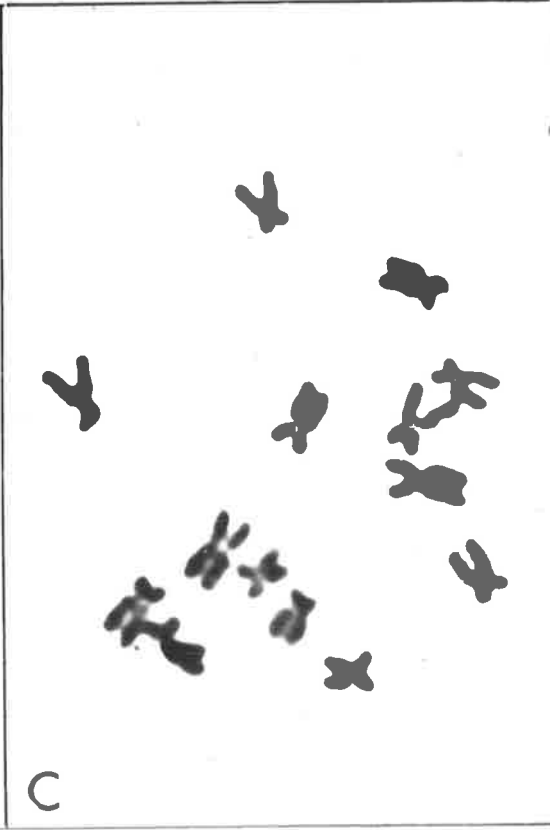University of Adelaide.
November 1970.

Frontispiece.

Root-tip metaphase chromosomes of

V. melanops (A), *V. hajastana* (B) and *V. tetrasperma* (C).

A

B

C

The investigations described in this thesis were carried out in the Botany Department, University of Adelaide, from August 1967 to February 1970.

To the author's belief and knowledge, this thesis contains no material previously submitted for a degree in any University by the author or by any other person, except where due reference is made in the text.

CHOOI WAI YEAN.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS.

Page.

Summary.

SUMMARY.

1. Cytophotometric determinations of the DNA content per cell made for 45 species of the genus *Vicia* show that there is a 6-fold variation in DNA content per cell throughout the genus. This variation occurs in diploid species with chromosome numbers of n = 5, 6 or 7.

2. Significant differences in DNA content per cell are found between taxonomic subspecies.

3. 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 has been accompanied by the loss of DNA.

4. The distributions of the 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. Distributions of average DNA content per chromosome are similar but in the sections Vicia and Faba the discontinuities are more marked and approximate to geometric series (1:2:4).

5. An analysis of the karyotypes of the 45 species was also carried out. In general, it appears that morphological advancement is accompanied by increasing asymmetry of the karyotype but this appears to be independent of increase in DNA content per cell.

6. Although a few observed changes could be interpreted as being the result of pericentric inversion or translocation, most changes involve change of DNA content per cell, usually without change in chromosome number. The change in DNA content per cell affects 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.

7. The cytological data (above) do not clearly discriminate between the local and lateral multiplicity hypotheses. Most of the cytological data appears, however, to favour the local multiplicity hypothesis but it is not easily compatible with two observations viz. the uniformity of chromosome sizes within a genome and the disjunct distributions of DNA values in the sections Vicia and Faba. For the local multiplicity hypothesis to explain these, the number of loci multiplied would have to be large and evenly scattered throughout the genome and, in the sections, Vicia and Faba, natural selection must act to produce adaptive peaks at or near multiples in a geometric series.

8. The DNA of six selected *Vicia* species (*V. faba*, *V. melanops*, *V. narbonensis*, *V. sativa*, *V. benghalensis* and *V. atropurpurea*) which have up to a 5.5-fold variation in DNA content per cell were compared with respect to nucleotide sequence homology and degree of repetition of nucleotide sequences using the method of DNA-DNA hybridisation (competition and reassociation rate experiments). Five of the six species appear to have similar nucleotide sequences but in *V. sativa* a major divergence seems to have occurred.

9. The degrees of repetition of nucleotide sequences are, however, different in the six species, a proportion between 15 and 38% of the total having been unevenly multiplied to form rapidly reassociating DNA. The remaining 62 to 85% has probably been evenly multiplied. The proportion of rapidly reassociating DNA does not appear to be related to nuclear DNA content.

10. The biochemical evidence shows that some increase in DNA can be accounted for by local multiplicity. Both the cytological and biochemical evidence suggest that the remainder of the DNA has increased more or less evenly but there is no clear evidence which shows whether this has been due to local multiplicity only or whether lateral multiplicity is also involved.

PART A.

CHAPTER 1.

INTRODUCTION.

1.1   Evolutionary change in DNA content per cell.

The DNA constancy hypothesis which states that the DNA content per cell is constant for the somatic cells of an organism and that this amount is twice that found in the sperm cells was formulated as a result of the work of Boivin, Vendrely and Vendrely (1948) and Mirsky and Ris (1949).   Since then, studies of DNA contents of organisms having different levels of taxonomic relationship have pointed to apparent paradoxes, particularly if one relates DNA content with complexity.

Generally, the evolution of the more complex forms from the simpler forms of life has been accompanied by a large increase in total amount of DNA per cell (Mirsky and Ris, 1951; Mirsky, 1950-51; review by McCarthy, 1969).   This probably reflects the increase in the total information content necessary for the development of increasing structural and behavioural complexity.

Although, in general, the amount of DNA per cell increases systematically with the complexity of the organism, large variations also occur independently of complexity, particularly among fish (Mirsky and Ris, 1951; Atkin and Ohno, 1967; Ohno and Atkin, 1966; Hinegardner, 1968), amphibia (Mirsky and Ris, 1951; Ullerich, 1966, 1967),lungfish (Mirsky and Ris, 1951) and higher plants (Sparrow and Evans, 1961; McLeish and Sunderland, 1961; Martin, 1966; Rees, Cameron, Hazarika and Jones, 1966; Rothfels, Sexsmith, Heimburger and Krause, 1966; Rothfels and Heimberger, 1968; Southern, 1967; Baetcke, Sparrow, Nauman and Schwemmer, 1967; Halkka, 1964).

In addition to the observation that the amount of DNA per cell does not always increase systematically with the complexity of the organism, it appears also that there is no general correlation between the amount of nuclear DNA and the degree of evolutionary advancement within a taxonomic group e.g. a family or a genus. In some groups (teleost fishes, *Lathyrus* species) general evolution and speciation appear to be accompanied by the loss of DNA (Hinegardner, 1968; Rees and Hazarika, 1969) while in others (e.g. *Lolium* species) the reverse takes place (Rees and Jones, 1967a).

Because it is reasonable to assume that species within a taxonomic group do not differ much in their arrays of enzymes and, consequently, do not have a greater diversity of DNA to code for these enzymes, it is now generally accepted by most authors that the amount of DNA per cell does not provide a simple index of either structural or functional complexity.

Increase in DNA per cell can be accounted for by replication of the same DNA polynucleotide sequences. Repetitious DNA has been associated with the presence of multiple copies of genes as suggested by the chromosome models of Callan (1967) and Whitehouse (1967). The nature of repetitious DNA will be dealt with in more detail in Chapters 6 and 7.

A phylogenetic reduction in DNA, however, is more difficult to explain. This is because a phylogenetic reduction is even more difficult to reconcile with the fact that even small deletions are often lethal or severely detrimental (Stebbins, 1966; Lewis and John, 1963). However, deletions are not always deleterious because there are exceptions where chromosome loss is tolerated and is a normal feature of the genetic system (Haga, 1968; Rutishauser, 1957).

1.2   <u>Trends in evolutionary change in DNA content</u>

        <u>per cell within taxonomic groups.</u>

        In the study of the variation in DNA content per
cell between related species, several interesting features have
emerged:

    a)   Appreciably different amounts of DNA may be found in
    species belonging to the same family and with approximately
    the same number of chromosomes e.g. *Bufo* species (Ullerich,
    1966); *Oedogonium* species (Hoffman, 1967); *Vicia, Lathyrus*
    and *Lolium* species (Rees *et al.*, 1966;   Martin and Shanks,
    1966); *Allium* (Jones and Rees, 1968);   *Eriocephalus* species
    (Rothfels *et al.*, 1966);   *Luzula* (Halkka, 1964).   A 1.27-fold
    range in DNA content was found between related species of *Allium*,
    a 1.4-fold range in *Lolium*, a 2-fold range in *Eriocephalus*,
    a 3.5-fold range in *Lathyrus* and a 5 or 7-fold range in *Vicia*.

    b)   In interspecific comparisons, a 2:1 ratio in chromosome
    number but a 1:1 ratio in DNA content per cell and a 1:1 ratio
    in chromosome number with an accompanying 2:1 ratio in DNA content
    per cell have been found between species in the same genus
    e.g. *Thyanta* species (Schrader and Hughes-Schrader, 1956);
    Banasa species (Schrader and Hughes-Schrader, 1958); *Bucholzia*
    and *Enchytraeus* species (Christensen, 1966).   Numerical
    polyploidy in *Thyanta calceata* (tetraploid) is not accompanied

by doubling of the DNA value. This species has exactly twice the number of chromosomes characteristic of the diploid species, except for the loss of a Y chromosome but it retains the same generic DNA value. Similarly, in the genus *Banasa*, the DNA value of the tetraploid species does not differ much from the diploid species. Also, the relative DNA values per diploid genome for *Drosophyllum* and diploid, triploid and higher polyploid *Drosera* were approximately 16:4:2:1 (Rothfels and Heimburger, 1968).

c) A mutation which resulted in a 2-fold increase in DNA content and which was not accompanied by an increase in chromosome number was found in *Tetrahymena pyriformis* under normal culture conditions (Alfert and Balamuth, 1957).

Darlington (1929) found a bud among many of *Tradescantia brevicaulis* in which chromosomes at the first pollen grain division were one-fifth the normal size. Thomas (1936) found an individual plant of *Lolium perenne* with chromosomes one-quarter the normal size and which when crossed to the normal gave progeny among which there were marked differences of chromosome size.

d) Small but significant intraspecific differences in DNA were found in flax (Evans, 1968) and some gymnosperms (Miksche, 1968). An intraspecific variation of a factor of 1.6 for *Picea glauca* and 1.5 for *Pinus banksiana* was found (Miksche, 1968).

e)   Geometric differences in DNA content of the same
cytological locus, as defined in terms of a specific band
in polytene chromosomes have been found between subspecies
of the same species, e.g. *Chironomus* subspecies *thummi* and
piger (Keyl, 1965).

In view of the above observations, the nature of
chromosomal structural changes which give rise to DNA variation
has been the subject of much speculation and debate.

1.3   Mechanisms by which repeated DNA sequences
           might evolve.

Polyploidy is clearly one means by which repetition of
DNA sequences might evolve, but in the genus *Vicia*, as in several
other genera, this is of very minor importance. Similarly, in the
genus *Vicia*, the 6-fold variation (see page 37) in DNA content per
cell occurs in species whose chromosome numbers are n = 5, 6 or 7 so
that anueploidy is also of minor importance. Ignoring change of
chromosome number, the following processes can, theoretically, lead
to repetition of DNA sequences.

a)   Local multiplicity, i.e., a short sequence of DNA
equivalent to a gene is multiplied; the new sequence is located
adjacent to the original one within a single stranded
chromosome.   Probably the most important ideas relevant to
this hypothesis are those of Keyl (1964, 1965) who made a
study of two subspecies of *Chironomus thummi*, viz. *thummi*
and *piger*.   He reported that certain bands in the polytene

Fig. 1.   Diagram to illustrate the local multiplicity

hypothesis.

Diagram below indicates the manner by which a 'gene' can

be duplicated during abnormal replication (after Keyl, 1965).

a, b, c, d and e denote individual 'genes'.

a b c d e

basic genome

local multiplicity

a a a a b c c c c c c c c d e e
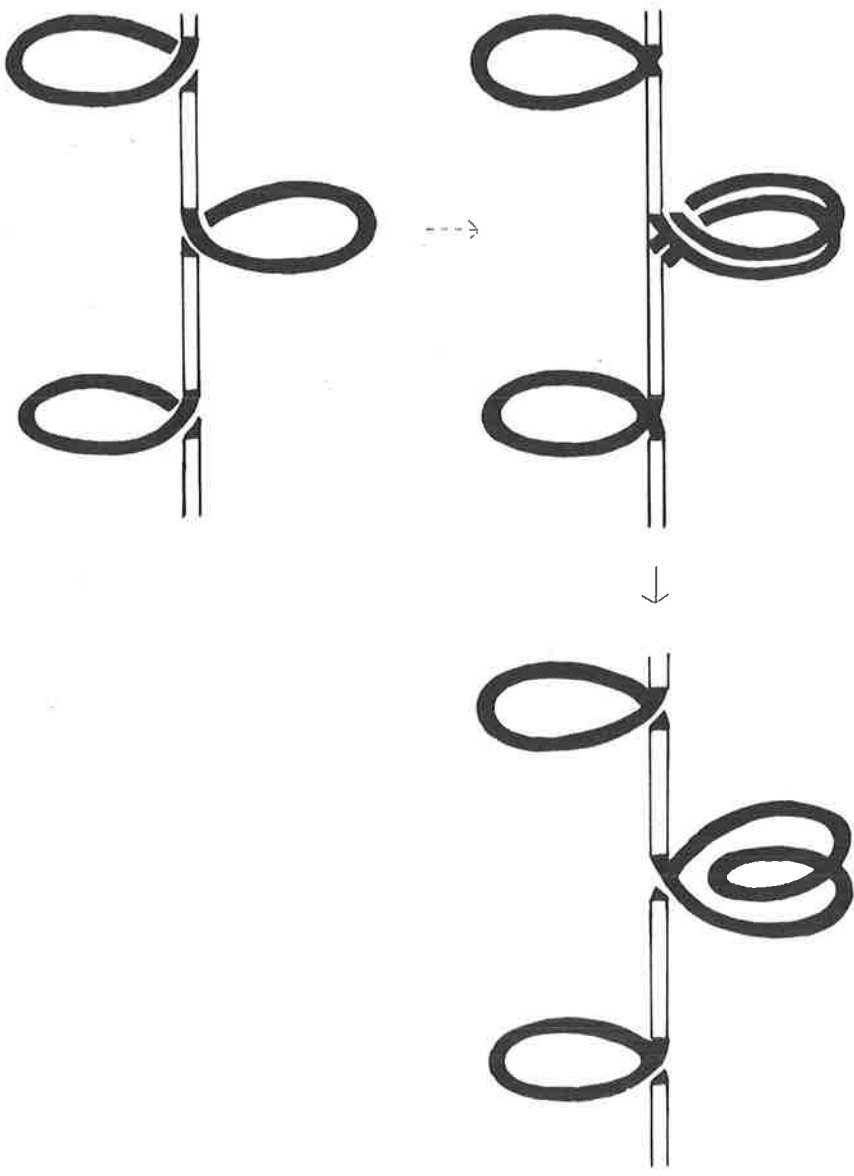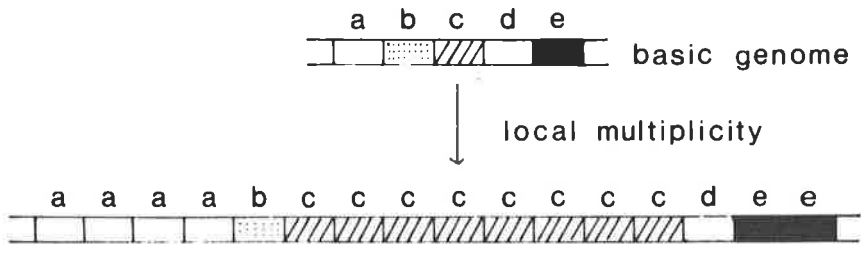
chromosomes of *thummi* differ in DNA concentration from those of *piger* by a factor 2-, 4-, 8-, or 16-fold. Intermediate values were not found. This suggests a progressive but localised doubling of DNA. The ratio (1.27) of the DNA content of the two subspecies is also identical in polytene nuclei and in meiotic cells. This implies that this is an evolutionary phenomenon and not a result of cellular differentiation. Keyl (1965) proposed a mechanism involving misreplication of a chromosome backbone which holds DNA loops. When replication of DNA situated in loops off the main axis is abnormal, either deleted or duplicated chromosomes result (Fig. 1). A subsequent similar event will lead to a quadrupling of the original amount and so on in a geometric series. The existence of DNA loops is substantiated by the demonstration of circular DNA molecules in the chromosomes of boar sperm (Hotta and Bassel, 1965) and calf thymus (Sonnenbichler, 1969).

Although the geometric increases observed by Keyl (1965) took place within single bands of salivary gland chromosomes, for the purpose of this thesis it is convenient to also classify as 'local multiplicity' duplications of single bands which are the result of unequal crossing-over at meiosis. Normal *Drosophila melanogaster* males have the 16A region of the salivary chromosome represented once, Bar males twice

and Bar-double males three times (Sturtevant, 1925).

Sturtevant (1925) showed that the frequency of reversion to
normal and to Bar-double are similar and their occurrence,
which takes place only in females, can be accounted for
through the phenomenon of unequal crossing-over.   Although
the situations in *Ch. thummi* and at the bar locus in
D. melanogaster are not identical, they are not distinguishable
by the methods used in this thesis and can be grouped as
'local multiplicity'.   Because 16-fold repetition was
described by Keyl (1965), this appears to be the more
powerful of the two mechanisms for giving rise to variation
in DNA content per cell;   when 'local multiplicity' is
referred to in future, this is the phenomenon which will be
emphasised.

b)   Segmental duplication, i.e., re-arrangement of a
chromosomal segment consequent upon breakage and rejoining,
followed by formation of gametes bearing the segment in
duplicate.   At pachytene in hybrids, such segments would
appear as unpaired loops.   These have been described in
hybrids between species with high and low DNA values in the
genus *Lolium* (Rees and Jones, 1967a) and *Allium* (Jones and
Rees, 1968).   Segments could be as small as those discussed
under the heading 'Local Multiplicity' but, as a way of

Fig. 2. Diagram to illustrate the lateral multiplicity hypothesis.

a, b, c, d and e denote individual 'genes'.
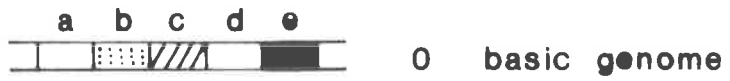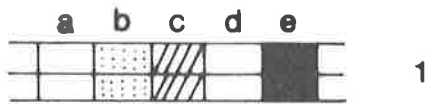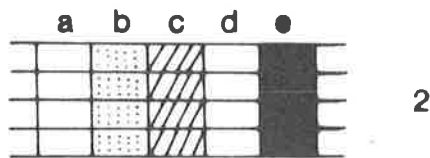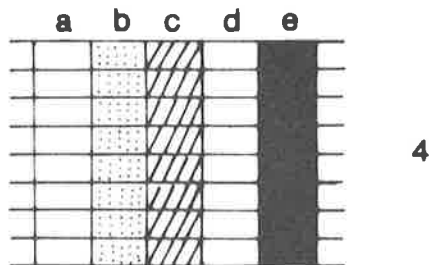
multiplying individual genes, it would be comparatively slow. However, if a whole duplicated segment including many different genes proved advantageous, a single event could lead to a substantial increase in DNA content per cell; the maximum approximating to one chromosome arm.

c) <u>Lateral multiplicity</u>, i.e. the total basic information in the chromosome is multiplied to produce a multistranded chromosome (Fig. 2). This undoubtedly occurs as a developmental phenomenon, e.g. in *Drosophila* salivary glands, and is characterised by a geometric increase in the number of strands. However, it has never been convincingly demonstrated as an evolutionary phenomenon. A modification of this hypothesis is the partial lateral multiplicity hypothesis, i.e., an arithmetic increase in only one or a few lateral strands.

It should be noted that while these mechanisms are different, they are not mutually exclusive, i.e, all might occur either in the same or in different organisms.

One major argument against both the local and lateral multiplicity hypotheses is that they do not meet all the requirements of genetical recombination, mutation and semiconservative replication. As these hypotheses involve genes being present in

replicate, they call for a reconsideration of the 'units' of function and mutation. Various models attempting to resolve these difficulties have been advanced. The models of local multiplicity (Keyl, 1965; Callan, 1967; Whitehouse, 1967), and the model of lateral multiplicity (Uhl, 1965) are the most feasible.

The model proposed by Callan (1967) involves serially repeated gene copies which include a terminal 'master' gene and a number of 'slave' genes. At prophase of meiosis, the polynucleotide chains of the 'slave' genes are matched with those of the 'master' gene, so that the 'slave' genes come to lie adjacent to those of the 'master'. If necessary, 'slave' genes are corrected so that their nucleotide sequences conform with those of the 'master'.

Whitehouse (1967) has modified Callan's model and suggested that during meiosis, 'slave' genes are removed from the chromatid by a crossover between the first and the last members of the linear series of identical genes and that only one polynucleotide chain of a 'slave' gene is matched to the 'master' gene.

While retaining a semiconservative mode of DNA replication, the hypothesis of Uhl (1965) is equally adaptable to single and multistranded chromosomes. It postulates that prior

to the DNA synthesis period (S period) of meiosis the chromosome
consists of segments of DNA double helices connected in series by
links.   The DNA, laterally, may consist of one or more double
helices but the links are, or act as, single units.   Prior to
DNA synthesis, the double helix (or helices) separates into its
constituent polynucleotide strands, with the links variously
remaining with one or the other of the strands, or groups of
strands.   This, in effect, fragments the DNA into polynucleotide
units, but does not fragment the chromosome as a whole, because
the links are randomly located in the separated strands and
presumably the associated histones and residual proteins maintain
the linear integrity.   Synapsis then occurs, after which the
links are formed to once again reunite the DNA throughout the length
of the chromosome.   In the process of formation of links, exchange
of chromatin can take place because the newly formed links can
connect pieces of DNA brought together by synapsis with, at the
same time, a re-establishment of the previous order of
nucleotide pairs.

None of these hypotheses is supported by incontrovertible
evidence.   Of the many that have been postulated, the above
hypotheses are the least harmful to the basic premises of
genetical recombination and semiconservative replication.

1.3.1   <u>Evidence in support of local multiplicity.</u>

Although there exists considerable data which favour
a certain degree of strandedness in chromosomes, there are,
nevertheless, some which are consistent with the local multiplicity
hypothesis (Keyl, 1965).   The most compelling observations,
perhaps, come from autoradiographic and radiation-induced breakage
studies (reviewed by Swift, 1965).

Detailed studies of the giant lampbrush chromosomes
in oocytes of the newt *Triturus* and the polytene chromosomes in
Diptera provide the main evidence for the local multiplicity
hypothesis.   The lampbrush chromosome consists of a backbone of
chromomeres containing most of the DNA and numerous closed loops
which extend from the chromomeres into the nucleoplasm.   Callan
(1967) equated sites of local multiplicity with visible
chromomeres.   Because chromomeres are also a feature of plant
chromosomes, it is suspected that some DNA increase in plants can
be explained by local multiplicity.   Stretching of the lampbrush
chromosome leads to splitting of the basal chromomere into two
parts held together by loops.   This shows that the longitudinal
strands do not pass directly along the backbone of the chromosome
but rather from chromomere regions into loop and back, then to the
next chromomere region, loop and so on (Gall, 1956).   Treatment
of unfixed chromosomes with enzymes (Callan and Macgregor, 1958;
Callan and Lloyd, 1960) also suggests that DNA is largely responsible
for the longitudinal continuity of chromosomes.   Lampbrush

chromosomes fragment when treated with DNAse but not with RNAse or proteases. The same is also true for polytene chromosomes of *Diptera* (Lezzi, 1965). Two other studies suggest further that the DNA in the loops consists of a single double helix. Gall (1963b) examined the kinetics of loop and chromosome breakage following treatment with pancreatic DNAse, which causes single strand breaks in DNA. His results suggested that the DNA in each loop contained a single DNA molecule, and that there were no further DNA strands running along the backbone of the chromosome. Miller (1965) studied the structure of the loops by electron microscopy after digesting away the RNA and proteins. He found that the remaining DNA material was of the order of 34 Å which showed that there could only be one molecule of DNA.

Additional support for the local multiplicity hypothesis is derived from the hybridisation studies on the DNA of the nucleolar organiser region in *Drosophila* and *Xenopus* (Ritossa, Atwood and Spiegelman, 1966; Wallace and Birnstiel, 1966; Brown and Weber, 1968). These studies showed that DNA sequences (or genes) specifying the synthesis of rRNA and transfer RNA are present in multiple copies. In the case of *Xenopus* there are 450 serially repeated copies of the genes coding for 28s and 16s ribosomal RNA.

The observation of a non-geometric DNA series, viz., a continuous one, in *Lathyrus* (Rees and Hazarika, 1969) and *Anemone* (Rothfels *et al.*, 1966) has been interpreted by the authors as being due to local multiplicity. Ullerich (1965, 1966) has also

interpreted the DNA differences among European toads, *Bufo calamita*, *B. bufo* and *B. viridis* (DNA ratios 1:1.07:1.49) as being due to local multiplicity.

The finding of Britten and Kohne (1967a) that many animal and plant DNA occur in classes with either small or large degrees of repetition can also be explained by the local multiplicity hypothesis.

In 1967, Kleinschmidt demonstrated that condensed chromosomes unravel considerably and often transform into extended fibres when spread at an air-water interphase. This spreading technique has been used to study chromatin of interphase nuclei from various plants, animals and protozoa (Gall, 1963a, 1966; Ris, 1966, 1967; Dupraw, 1965a,b; Wolfe, 1965a, b; J.Wolfe, 1967; Abuelo and Moore, 1969; McDermott, 1968) sperm nuclei (Gall, 1966; Ris, 1966; Solari, 1967), mitotic and meiotic chromosomes (Dupraw, 1965a, b, 1966a; Wolfe, 1965, a, b; Wolfe and John, 1965; Wolfe and Hewitte, 1966; Gall, 1966), salivary gland chromosomes (Rae, 1966) and lampbrush chromosomes (Miller, 1965). All the above authors are in favour of the folded fibre model, i.e., the chromosome is made up of parallel folding of a single strand.

The ^{3}H-thymidine labelling of DNA prior to subjecting them to the spreading technique, suggests that chromatin fibres consist of several tandemly joined replication segments (Cairns,1966; Sasaki and Norman, 1966; Huberman and Riggs, 1968). The extreme

length of some of these fibres as well as the paucity of free ends in favourable preparations are taken to indicate by some authors that chromosomes possibly contain a single microfibrillar strand only.   It is still unclear, however, how much such a macromolecular unit, even if folded back on itself according to some complex pattern, could give rise to coiled structures such as those seen at mitosis.

1.3.2   Evidence in support of segmental duplications.

Evidence in support of segmental duplications is found in closely related species with different DNA contents per cell. It has been reported that there is a 1.35-fold variation in DNA content per cell between *Lolium temulentum* and *L. perenne* (Rees and Jones, 1967a) and a 1.27-fold variation in DNA content per cell between *Allium cepa* and *A. fistulosum* (Jones and Rees, 1968).   From a study of the pairing behaviour of the 'homologous' chromosomes in the hybrid formed between *L. temulentum* and *L. perenne* and *A. cepa* and *A. fistulosum*, these ~~two~~ authors found that:

a)   the difference in DNA content per cell between the two *Lolium* species is due, in part, to lengthwise replication or loss of chromosome segments,

b)   the difference in DNA content per cell between the two *Allium* species is due entirely to lengthwise incorporation or loss of chromosome segments.

1.3.3   Evidence in support of lateral multiplicity.

The lateral multiplicity hypothesis is derived largely from findings that geometric increases in DNA content per cell are not accompanied by corresponding geometrical increases in chromosome number.   For example, diploid genome for *Drosophyllum lusitanicum* and the related diploid, triploid and higher polyploid *Drosera* species are approximately 16:4:2:1.   These values are compatible with a multistranded interpretation of chromosome structure having the observed multiplicities of $2^4:2^2:2^1:2^0$ parallel chromosome strands (Rothfels and Heimburger, 1968).   The average chromosome sizes of *Vicia* species have also been reported to fall into a 4:2:1 ratio (Martin, 1968).

One approach to the problem has been a study of hybrids formed between species with different amounts of DNA.   However, no clear picture has emerged.   In fact, evidence seems to indicate that DNA increase or decrease could have originated by local multiplicity, lateral multiplicity or both.   Evidence for local multiplicity is presented on pages 12–15.   If it is assumed that total chromosome size is proportional to DNA content, then, evidence in support of lateral multiplicity is provided by the pairing behaviour of the hybrid between *Crepis neglecta* and *C. fuliginosa*.   The metaphase chromosomes of these species have a 4-fold difference in volume.   Despite this, at pachytene of meiosis synapsis was almost complete (Togby, 1943).

Other approaches include electron micrographs of whole chromosomes (Ris, 1959, 1961; Ris and Chandler, 1963; Gall, 1963a; Osgood, Jenkins, Brooks and Lawson, 1964; Marin and Prescott, 1964; Dupraw, 1965a,b,c, 1966 a,b; Wolfe, 1965a; Solari, 1965; Ris, 1966; Barnicot, 1967; Abuelo and Moore, 1969; Dupraw, 1968; Wolfe and Martin, 1968), and construction of three dimensional models from consecutive thin slices of nuclei (Harris, 1965; Ris, 1955, 1956; Gay, 1956; Kaufmann and McDonald, 1956; Davies and Tooze, 1964; Dales, 1960; Barnicot and Huxley, 1965; Sparvoli, Gay and Kaufmann, 1965).

The examination under the light microscope of isolated mammalian and plant metaphase chromosomes treated with enzymes or uncoiling agents shows the presence of at least two subunits within the chromatids (Ris, 1959; Ris and Chandler, 1963; Osgood *et al.*, 1964; Trosko and Wolff, 1965; Sparvoli *et al.*, 1965). In *V. faba*, each chromatid consists of at least two separate strands, each of which bifurcates further. In favourable preparations, up to four such strands may be demonstrated (Brooks, Jenkins, Lawson and Osgood, 1962; Gimenez-Martin, Lopez-Saez and Gonzales-Fernandez, 1963; Trosko and Wolff, 1965; Trosko and Brewen, 1966). Several authors believe, from examination of thin sections, that these latter subunits in turn consist of several microfibrils (Kaufmann, Gay and McDonald, 1960; Ris, 1957; Barnicot, 1967; Rae, 1966; Govarts and Dekegel, 1966; Osgood *et al.*, 1964). Owing to the difficulties of

interpretation, evidence gathered from such preparations is, however, far from conclusive.

A comparison of the chromosomes of *V. faba* and *V. sativa* which has one-fifth the DNA content of *V. faba* has been made (Wolfe and Martin, 1968). These authors reported that while there was only one subdivision of the chromatid of *V. faba* and no marked subdivision of the chromatid of *V. sativa*, the centromeres in both had a stranded appearance. This points to the difference in DNA content being reflected in the degree of strandedness.

Most of the comparisons made between chromosomes with high DNA content and those with low DNA content have been made on metaphase chromosomes although their great density and complex architecture have proved most difficult to study under the electron microscope. Electron micrographs of whole chromosomes generally reveal little more than a random configuration of chromatin fibers which could be interpreted as representing a multistranded or a single stranded structure folded back on itself. Numerous very fine fibrils of varying diameters are repeatedly encountered and have been considered to be the basic longitudinal unit of the chromosome. Admittedly, all authors agree that chromosomes consist of similar microfibrillar units at all stages of mitosis (Ris, 1966, 1967, 1969). The difficulty in interpreting available ultrastructural data in chromosomes stems not only from their complex organisation but also from our persisting ignorance of

the number of basic strands they contain.

The fine structure of the chromosome and the difference in organisation between a large and a small chromosome at the molecular level has still to be elucidated.

Further evidence from other species that each chromatid at metaphase is subdivided laterally once or possibly several times in larger chromosomes is supported in part both by X-ray and cytological studies.

If chromosomes are irradiated by X-rays prior to DNA synthesis, the aberrations produced are of the chromosomal type; they involve the entire chromosome across it's full diameter rather than some finer longitudinal subdivision of it.   If irradiated during or after DNA synthesis, the aberrations are of the chromatid type: the chromatid rather than the whole chromosome is the unit of breakage.

Subchromatid or side-arm bridges have been observed in large chromosomes of *Scilla siberica* (La Cour and Rutishauser, 1954), *Lilium longiflorum* (Mitra, 1958; Crouse, 1954), *Trillium erectum* (Wilson, Sparrow and Pond, 1959), *Scilla campanulata* (Rees, 1953), *V. faba* (Heddle, 1969).   These observations lend morphological support to the theory that side-arm bridges are, in fact, exchanges involving a subchromatid unit.   These observations have been interpreted as being consistent with a partial or total lateral multiplicity model.   Alternative

interpretations, however, are not entirely ruled out by this study. Thus, side-arms could form if each chromatid consisted of a single folded-fiber (Dupraw, 1966a). Such an interpretation appears unlikely when the data concerning chromosome replication following the induction of side-arm bridges are considered (Brinkley and Humphrey, 1969).

Cytological evidence obtained by autoradiographic techniques also support the possiblity that the chromatids are multistranded. Peacock (1963) reported that although the majority of the chromosomal label in *V. faba* is distributed to the daughter chromatids semiconservatively (Taylor, 1957; Taylor, Woods and Hughes, 1957; Prescott and Blender, 1962; Taylor 1958, 1959, 1965), very frequently two chromatids at the second division are isolabelled. Deaven (1968) showed that isolabelled chromatids also appeared at second and subsequent mitoses after labelling.

Another line of evidence in favour of multistranded chromosomes comes from the microcine film of Bajer (1965). Half chromatid units were observed both in normal chromosomes and in side-arm bridges of living (unfixed) cells.

At present, the molecular organisation of chromosomes and the 'structural' manner by which DNA increase takes place in chromosomes are still unresolved.

1.4   Parallel trends in evolution.

Correlations have been found between evolutionary change in the amount of DNA per cell in some plants and animals and their

    a)   nuclear volumes,

    b)   adaptations to their habitats,

    c)   breeding systems.

    a)   nuclear volumes.

Several authors have reported that there is a close correlation between the amount of DNA per cell and nuclear volume (Baetcke *et al.*, 1967; Pogo, Pogo and Funes, 1960; Sparrow, Schairer and Sparrow, 1963; Van't Hof and Sparrow, 1963). An inverse correlation was found between DNA content per chromosome and radiosensitivity (Baetcke *et al.*, 1967; Sparrow, Baetcke, Shaver and Pond, 1968).

    b)   adaptations to their habitats.

In several groups of higher plants, a definite correlation exists between DNA content, nuclear volume and ecological adaptation. Tribes and genera of the grasses *(Graminea)* centred in tropical regions have small to medium-sized chromosomes and nuclei whereas most of the grasses that grow chiefly in cool temperate regions have large chromosomes and nuclei (Avdulov, 1931). Genera of the *Liliales* which

are primarily tropical or subtropical such as *Sanservieria*,
Asparagus and *Smilax* have smaller chromosomes (Sato, 1942)
and presumably lower DNA contents (Baetcke *et al.*, 1967;
Miksche, 1968). There are, however, exceptions to these.
Rheo in the *Commelinaceae*, members of the *Loranthaceae*
and the *Proteaceae* which are strictly tropical have relatively
large chromosomes (Stebbins, 1966). All tropical members of
the *Leguminosae* have small to medium-sized chromosomes as do
most of the temperate-climate genera in this family (Stebbins,
1966).

Gymnosperms with small DNA contents are reported to
display greater geographic distribution and faster growth
rates than species with large DNA contents (Miksche, 1967).

Assuming that nuclear volume is proportional to cell
size, it has been proposed that cell size may have some
adaptive value (Martin, 1968; Szarski, 1970) and is accordingly
influenced by natural selection.

c) breeding systems.

In general, inbreeding species are descendents of
outbreeders. In *Lathyrus* (Rees and Hazarika, 1969) and
Crepis (Babcock, 1947), inbreeding annuals generally have
lower DNA values than outbreeders. However, in *Lolium*
(Rees and Jones, 1967a), inbreeding species show an increased
nuclear DNA in comparison with outbreeders.

1.5   Selection of study material.

For this thesis, the author tried to obtain as large a number of *Vicia* species as possible spread throughout all the four sections of the genus.   A total of 45 species was obtained.

Martin and Shanks (1966) have reported a 5-fold variation in DNA content per cell between six species from the section Vicia.   Rees *et al*. (1966) reported a 7-fold variation in the same species.   Martin and Shanks (1966) postulated that the differences in DNA content per cell between these species could lie in lateral multiplicity.

The present study involves:

a)   a re-examination of the DNA content of those species examined by Martin and Shanks (1966), Rees *et al*. (1966) and other species (Chapter 3),

b)   a more thorough study of the genus to determine whether there is an evolutionary increase or decrease in DNA content per cell and if there is a disjunct pattern in the distribution of DNA content per cell in all the four sections of the genus (Chapter 3),

c)   a study of the evolution of the karyotype and how this is related to the evolutionary change in DNA content per cell (Chapter 3),

d)   a cytological study of the nature of the evolutionary change in DNA content per cell to determine if the quantitative differences in DNA could be attributed to local or lateral multiplicity (Chapter 3),

e)   a biochemical study of the nature of the differences in DNA content between species to determine whether the differences lie only in repetitious DNA and if the same degree of repetition occurs in all species (Chapters 6 and 7).

CHAPTER 2.

MATERIALS AND METHODS.

2.1   Source of *Vicia* seeds.

Forty-five species of *Vicia* were used in this investigation.   Herbarium specimens of each of the species are to be found in the herbarium of the establishment from which the seeds were obtained.   Seeds of the following species were obtained from

a)   the Waite Agricultural Research Institute, Adelaide, South Australia.

Species	Herbarium specimen number
V. atropurpurea Desf.	WARI 5131
V. dasycarpa Tenore	WARI 5087
V. disperma DC.	WARI 5094
V. ervilia (L.) Willd.	WARI 5392
V. galeata Boiss.	WARI 5100
V. hybrida L.	WARI 5095
V. lutea L.	WARI 5103 (Portugal)
V. narbonensis L.	WARI 5398
V. sativa L.	WARI 5403 (Rhodes)
V. tetrasperma (L.) Schreber.	WARI 5102

b)   Division of Plant Industry, C.S.I.R.O., Canberra City,

A.C.T., Australia.

Species	Herbarium specimen number
V. hirsuta (L.) S.F. Gray	CPI 10488
V. pannonica Crantz var.	CPI 22907
pannonica	

c)   the Institut für Kulturpflanzenforschung der Deutschen

Academie der Wissenschaften zu Berlin, Gatersleben, Krs.

Aschersleben.

Species	Herbarium specimen number
V. angustifolia L. ssp.	VIC 586/64 2347=A67
angustifolia	
V. articulata Hornem	VIC 298/65
V. benghalensis L.	VIC 301/65
V. biennis L.	VIC 476/65
V. bithynica (L.) L.	VIC 305/65
V. cassubica L.	L. 240/65
V. cordata (Wulfen ex Hoppe)	VIC 465/63
Ascherson & Graebner.	
V. cracca L.	L 532/67
V. dumetorum L.	L 94/65
V. eriocarpa (Hausskn.) Halacsy	VIC 629/65
V. grandiflora Scop. var.	VIC 472/66 A67
grandiflora	
V. graminea SM.	L.570/66 A68
V. hajastana Grossh.	VIC 101/67 A68

V. hyrcanica Fisch. et Mey.	VIC 640/66
V. incisaeformis Stef.	VIC 123/67
V. lathyroides L.	VIC 641/65
V. macrocarpa (Moris) Arcangeli	V 4/66
V. melanops Sibth & Sm var. melanops	VIC 474/65
V. meyeri Boiss.	VIC 643/66 A68
V. michauxii Spreng.	VIC 644/65 A68
V. neglecta Hanelt & Mettin	VIC 645/65
V. orobus DC.	L 248/65
V. peregrina L.	VIC 317/65
V. pilosa Bieb.	V 39/66
V. pisiformis L.	VIC 1/63
V. pubescens (DC.) Link.	VIC 646/65 A68
V. ramuliflora (Maxim) Ohwi.	L 450/67
V. sepium L.	L 400/62 A67
V. sylvatica L.	L 533/66
V. tenuifolia Roth.	L 536/65
V. unijuga A. Br.	L 250/65
V. villosa Roth. cultivar 'Minikowska'	VIC 509/64 2305/64

d)   M.F. Hodge & Sons. Pty. Ltd., Adelaide, South Australia.

Species

V. faba L. cultivar 'Seviele'.   As this is a commercial
variety, a herbarium specimen was not kept.

2.2   Measurement of DNA content per cell by

           Feulgen microspectrophotometry.

Seeds were germinated in vermiculite in perspex boxes
(20 cm x 20 cm x 8 cm) at room temperature and kept moist with tap
water.   In winter, the final percentage germination ranged from
70 to 90%.   In summer, however, a high percentage germination
could only be obtained when the seed coats were broken.

Seedlings were selected in which the 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.

Slides were prepared by a modification of the method
of Martin and Hayman (1965).   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.   The roots
were then hydrolysed in 1N HCl at 60°C for 10 min.   A root-tip
squash of the control species in 45% acetic acid was made three-
quarters of an inch from one end of the microscope slide, ensuring
that the cells were well separated and flat.   Similarly, a root-
tip squash of the species with which it was being compared was made
three-quarters of an inch from the other end of the same slide.

TABLE 1.

Time schedule of Feulgen-staining procedure.

Bath.	Time (min.)
Absolute alcohol	2
90% alcohol	2
70% alcohol	2
50% alcohol	2
30% alcohol	2
Distilled water 1	2
Distilled water 2	2
1N HCl $60^{o}C$	10
Leuco-basic fuchsin	120
SO_2 water 1	10
SO_2 water 2	10
SO_2 water 3	10
Distilled water 1	2
Distilled water 2	2
30% alcohol	2
50% alcohol	2
70% alcohol	2
90% alcohol	2
Absolute alcohol 1	2
Absolute alcohol 2	2
Xylene 1	2
Xylene 2	2

Mounted in Xam, neutral mounting medium.

After the slide had been freeze-dried with dry ice, the cover slips were removed and the slide was transferred to absolute ethyl alcohol, followed by a descending series of alcohols to water. The slide was then stained in leuco-basic fuchsin (Darlington and La Cour, 1960) for 2 hr. and washed in sulphur dioxide water (three changes of 10 min. each) followed by two changes of distilled water. After dehydration through an increasing series of alcohols and finally two changes of xylene, the slide was mounted in neutral mounting media with number 1 cover slips. This procedure is summarised in Table 1.

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 GN 2 (Deeley, 1955). The wavelength used was $5480^{\circ}A$. Telophase nuclei were measured in *V. faba* because most of the prophase nuclei were too large for the first field stop used (10X) and the values were doubled to obtain the value of the prophase nuclei. The prophase nuclei of the species with which it was compared were measured directly. 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 ± standard error. The method used for the calculation of standard error is shown in the appendix (page 125).

2.3   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 three

to three and three-quarter 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

hydrolysed in 1N HCl at 60°C for about 10 min.

            Root-tips (2 mm) were squashed in acetic orcein (1%

synthetic 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 per

cent length and relative DNA content.   In this study, mean per cent

area, rather than mean per cent 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 minimised 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 characterise the individual pairs.   In these cases the chromosomes were grouped.

The amount of DNA in a chromosome arm was calculated by multiplying its mean per cent area by the relative DNA content per cell.   The assumptions involved were recognised 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.   This is justified because, except for *V. melanops* and *V. faba*, the species examined appeared to have chromosomes of fairly uniform size.

Sometimes, a test of significance was carried out between the areas of the chromosome arms of two species.   The method used is as described in the appendix (page 126).   Two chromosome arms are considered to be the same (or 'shared') if they are not significantly different at the level of probability $P = 0.05$.

2.4   Species hybridisation.

Plants were grown either in pots in the glasshouse or in the open garden.

At the time of flowering, the viability of the pollen grains and the time of occurrence of meiosis in the pollen mother cells of each species were examined.   Crosses were made by emasculating the flowers with forceps, as far as possible leaving the

corolla and the calyx untouched. The stigma was pollinated immediately with fresh pollen from the male parent. Pollinations were repeated three to four times at daily intervals. The periods between flowering and emasculation and between emasculation and pollination were also varied. The freshly pollinated stigmas were covered with gelatin capsules which reduced evaporation without condensation.

Reciprocal crosses were made between species with

a)   large differences in DNA content per cell but taxonomically very closely related,

b)   small differences in relative DNA content per cell (e.g. the cross between *V. faba* and *V. melanops*),

c)   similar karyotypes (see page 44) but different DNA contents per cell.

CHAPTER 3.

DNA CONTENT PER CELL AND KARYOTYPES OF *VICIA* SPECIES.

RESULTS AND DISCUSSION.

3.1   Morphological evolution.

Because there is no monograph of the genus *Vicia*,
the data on taxonomy and evolution of the genus *Vicia* quoted in
this thesis are obtained from several sources.   They are:

a)   Flora Europeae, Volume 2 (Number 49. *Vicia* L., by
Ball) (edited by Tutin, Heywood, Burges, Moore, Valentine,
Walters and Webb, 1968).

b)   Flora von Mittel-Europa (*Vicia* L., by Gams) (edited by
Hegi, 1924).

c)   Communications of Mettin (1961), Hanelt and Mettin
(1966, 1970), Mettin and Hanelt (1968).

d)   Hanelt (personal communications).

The genus *Vicia*.

Vicia is a genus of the tribe *Fabeae* of the family
Leguminosae.   The genus consists of about 150 species, most of
them occurring in the northern temperate zone of the old and new
world though there are a few in S. America.

TABLE 2.

Some characteristics of *Vicia* species
in the four sections of the genus.

Section	Ervum	Cracca	Vicia	Faba
Habit	Mostly annuals	Annuals and perennials	Annuals except *V. sepium*	Annuals
Leaflets	Numerous > 4 pairs	Numerous > 5 pairs	> 3 pairs	1-3 pairs
Flowers	Few. In long pedunculate racemes. Calyx not gibbous at base. Corolla ⩽10 mm. Style glabrous or equally pubescent all round. Autogamy especially in small flowered species.	Numerous. In long pedunculate racemes. Calyx bilabiate, gibbous at base. Corolla large, >10 mm. Style equally pubescent all round. Insect pollinated.	Solitary, axillary or in few flowered sessile or shortly pedunculate racemes. Corolla large, >10 mm. Style pubescent on the lower side beneath stigma. Insect pollinated. Tendency towards autogamy.	As in Vicia.
Evolutionary status with regard to morphological characteristics	Retained relatively many primitive characteristics. Broke away from ancestral stock early and underwent independent differentiation.	Intermediate between Ervum and Vicia.	A more derived group.	As in Vicia.

Gams (1924) divided the genus *Vicia* into three subgenera, Vicia, Cracca and Ervum. Ball (1968), however, recognises four sections, Ervum, Cracca, Vicia and Faba in his treatment of the flora of Europe. This division is used in this thesis. According to Ball (1968) the section Faba consists of only three species, *V. bithynica*, *V. narbonensis* and *V. faba*.

The four sections of the genus *Vicia* were set up primarily on the basis of morphological similarities and discontinuities together with the aid of evidence from geographic distribution and in a few cases biochemical characteristics. Karyotype similarity has been used by a few authors in a few cases in deciding to which of two closely related sections a particular species belonged; that is, karyotype evidence has been used only to a limited extent in giving weight to morphological discontinuities between the sections. This restricted use of karyotype evidence in delimiting the sections thus has little effect in accentuating or obscuring the evolutionary trends in karyotype changes which will be described here.

To date, no attempt has been made by taxonomists to arrange species within sections in order of their evolutionary advancement. Some of the taxonomic characteristics of each section of the genus is tabulated in Table 2. The morphological characteristics shown in Table 2 are taken from Ball (1968). According to Hanelt and Mettin (1970), the most general morphological

TABLE 3.

A comparison between the observed means and the expected means of three *Vicia* species.

	Observed mean ± standard error	Expected mean from the other two comparisons
1. *V. narbonensis* versus *V. faba*	54.5 ± 1.8	52.1 (19.8 ÷ 38.0) x 100
2. *V. sativa* versus *V. faba*	19.8 ± 1.0	20.7 (0.38 x 0.545 x 100)
3. *V. sativa* versus *V. narbonensis*	38.0 ± 0.7	36.3 (19.8 ÷ 54.5) x 100

evolutionary trends in the genus *Vicia* are decrease in the number
of primary scale-leaves, reduction of the inflorescense, shift
to autogamy and change in habitat pattern (from the cold temperate
to the warm temperate).   If this is true, it appears then that
the section Ervum has retained a large number of primitive
characteristics while the sections Vicia and Faba appear to have
more advanced characteristics and may be said to be more derived.
The section Cracca appears, however, to have some morphological
characteristics that are intermediate between the sections Ervum
and Vicia.   In this context, Ervum may be regarded as being the
most primitive, then Cracca, Vicia and Faba the most advanced.
It should be noted, however, that there is no taxonomic evidence
to show that there is a direct evolutionary trend from
Ervum→ Cracca→ Vicia→ Faba.

3.2   DNA content per cell of *Vicia* species.

3.2.1   Variation in DNA content per cell between
             Vicia species.

The relative DNA content per cell of 45 species of
the genus *Vicia* were estimated as described in Methods.   To test
the reliability of the method of estimation, DNA comparisons were
made between three species of *Vicia* (*V. faba*, *V. narbonensis* and
V. sativa).   The data shown in Table 3 indicate that there is

TABLE 4.

Comparison of DNA contents per cell for some *Vicia* species
reported independently by three different workers.

Species	In this thesis	Martin (1968)	Rees *et al.* (1966)[#]
V. lathyroides	19.7 ± 1.2	22.3 ± 1.2	
V. angustifolia	23.0 ± 1.0		17.2
V. grandiflora	24.9 ± 0.8	25.5 ± 0.7	
V. sativa	19.8 ± 1.0	18.5 ± 0.5	17.2
V. cordata*	17.2 ± 0.9	16.7 ± 0.4	
V. sepium	35.4 ± 0.7	27.6 ± 1.1	57.0
V. hybrida	51.1 ± 1.3	50.9 ± 1.4	
V. pannonica	50.9 ± 1.0	51.7 ± 0.9	
V. lutea	55.6 ± 1.3	59.1 ± 1.7	46.7
V. narbonensis	54.5 ± 1.8	56.8 ± 1.3	46.7
V. hirsuta	30.01 ± 0.7		37.9
V. melanops	86.1 ± 1.5	82.8 ± 0.7	
V. faba	100	100	100

* Mistaken for *V. amphicarpa* in Martin and Shanks (1966)
and Martin (1968).

\# Rees *et al.* (1966) did not publish actual DNA values. The
values shown in this column were calculated from a graph
published in their communication.

TABLE 5.

Comparison of the karyotypes of six *Vicia* species reported by Martin and Shanks (1966) and those reported here.

Species	Chromosome number	Differences in chromosome arm areas obtained	
		Results reported by Martin and Shanks (1966)	Results reported here
V. faba	1	Satellite larger by 0.46 units. Distal arm smaller by 0.65 units.	
	2	Short arm smaller by 0.28 units. Long arm smaller by 1.38 units.	
	3	Not grouped Short arm larger by 0.19 units.	3, 4 and 5 grouped
	5	Short arm larger by 0.25 units.	
V. lutea	1	Satellite larger by 0.74 units. Proximal arm larger by 0.27 units. Distal arm smaller by 0.42 units.	
V. narbonensis			
	1	Satellite larger by 0.92 units. Proximal arm larger by 0.31 units. Distal arm shorter by 0.35 units.	
	4	Metacentric	Metacentrics absent
	7	Metacentric	
		Grouping different	
V. hybrida		Only one satellite chromosome	Two satellite chromosomes
	3	Long arm smaller by 1.04 units.	
	4, 5, 6.	Short arm of 4, 5 and 6 larger by 0.16 units	
		Grouping different	
V. cordata *	2	Long arm smaller by 0.75 units	
	3, 4, 5.	3, 4 and 5 grouped	No grouping
V. sativa	2	Long arm smaller by 0.45 units	
	3, 4.	Short arms larger by 0.12 units. Long arms smaller by 0.56 units. 3 and 4 grouped	No grouping
	5	Metacentric	Metacentrics absent

* Mistaken for *V. amphicarpa* in Martin and Shanks (1966).

TABLE 6.

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

Section	DNA value ± standard error (arbitrary units)	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

TABLE 6 Cont.

Section	DNA value ± standard error (arbitrary units)	Haploid chromosome number	Average DNA per chromosome	Annual (a) or perennial (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	3.6	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*	35.5 ± 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*	27.8 ± 0.8	7	4.0	a

a/p = annual, sometimes perennial.

* = species placed in section Vicia by Hanelt (personal communication).

reasonably good agreement between the observed means and the
expected means.   The expected mean for any one comparison is
derived from the observed means of the other two comparisons.

Rees *et al.* (1966) and Martin (1968) have independently
reported the DNA contents per cell of seven and twelve *Vicia*
species, respectively.   Except for *V. sepium* (reported by both
the above authors), *V. narbonensis* and *V. hirsuta* (reported by Rees
et al., 1966), the DNA values reported here are, in general, in
agreement with those reported by the above two authors.   The
differences found may be due to differences in method, to error,
to intraspecific variation or to differences in taxonomic
identification.   Table 4 shows a comparison of the DNA contents
per cell obtained by the author, Rees *et al.* (1966) and Martin
(1968).

The karyotypes of six species of *Vicia* have also been
reported independently by Martin and Shanks (1966).   Although the
same species were also studied in this work, substantial differences
in karyotype were obtained (Table 5).

The salient facts concerning the basic chromosome
number, relative DNA content per cell and average DNA content per
chromosome of the 45 species of *Vicia* studied in this thesis are
shown in Table 6.   From the table 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

Fig. 3.   Graph showing the relationship between relative

DNA content per cell and average relative DNA per chromosome

for 45 *Vicia* species.

      ○   – denotes relative DNA content per cell of one

           species

      O   – denotes relative DNA content per cell of two

           species
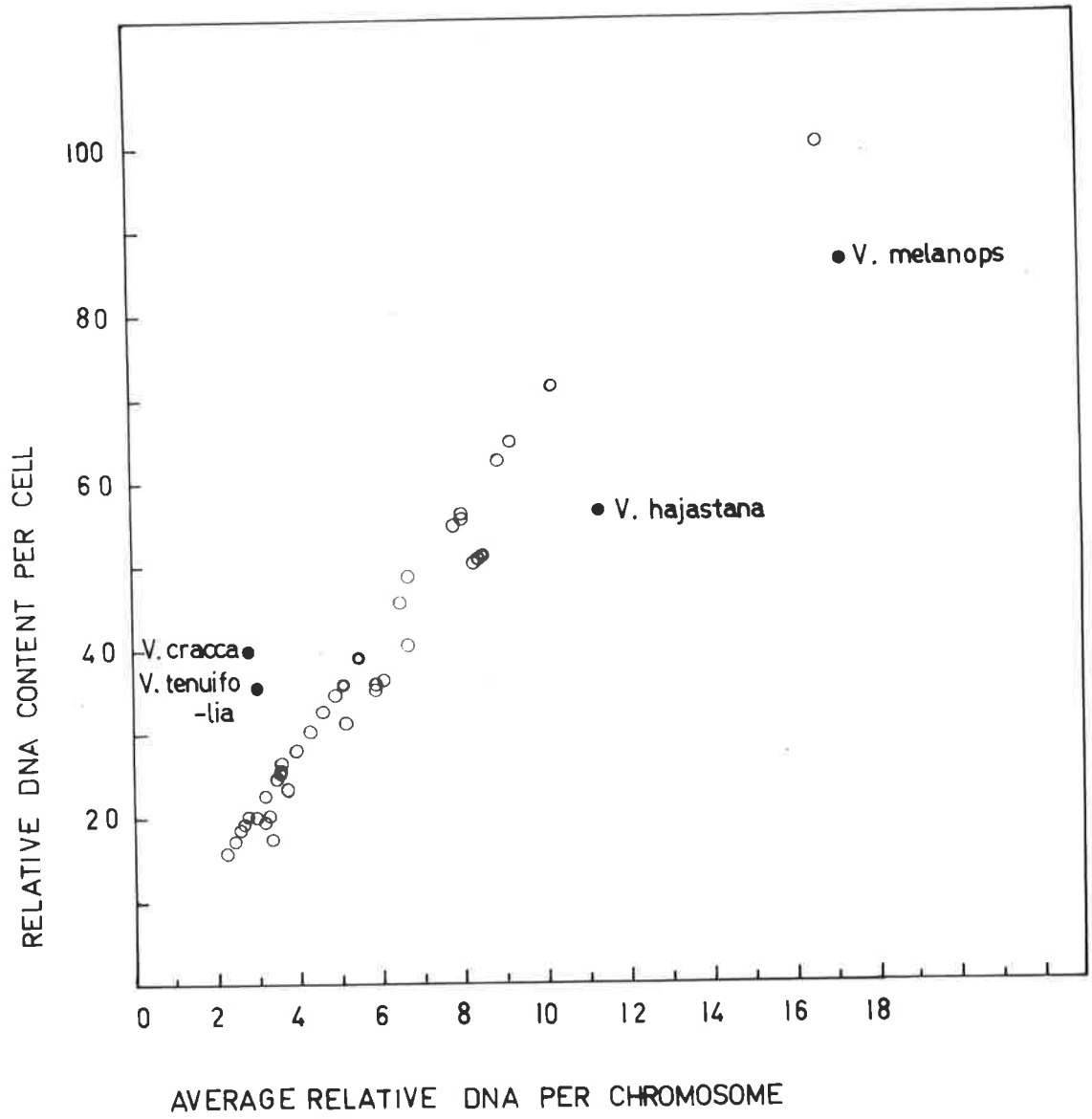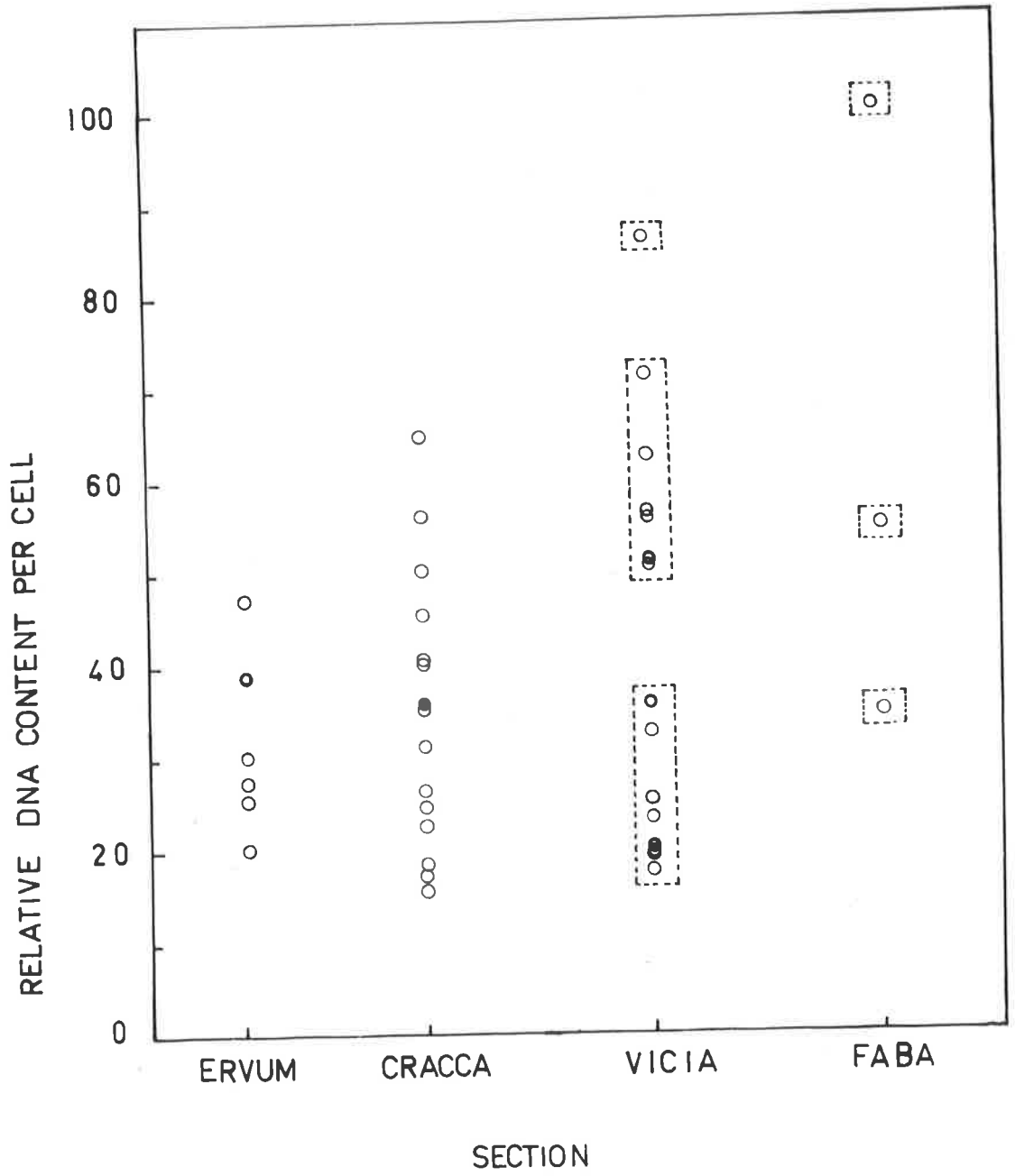
      ●   – see text, page 38.

Fig. 4.   Graph showing the distribution of DNA contents
per cell of 45 *Vicia* species in the four sections of the
genus *Vicia*.

          ○   – denotes DNA content per cell of one species.

          ○   – denotes DNA content per cell of two species.

          ●   – denotes DNA content per cell of three species.

DNA content per cell varies approximately 6-fold from 15.7 in

V. eriocarpa to 100 in *V. faba.*

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 distribution pattern (Fig. 3).   However, if the genus

is split up into its four component sections, 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 (Fig. 4).   There seem to be three disjunct groups in the two

sections Vicia and Faba (this rather subjective judgement will be

re-inforced on pages 39-40).   In the section Vicia, the first

disjunct group (comprising ten species) centres around an average

relative DNA content per cell of 25;   the second disjunct group

(comprising seven species) centres around an average relative

DNA content per cell of 60, and the third disjunct group

(comprising only one species) has a value of 86.1.   In the section

Faba which includes only three species, there is one species with

a relative DNA content per cell of 34.3, another of 54.5 and a

third of 100.

There appears to be some correlation between the

relative DNA content per cell and the taxonomic division of the

genus into its four sections.   Larger variations appear to be

found in the 'more advanced' sections (Fig. 4).   The relative

DNA content per cell of species in the 'most primitive' section

Ervum has the smallest variation from 19.8 to 47 (approximately

2.5-fold). Species in the section Cracca have an approximately

3.7-fold variation in relative DNA content per cell, from 15.7 to

55.8. A 3-fold variation from 34.3 to 100, in relative DNA

content per cell is found between species in the section Faba.

The largest variation in relative DNA content per cell

(approximately 4.5-fold, from 17.2 to 86.1) is found in the

section Vicia.

     Fig. 3 shows a graph of average relative DNA content

per chromosome against relative DNA content per cell. It is

apparent that there is a linear relationship between relative

DNA content per cell and average relative DNA content per

chromosome. With four exceptions (*V. melanops*, *V. hajastana*,

V. cracca and *V. tenuifolia* which are denoted by black dots in

Fig. 3), species with more DNA per cell have correspondingly

more average DNA per chromosome, i.e., larger chromosomes.

Fig. 3 shows that *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, see Fig. 7) is postulated to have been

derived from the fusion of two smaller acrocentrics. 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 diploid species. The

Fig. 5.   Graph showing the distribution of chromosome sizes
(average relative DNA per chromosome) of 45 *Vicia* species in
the four sections of the genus *Vicia*.

        ○   – denotes average relative DNA per chromosome

                of one species.

        O   – denotes average relative DNA per chromosome

                of two species.

AVERAGE RELATIVE DNA PER CHROMOSOME
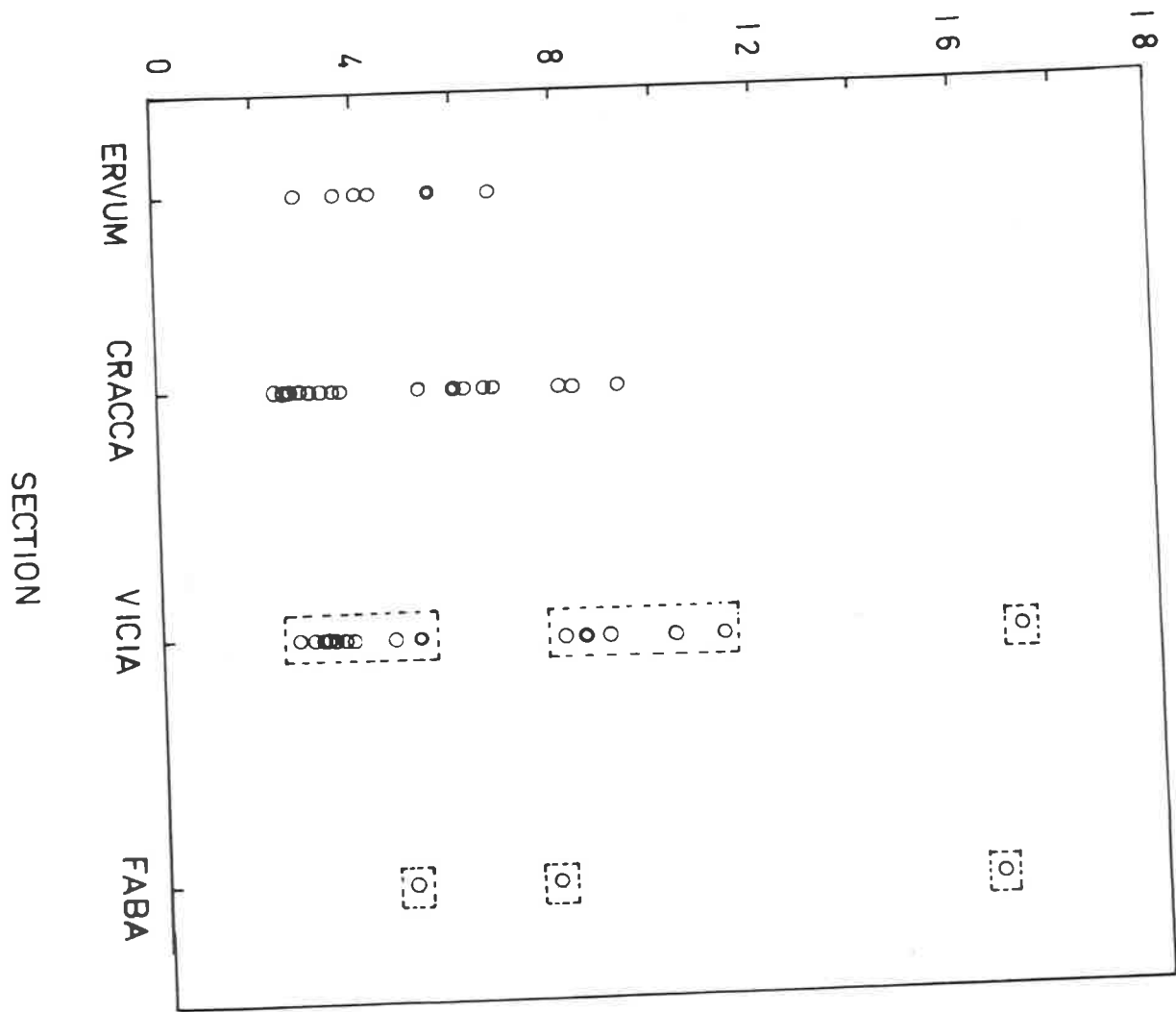
same explanation can also be given to the case of *V. hajastana*
if its large satellite chromosome (chromosome 1, see Fig. 7) is
postulated to have been derived from the fusion of two other
smaller chromosomes (see page 59). Again if the relative DNA
content per cell is divided by a haploid chromosome number of 6
instead of 5, the relative DNA content per cell would then bear
the same relationship to the average DNA content per chromosome as
the rest of the *Vicia* species. 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 polyploidy
results in 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 these two hypotheses is correct is uncertain.

Fig. 5 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*) to 17.2 (in *V. melanops*). It is apparent
that the evolution in *Vicia* species has been accompanied by a change
in chromosome size. In the section Ervum, the DNA content per
chromosome varies only 2.4-fold, from 2.8 to 6.7. In the section
Cracca the DNA content per chromosome varies over a 4-fold range,
from 2.3 to 9.2. In the section Vicia there is a 6-fold range in
DNA content per chromosome, from 2.7 to 17.2. In the section Faba

TABLE 7.

Chromosome size (amount of DNA in arbitrary units) in

45 *Vicia* species.

Analysis of variance

	Sums of squares	Degrees of freedom	Variance
Variation between species	3035.78	44	70.60
Variation within species	141.32	263	0.5374
Total	3177.12	306	

Variance ratio = 130.74 $P < 0.001$

there is a 3.5-fold range in DNA content per chromosome, from 4.9 to 16.7. If the genus is considered as a whole the values for chromosome sizes form a continuous series. If the genus is split into its four sections, however, chromosome sizes fall into three disjunct groups in the sections Vicia and Faba but form continuous series in the sections Ervum and Cracca. In the section Faba, the three disjunct values formed by the three component species of the section are 4.9, 7.8 and 16.7. In the section Vicia, the first group consists of values of approximately 4, the second of approximately 9.5 and the third of 17.2. The significance of disjunct distributions of DNA contents per chromosome with regard to the mechanism of increase in DNA in chromosomes is discussed on pages 66-68. It is noteworthy that

a) in all sections (except in the section Faba which, because of its small size, can be ignored) there are species with very small chromosomes,

b) there is little variation in size between chromosomes constituting a chromosome complement. Table 7 shows that variation in chromosome sizes (DNA per chromosome in arbitrary units as displayed in Fig. 7) within a species is very significantly smaller than variation in chromosome sizes between species; the ratio of the 'between species' variance to that within species is 130.

TABLE 8.

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	P<0.001
Between species	1	406.7	406.7	78.1	P<0.001
Interaction	1	4.3	4.3	0.8	P>0.01
Within samples	76	396.0	5.2		
Total	79	846.5			

Mean of *V. dasycarpa* = 18.8

Mean of *V. villosa* = 14.2

3.2.2   Variation in DNA content per cell between
         taxonomically synonymous species and taxonomic
         subspecies.

According to Ball (1968) *V. dasycarpa*
(RDC/cell[1] = 24.4 ± 1.4) and *V. eriocarpa* (RDC/cell = 15.7 ± 0.3)
are subspecies of *V. villosa* (RDC/cell = 17.1 ± 0.5).   The
relative DNA content per cell of *V. dasycarpa* appears, however,
to be significantly different from that of *V. villosa*.   A
similar result is obtained when a direct comparison is made between
V. dasycarpa and *V. villosa* (Table 8) (i.e. instead of
indirectly via *V. faba*).

         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 (similar to the one made between
V. dasycarpa and *V. villosa*) between *V. angustifolia*
(RDC/cell = 23.0 ± 1.0) and *V. sativa* shows that their relative
DNA contents per cell are significantly different.

---

[1] RDC/cell = abbreviation for relative DNA content per cell.   The
same abbreviation is used throughout the text and figures.

Fig. 6.   Graph showing the distribution of DNA contents per cell of annual (○) and perennial (△) species in the four sections of the genus *Vicia*.

> ○   – denotes relative DNA content per cell of one annual species.
>
> ●   – denotes relative DNA content per cell of two annual species.
>
> ●   – denotes relative DNA content per cell of three annual species.
>
> △   – denotes relative DNA content per cell of one perennial species.
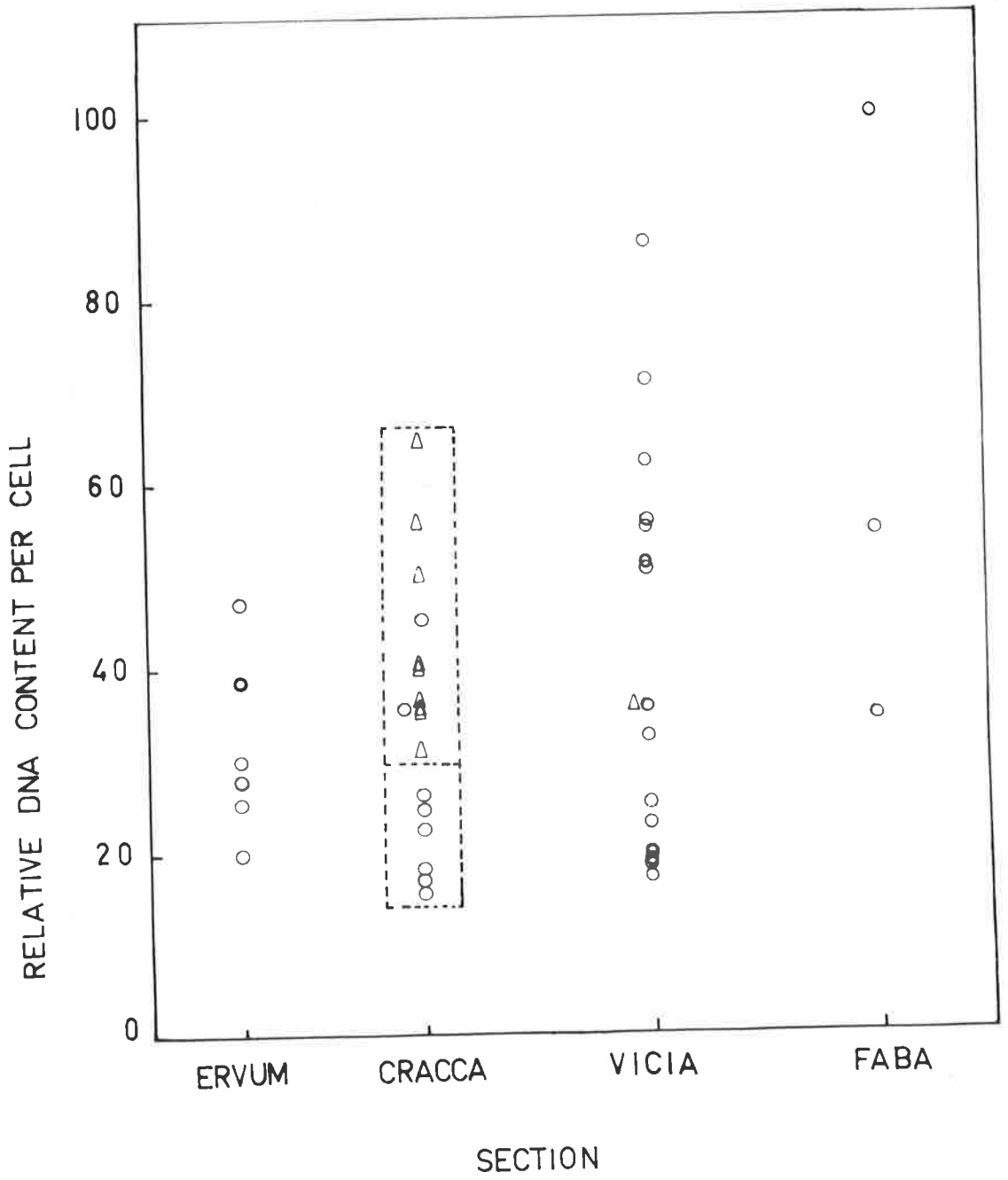
According to Mettin and Hanelt (1968) *V. atropurpurea* (RDC/cell = 18.2 ± 0.9) is taxonomically synonymous with *V. benghalensis* (RDC/cell = 26.2 ± 0.2). However, *V. benghalensis* appears to have 44% more DNA per cell than *V. atropurpurea*. A direct comparison (similar to the one between *V. dasycarpa* and *V. villosa*) between the two species shows that their relative DNA contents per cell are significantly different.

These examples show that variation in DNA content per cell occurs not only between species, but also between subspecies and between species that are considered as taxonomically synonymous. 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.

3.2.3   Correlation between reduction in DNA content

         per cell and reduction in life cycle.

In the genus *Vicia*, the 'most primitive' (Ervum) and the 'most advanced' sections (Vicia and Faba) are dominated by annuals. The section Cracca, however, consists of both annuals and perennials. In the section Cracca, with two exceptions, perennials have more DNA per cell than annuals (Fig. 6). Because

a)   Hanelt and Mettin (1970) have suggested that evolution

      within the section Cracca has proceeded from a perennial

      habit to an annual habit,

   b)   the finding that, with two exceptions, perennials in

   the section Cracca have more DNA per cell than annuals,

it is probable that evolution from a perennial habit to an annual

habit within the section Cracca is accompanied by loss in DNA per

cell.   The morphological, cytological and biochemical data of

Hanelt and Mettin (1970) have strongly indicated that *V. sepium*

(perennial) is very closely related to *V. grandiflora* (annual)

and that 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 6).   It appears, therefore, that a shortening

of the life cycle is accompanied by a loss in DNA content per cell.

The observation 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 early from

the ancestral stock of species and have undergone independent

differentiation (Hanelt and Mettin, 1970).   This correlation

between a low DNA content per cell and an annual life cycle has

also been observed in the genus *Crepis* where the specialised

short-lived annuals appear to have undergone a reduction in

chromosome size (Babcock and Jenkins, 1943).   This correlation

also suggests that perennial species may possess internal

duplications of genetic material which can be sacrificed without

lethal results.   It is perhaps reasonable to assume that the

Fig. 7.   The karyotypes of 45 *Vicia* species.

RDC/cell = relative DNA content per cell.   The same

abbreviation is used throughout the text and Figures.

aar = average arm ratio of the chromosomes of the

complement.

⌞____⌟ denotes chromosomes are grouped (see text,

page 31).

The satellite of a satellite chromosome is shown
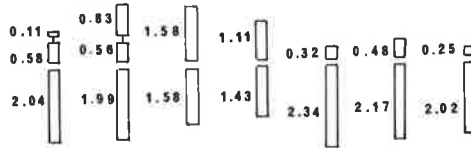
attached to one of the chromosome arms.

Note: All the karyotypes in this Figure are drawn

to the same scale so that visual comparison is
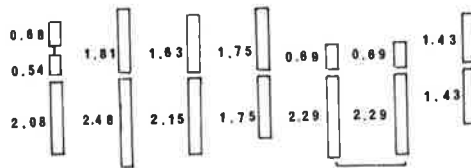
possible.

V. graminea

RDC/cell = 38.78 ± 0.30

2n = 14   aar = 3.8

0.11 0.83 1.58 1.11 0.32 0.48 0.25

0.58 0.56

2.04 1.99 1.58 1.43 2.34 2.17 2.02

V. meyeri

RDC/cell = 46.95 ± 0.27

2n = 14   aar = 1.9

0.68 1.81 1.63 1.75 0.89 0.69 1.43

0.54

2.08 2.48 2.15 1.75 2.29 2.29 1.43

SECTION CRACCA

V. eriocarpa

RDC/cell = 15.66 ± 0.31
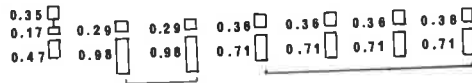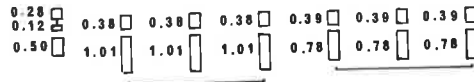
2n = 14   aar = 2.3

0.35 0.29 0.29 0.36 0.36 0.36 0.36

0.17

0.47 0.98 0.98 0.71 0.71 0.71 0.71

V. villosa

RDC/cell = 17.12 ± 0.49

2n = 14   aar = 2.2

0.28 0.38 0.38 0.38 0.39 0.39 0.39

0.12

0.50 1.01 1.01 1.01 0.78 0.78 0.78

V. atropurpurea

RDC/cell = 18.20 ± 0.94

2n = 14   aar = 2.5

0.33 0.34 0.34 0.41 0.35 0.39 0.39

0.12

0.58 1.18 1.18 0.92 1.05 0.78 0.78
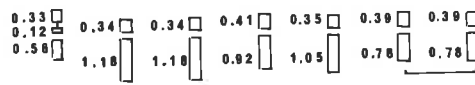
V. dasycarpa

RDC/cell = 24.38 ± 1.39

2n = 14   aar = 2.1

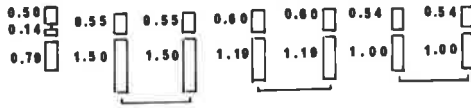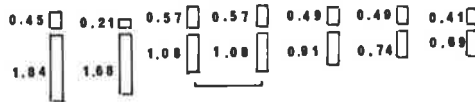**V. benghalensis**

RDC/cell = 26.16 ± 0.24

2n = 14   aar = 2.3

V. biennis

RDC/cell = 22.44 ± 0.39

2n = 14   aar = 3.0

V. tenuifolia

RDC/cell = 35.52 ± 0.38

2n = 24   aar = 3.0
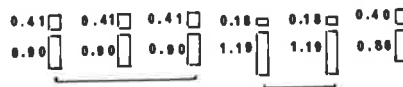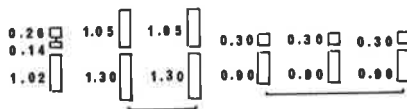
V cracca

RDC/cell = 39.76 ± 0.38

2n = 28   aar = 3.7

V. cassubica

RDC/cell = 30.05 ± 0.23

2n = 12   aar = 2.0

0.31
0.38   1.26   1.09   1.09   0.64   0.64

1.56   2.10   1.43   1.43   1.77   1.77
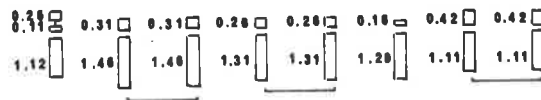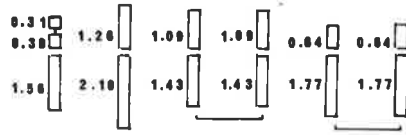
V. orobus

RDC/cell = 40.31 ± 0.28

2n = 12   aar = 2.0
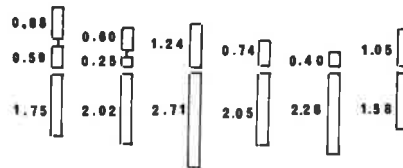
0.46
0.52   1.61   1.66   1.31   0.78   0.78

1.89   2.64   2.11   1.61   2.28   2.28

V. unijuga

RDC/cell = 36.30 ± 0.29

2n = 12   aar = 2.6

0.88   0.60   1.24   0.74   1.05
0.59   0.25   0.40

1.75   2.02   2.71   2.05   2.28   1.58

V. ramuliflora

RDC/cell = 35.13 ± 0.13

2n = 12   aar = 3.2

0.61
0.65   0.52   1.19   0.61   0.33   0.96

1.80   2.01   2.60   2.05   2.26   1.78

V. dumetorum

RDC/cell = 55.78 ± 0.47

2n = 14   aar = 2.0

0.31
0.68   1.86   1.51   1.51   1.51   1.73   0.79

2.90   2.96   2.48   2.48   2.48   1.73   2.07

V. neglecta

RDC / cell = 35.39 ± 0.31

2n = 12   aar = 4.1

0.29 1.78   0.56   0.35   0.54   0.68

0.46

1.89 2.30 2.64 2.43 2.10 1.82
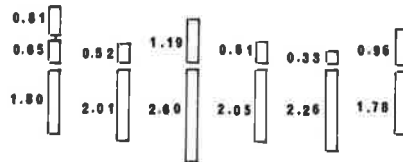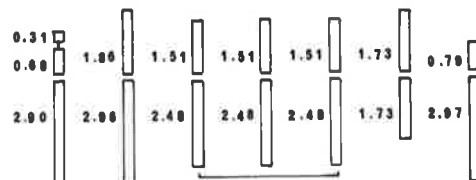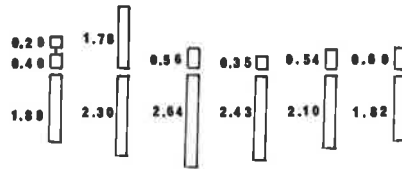
V. articulata

RCD/ cell = 45.33 ± 1.52

2n = 14   aar = 5.2
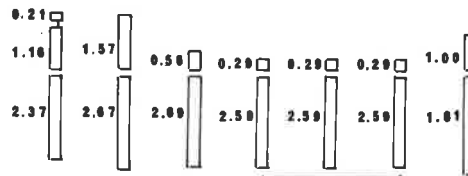
0.21

1.16 1.57   0.56   0.29   0.29   0.29   1.00

2.37 2.67 2.69 2.50 2.59 2.59 1.81

V. pisiformis

RDC/cell = 49.92 ± 0.29

2n = 12   aar = 1.8

0.30   2.81   2.08   2.21   0.91   1.12

0.66

2.66 2.81 2.37 2.21 2.85 1.98

V. sylvatica

RDC/cell = 64.56 ± 0.84

2n = 14   aar = 2.3

1.87 1.25 1.25 1.25 1.25 1.43 1.73
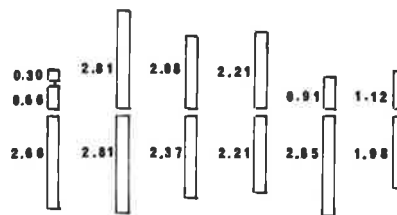
4.03 3.46 3.46 3.46 3.46 2.67 1.73
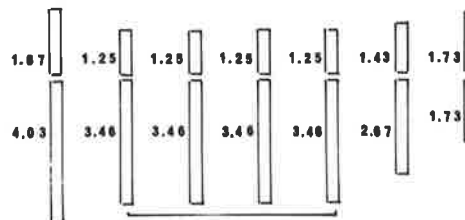
SECTION VICIA

V. sativa

RDC/cell = 19.60 ± 0.98

2n = 12   aar = 16.6

V. angustifolia

RDC/cell = 22.97 ± 1.45

2n = 12   aar = 14.5

V. cordata

RDC / cell = 17.22 ± 0.90

2n = 10   aar = 16.5

V. pilosa

RDC/cell = 18.58 ± 0.85

2n = 14   aar = 7.5

V. macrocarpa

RDC/cell = 19.34 ± 0.52

2n = 12   aar = 7.3

V. hybrida

RDC/cell = 51.10 ± 1.30

2n = 12   aar = 3.5

1.03

1.85   0.83
        0.48   1.03   0.76   0.76   0.76

1.49
        2.70   4.14   3.26   3.26   3.26

V. pannonica

RDC/cell = 50.87 ± 0.97

2n = 12   aar = 3.2

1.44
        1.01
1.10   0.49   1.16   0.79   0.84   0.79

1.39
        2.80   4.42   3.43   3.05   2.95

V. hyrcanica

RDC/cell = 50.50 ± 0.30

2n = 12   aar = 3.8

2.42

0.55   1.24   0.85   0.74   0.74   0.74
1.07
        4.26   3.45   3.07   3.07   3.07

V. lutea

RDC/cell = 55.84 ± 1.27

2n = 14   aar = 3.2

1.32
0.28   1.57   0.81   0.81   0.81   0.81   0.81

1.89   3.10   3.12   3.12   3.12   3.12   3.12

V. hajastana

RDC/cell = 56.22 ± 1.73

2n = 10   aar = 3.3

1.23

1.92

3.32

1.63

1.09   0.94   0.93

1.01

3.70

4.69   3.69   3.18
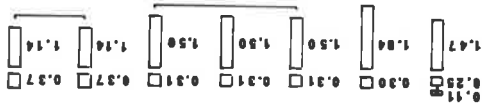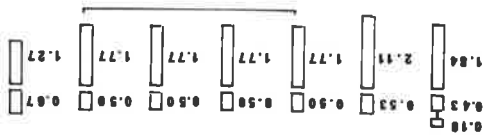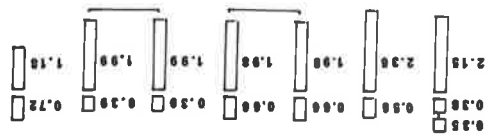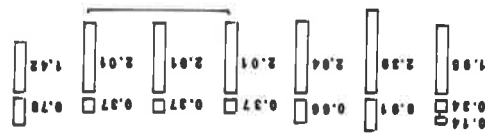
V. michauxii

RDC/cell = 62.27 ± 0.31

2n = 14   aar = 16.5

1.82

2.10

1.80

0.41   0.33   0.33   0.14   0.14   0.14

0.54

3.25   4.22   4.22   3.82   3.82   3.82

V. peregrina

RDC/cell = 71.10 ± 0.56

2n = 14   aar = 15.5

2.24

2.44

2.21

0.53   0.38   0.38   0.29   0.29   0.09

0.29

4.09   4.81   4.81   4.44   4.44   3.85
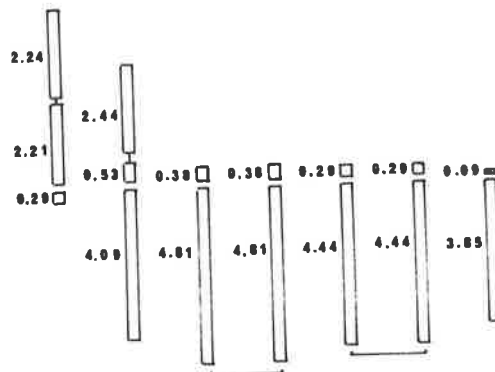
V . melanops

RDC / cell = 86.13 ± 1.48

2n = 10   aar = 3.1

1.77

3.53   5.94

1.43   1.25   1.25

3.80   6.93   6.41   5.38   5.38
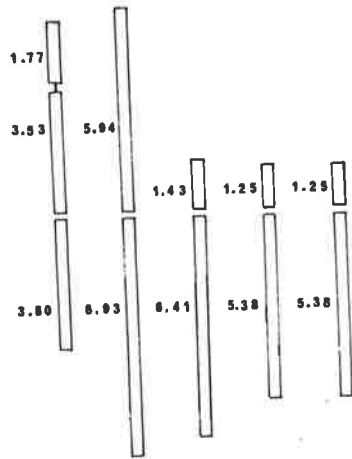
SECTION FABA

V . bithynica

RDC / cell = 34.32 ± 1.26

2n = 14   aar = 15.0

0.16   0.21   0.06   0.18   0.18   0.18   0.35

2.46   2.62   2.03   2.23   2.23   2.23   1.66

V . narbonensis

RDC / cell = 54.50 ± 1.82

2n = 14   aar = 2.0

0.83

0.41   1.11   1.11   1.49   1.49   1.35   1.35

1.45   2.55   2.55   2.67   2.67   2.92   2.92

V. faba

RDC/cell = 100

2n = 12   aar = 13.0

2.43

4.53

0.57   0.41   0.41   0.41   0.37

8.60   7.60   6.38   6.38   6.38   5.55
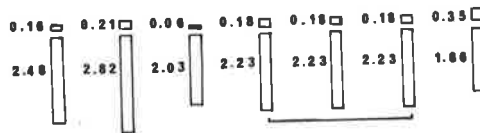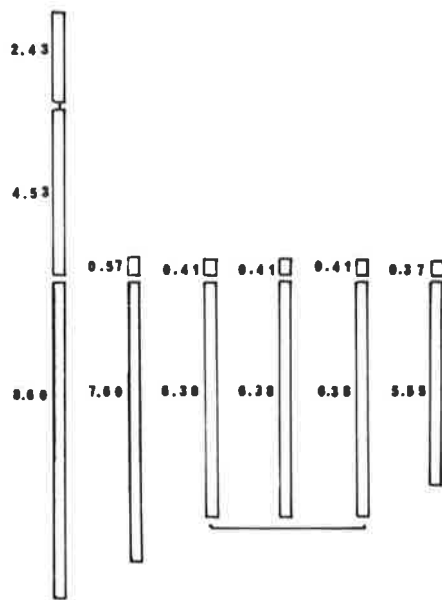
deleted portions of the genome have the effect of restricting the species to special environments, thus seriously limiting their adaptability.

An alternative explanation for the correlation between high DNA value and being perennial within the section Cracca is that species with more DNA per cell tend to become perennials. Which of these two hypotheses is correct is uncertain but the morphological evidence apparently favours the former.

3.3   Karyotypes of diploid *Vicia* species.

The karyotypes[2] of 45 species of *Vicia* which were analysed as described in Methods are shown in Fig. 7.   The DNA contents in chromosome arms are such that, if added, they would give the DNA content of a haploid cell relative to a diploid content per cell of 100 in *V. faba*.

Similarity of chromosomes between species is taken as an indication of relationship.   Homology of chromosomes in this genus can only be suggested, not determined, by morphology of the mitotic metaphase chromosomes and the quantitative estimation of DNA in chromosome arms.   The pairing behaviour of chromosomes in

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[2]   The term karyotype used in this thesis is defined as the sum of the characteristic properties of the chromosome complement, i.e., i) number of chromosomes, ii) relative sizes of chromosomes within the set, iii) arm ratios, but NOT iv) absolute sizes as measured by DNA content per chromosome.

Fig. 8.   Graph showing the distribution of average arm ratio

values of 45 *Vicia* species in the four sections of the genus

Vicia.

    O   – denotes average arm ratio of one species.

    O   – denotes average arm ratio of two species.

    ●   – denotes average arm ratio of three species.
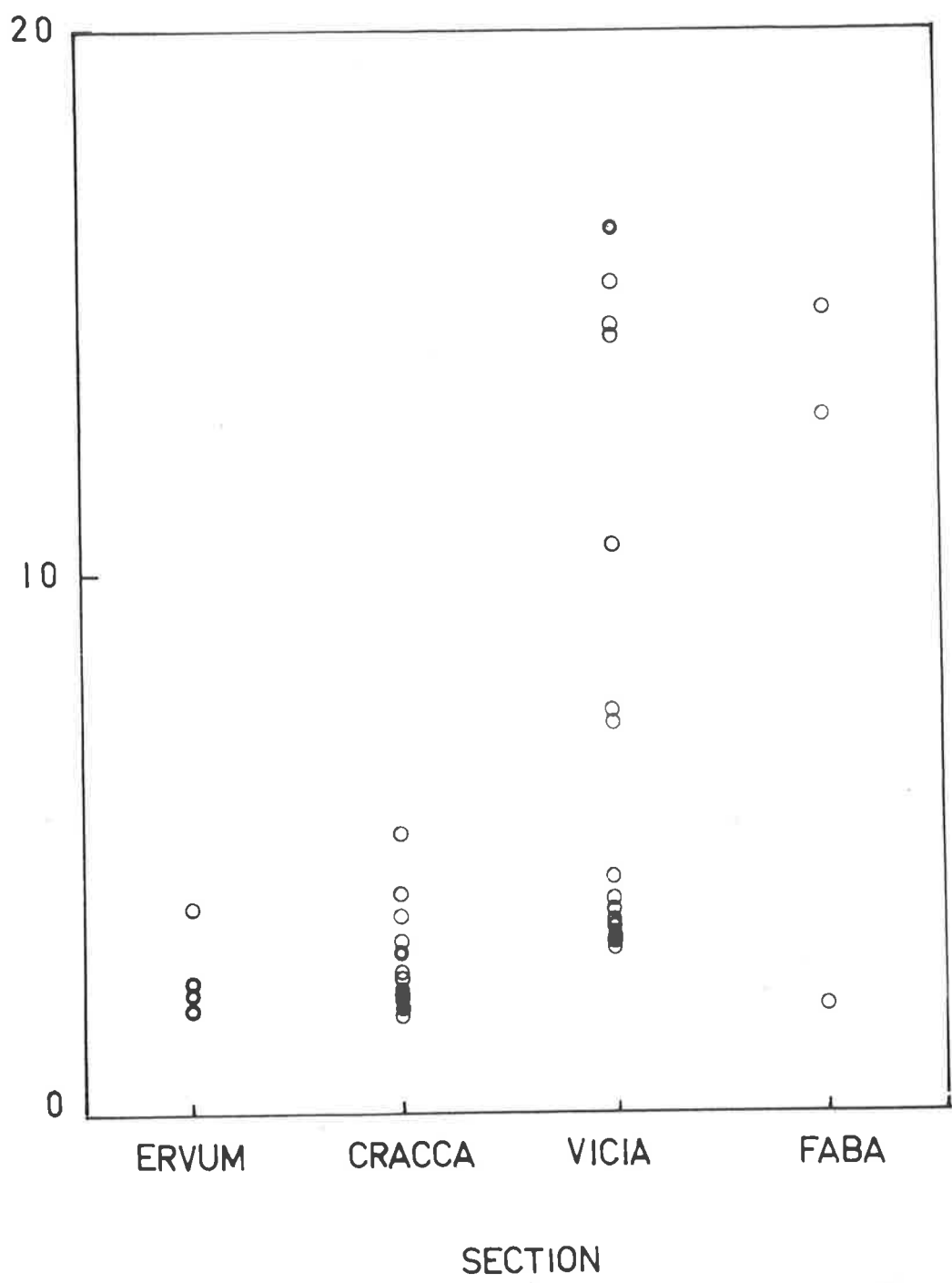
meiosis of hybrids would give more precise information but this is not available (see page 63). An added difficulty to interpreting the direction of karyotype change has been the fact that species within this genus have not been placed in a taxonomic phylogenetic sequence.

In order to study the nature of changes in DNA content per cell, nine small groups of species will be studied. These groups have been chosen either because the members are considered to be very closely related taxonomically or because they have such similar karyotypes and DNA contents that they may well be closely related. These groups will be dealt with section by section.

3.3.1   Section Ervum.

The karyotypes of species in the section Ervum are characterised by the symmetrical[3] form of most of the chromosomes. With one exception (*V. graminea*, average arm ratio = 3.8), the average arm ratios[4] of all the karyotypes in this section are less than 2.5 (they range from 1.9 to 2.4). Such karyotypes are in sharp contrast to most of those in the section Vicia in which individual chromosomes are mainly acrocentric or subtelocentric (Fig. 8, also see page 53). It appears, therefore, that the

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[3] symmetrical is defined as being metacentric or near metacentric.

[4] The arm ratio is defined as the ratio of the long arm to the short arm of a chromosome. The average arm ratio is defined as the average of the arm ratios of all the chromosomes constituting a chromosome complement.

Fig. 9. A comparison of the karyotypes of *V. hirsuta*
and *V. meyeri*.

        ar = arm ratio of the chromosome.

        aar = average arm ratio of the chromosomes of
        the complement.

V. hirsuta

RDC/cell = 30.01 ± 0.05

2n = 14   aar = 2.4

0.90 [] 0.67 [] 1.06 [] 0.91 [] 0.91 [] 0.36 [] 0.36 []

0.90 [] 0.67 [] 1.76 [] 1.51 [] 1.51 [] 1.74 [] 1.74 []

ar = 1.0   ar = 1.0   ar = 1.7   ar = 1.7   ar = 4.8

V. meyeri

RDC/cell = 46.95 ± 0.27

2n = 14   aar = 1.9

0.68 []

0.54 [] 1.81 [] 1.63 [] 1.75 [] 0.69 [] 0.69 [] 1.43 []

2.08 [] 2.48 [] 2.15 [] 1.75 [] 2.29 [] 2.29 [] 1.43 []

ar = 1.6   ar = 1.4   ar = 1.3   ar = 1.0   ar = 3.3   ar = 1.0
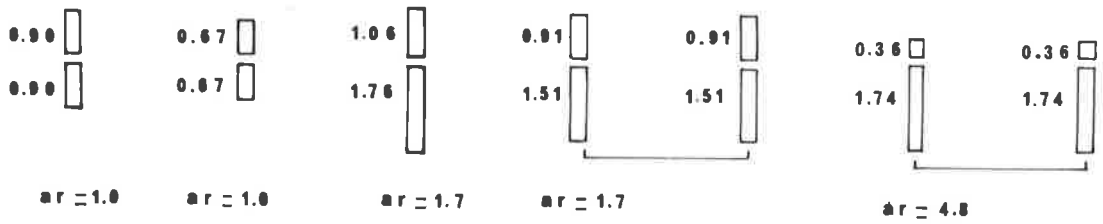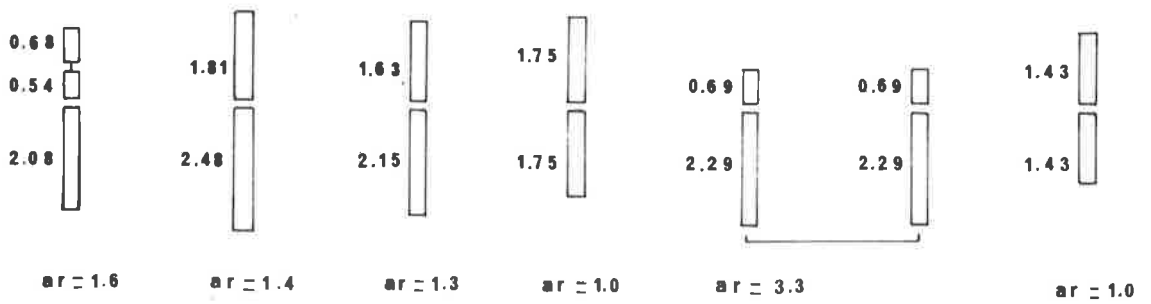
'primitive' species of *Vicia*, as determined from plant morphology also have the most nearly symmetrical chromosomes.   Although there are many similar chromosomes as well as general similarities in the karyotypes of some of the species in the section Ervum, each is nevertheless distinct and there is no basis for arranging these species in subgroups with respect to their karyotypes.   The structural changes involved in karyotype evolution in this section are not obvious from the morphology of the karyotypes.   Two pairs of species which are regarded as being taxonomically very closely related in the section Ervum are placed in two karyotype groups. Group I consists of *V. meyeri* and *V. hirsuta* while group II consists of *V. tetrasperma* and *V. pubescens*.

Group I.

V. meyeri and *V. hirsuta*.

Taxonomically, *V. meyeri* (RDC/cell = 47.0 ± 0.3) is very closely related to *V. hirsuta* (RDC/cell = 30.0 ± 0.7) (Mettin and Hanelt, 1968).   Karyotypically, however, they are different (Fig. 9).   In addition, *V. meyeri* has 17.0 units of DNA per cell more than *V. hirsuta*.   This 17.0 units of 'additional' DNA in *V. meyeri* is located in both the long and short arms of all the chromosomes in its genome but it is not distributed evenly or proportionally throughout all the chromosome arms.   The short arms in *V. meyeri* could have increased in area relatively more since the

Fig. 10.   A comparison of the karyotypes of *V. pubescens*
and *V. tetrasperma.*

ar = arm ratio of the chromosome.

aar = average arm ratio of the chromosomes of

the complement.

V. pubescens

RDC / cell = 19.77 ± 0.24

2n = 14   a ar = 1.9

0.44 ⬜   0.44 ⬜   0.44 ⬜   0.53 ⬜   0.53 ⬜   0.50 ⬜   0.62 ⬜

0.95 ⬜   0.95 ⬜   0.95 ⬜   0.70 ⬜   0.70 ⬜   1.03 ⬜   1.10 ⬜

　　ar = 2.2   ar = 1.3   ar = 2.1   ar = 1.8
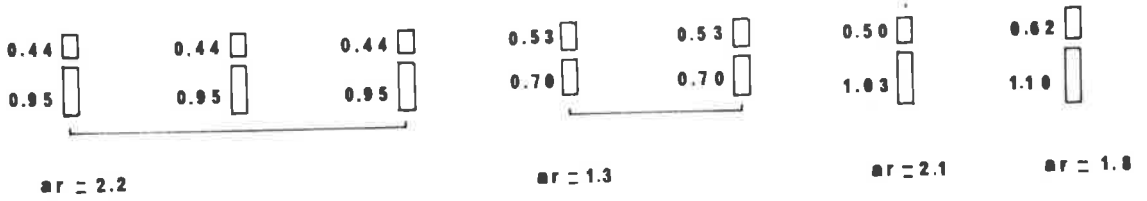
V. tetrasperma

RDC / cell = 27.80 ± 0.75

2n = 14   a ar = 2.2

0.99 ⬜   0.61 ⬜   0.61 ⬜   0.66 ⬜   0.52 ⬜   0.52 ⬜   0.52 ⬜

1.58 ⬜   1.31 ⬜   1.31 ⬜   0.97 ⬜   1.43 ⬜   1.43 ⬜   1.43 ⬜

　　ar = 1.6   ar = 2.2   ar = 1.5   ar = 2.8
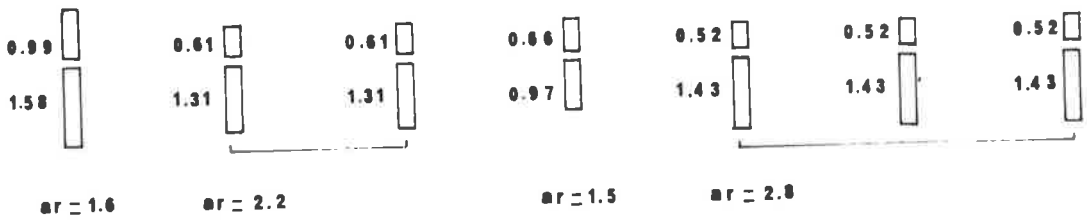
arm ratios of five pairs of its chromosomes are smaller.

Although *V. meyeri* has more DNA per cell than *V. hirsuta*,

its karyotype is not significantly more advanced since the

average arm ratio (1.9) of the chromosomes in *V. meyeri*

is smaller than that (2.4) in *V. hirsuta*.   The close morphological

relationship shared by these two species is not paralleled by

similarity in their karyotypes.

Group II.

V. tetrasperma and *V. pubescens*.

Taxonomically, *V. tetrasperma* (RDC/cell = 27.8 ± 0.8)

is very closely related to *V. pubescens* (RDC/cell = 19.8 ± 0.2)

(Mettin and Hanelt, 1968).   However, besides having 8.0 units of

DNA per cell more than *V. pubescens*, *V. tetrasperma* has chromosomes

with larger arm ratios (Fig. 10).   Although the 'additional' 8.0

units of DNA per cell found in *V. tetrasperma* is distributed to

all its chromosomes, it is neither evenly distributed to all the

chromosome arms nor is it proportionally distributed between the

two arms of any one chromosome.   As a result, the arm ratios are

different from those of *V. pubescens*.

3.3.2   Section Cracca.

If the four sections of the genus *Vicia* are placed in

a phylogenetic sequence using morphological characteristics

(i.e. non-chromosomal characteristics) the section Cracca is found

as a connecting link between the sections Ervum and Vicia (see

page 35). The species in this section may, in general, be

considered transitional. Chromosomally, they show definite

relationships to the 'primitive' section Ervum since many (fifteen)

species have average arm ratios similar to those in the section

Ervum (average arm ratios vary from 1.9 to 3.8, Fig. 8). At the

same time, a number of advanced karyotypic characteristics such

as acrocentric chromosomes are also present in some species.

Four species in the section Cracca (average arm ratio in the

section Cracca = 1.8 to 5.2) have average arm ratios that are

similar to some of those in the section Vicia (average arm ratio =

3.1 to 16.5). The average arm ratios of the majority (thirteen)

of the species in the section Cracca, are, however, smaller than

those in the section Vicia.

Of the seventeen species examined from the section

Cracca, two can be placed in one karyotype group (I), three can be

placed in a second karyotype group (II) and another two into a

third (III). Although there are general similarities in the

karyotypes of the remaining species they are too distinct to be

grouped.

Group I consists of *V. atropurpurea* and *V. benghalensis*.

Group II consists of *V. villosa*, *V. dasycarpa* and *V. eriocarpa*

while Group III consists of *V. unijuga* and *V. ramuliflora*.

Fig. 11.   A comparison of the karyotypes of *V. atropurpurea*
and *V. benghalensis*.

ar = arm ratio of the chromosome.

aar = average arm ratio of the chromosomes of
the complement.

'Additional' DNA is shaded.

V. atropurpurea

RDC $/$ cell $\simeq$ 18.20 $\pm$ 0.94

2n $\simeq$ 14   a ar $\simeq$ 2.5

0.33
0.12

0.56

0.34   0.34

1.18   1.18

0.41   0.35   0.39   0.39

0.92   1.05   0.78   0.78

ar $\simeq$ 1.2   ar $\simeq$ 3.5   ar $\simeq$ 2.2   ar $\simeq$ 3.0   ar $\simeq$ 2.0

V. benghalensis

RDC $/$ cell $\simeq$ 26.16 $\pm$ 0.24

2n $\simeq$ 14   a ar $\simeq$ 2.3

0.38
0.18

0.42

0.63   0.63

1.30   1.30

0.69   0.69   0.45   0.45

1.66   1.66   1.32   1.32

ar $\simeq$ 1.3   ar $\simeq$ 2.1   ar $\simeq$ 2.4   ar $\simeq$ 2.9
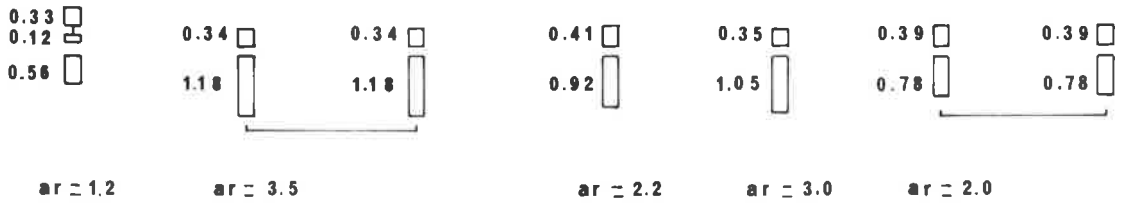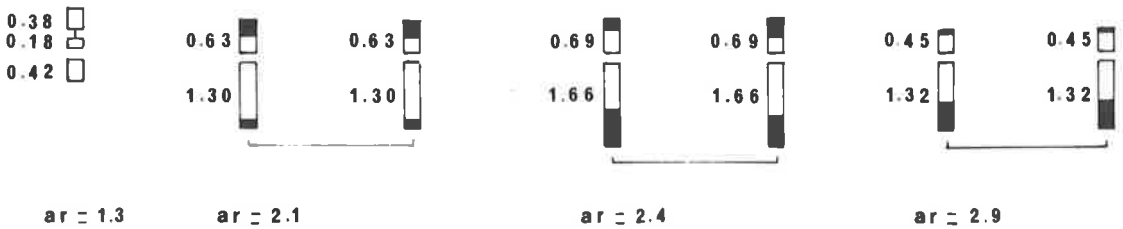
Group I.

V. atropurpurea and *V. benghalensis*.

Although *V. atropurpurea* is regarded as being taxonomically synonymous with *V. benghalensis* (Mettin and Hanelt, 1968), the only karyological similarities lie in the diploid number of chromosomes per cell and in the morphology of their satellite chromosomes (Fig. 11). It was shown (page 42) that *V. benghalensis* has 8.0 units of DNA per cell more than *V. atropurpurea*. It appears that the 'additional' 8.0 units of DNA per cell in *V. benghalensis* (shaded in Fig. 11) is distributed throughout six of the seven pairs of chromosomes[5] and is not distributed proportionally to the two arms of each chromosome. This has resulted in a change in the arm ratios of the chromosomes in *V. benghalensis*.

Group II.

V. villosa and subspecies *V. dasycarpa* and *V. eriocarpa*.

V. dasycarpa (RDC/cell = 24.4 ± 1.4) and *V. eriocarpa* (RDC/cell = 15.7 ± 0.3) are regarded as subspecies of *V. villosa* (RDC/cell = 17.1 ± 0.5) (Ball, 1968). It has been shown (page

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[5] The numbering of chromosomes is arbitrary but the increase in DNA will still be found distributed throughout all the chromosome arms of the six pairs of chromosomes no matter how the chromosomes are arranged.

Fig. 12. A comparison of the karyotypes of *V. villosa*, *V. eriocarpa* and *V. dasycarpa*.

ar = arm ratio of the chromosome.

aar = average arm ratio of the chromosomes of the complement.

The number written above a chromosome denotes its total area.

V. eriocarpa

$RDC/cell = 15.66 \pm 0.31$

$2n = 14$ $aar = 2.3$

0.99
0.35
0.17   1.27   1.07
0.47   0.29   0.29   0.36   0.36   0.36   0.36
       0.98   0.98   0.71   0.71   0.71   0.71

ar = 0.9   ar = 3.4   ar = 2.0
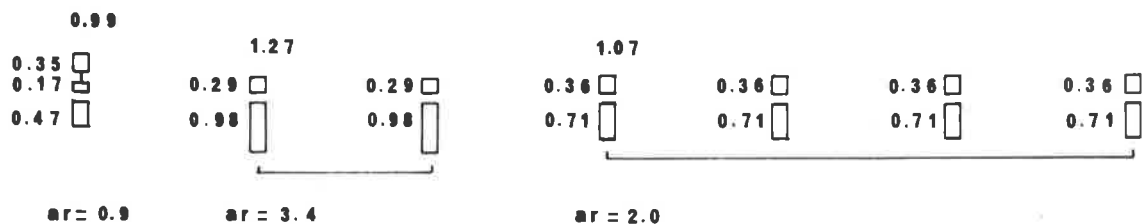
V. villosa

$RDC/cell = 17.12 \pm 0.48$

$2n = 14$ $aar = 2.2$

0.90
0.28
0.12   1.39   1.17
0.50   0.38   0.38   0.38   0.39   0.39   0.39
       1.01   1.01   1.01   0.78   0.78   0.78

ar = 1.2   ar = 2.7   ar = 2.0

V. dasycarpa

$RDC/cell = 24.38 \pm 1.39$

$2n = 14$ $aar = 2.1$

0.50
0.14
0.79   0.55   0.55   0.60   0.60   0.54   0.54
       1.50   1.50   1.19   1.19   1.00   1.00

ar = 1.2   ar = 2.7   ar = 2.0   ar = 1.9
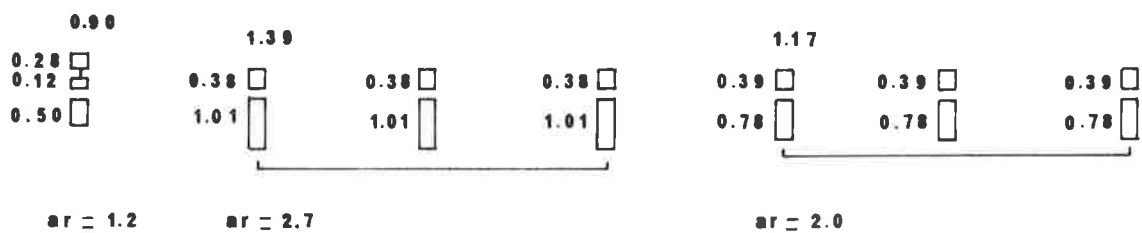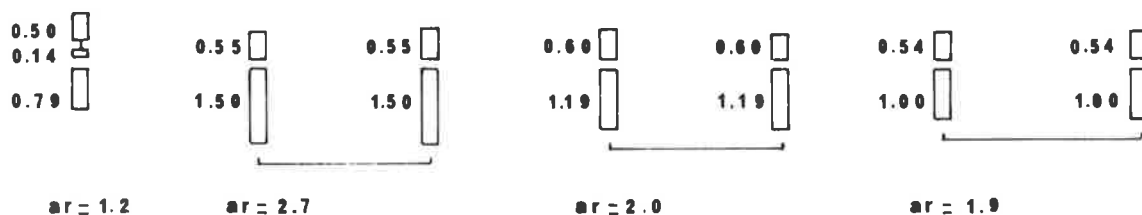
41) that *V. dasycarpa* has significantly more DNA per cell than *V. villosa* while *V. villosa* and *V. eriocarpa* have similar DNA contents per cell.   From an inspection of their karyotypes (Fig. 12), the three species appear to have similar karyotypes. Statistically[6], however, these three karyotypes are significantly different.

V. eriocarpa* and *V. villosa* have similar DNA contents per cell and there are no large differences in their chromosomes. However, the other subspecies *V. dasycarpa* has a considerably larger DNA content per cell.   The karyotype of *V. dasycarpa* is more similar to that of *V. villosa* than that of *V. eriocarpa*. Chromosomes 1, 2, 3, 4 and 5 in *V. dasycarpa* have similar arm ratios as chromosomes 1, 2, 3, 5 and 6 respectively in *V. villosa*. Chromosomes 6 and 7 in *V. dasycarpa*, are, however, larger in total area and have smaller arm ratios than chromosomes 4 and 7 in *V. villosa*.

A consideration of the karyotypes of these three species (*V. eriocarpa*, *V. villosa* and *V. dasycarpa*) show that

a)   in *V. dasycarpa*, the 'additional' DNA is not distributed evenly or proportionally to all the chromosome arms,

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[6] The hypothesis was tested as follows: The DNA contents per cell of the three species were each converted to 100.   The mean per cent areas of the chromosome arms of the three species was then compared using the method described on page 126.

Fig. 13.   A comparison of the karyotypes of *V. unijuga* and *V. ramuliflora*.

aar = average arm ratio of the chromosomes of the complement.

Postulated interchange units are arrowed.

V. unijuga

RDC / cell = 36.30 ± 0.29

2n = 12   aar = 2.6

0.88
0.59
1.75

0.80
0.25
2.02

1.24
2.71

0.74
2.05

0.40
2.28

1.05
1.58

V. ramuliflora

RDC / cell = 35.13 ± 0.13

2n = 12   aar = 3.2

0.81
0.65
1.80

0.52
2.01

1.19
2.60

0.61
2.05

0.33
2.28

0.96
1.78

reciprocal
translocation
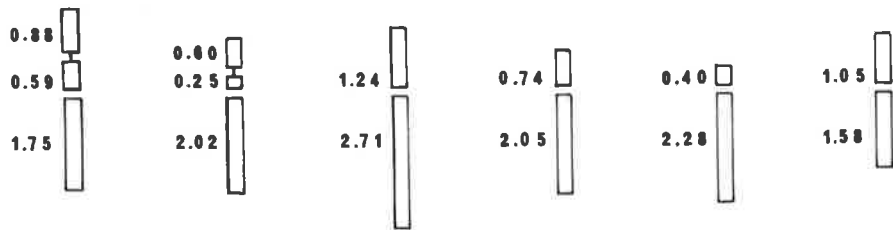
← 0.2
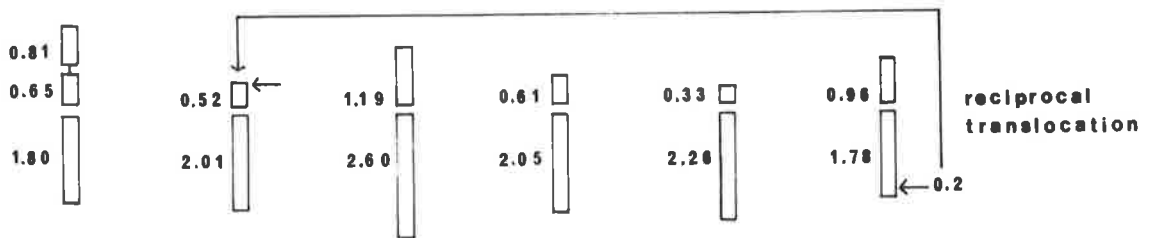
PLATE   1

Root-tip metaphase chromosomes of *V. ramuliflora*

(diploid).

10μ

b)   structural changes, the nature of which are not known, could have taken place in either *V. villosa* or *V. eriocarpa*.

It is probable, therefore, that as a result of these, significant differences are found between the karyotypes of the three species.

Group III.

V. unijuga and *V. ramuliflora*.

V. unijuga (RDC/cell = 36.3 ± 0.3) is very similar in karyotype to *V. ramuliflora* (RDC/cell = 35.1 ± 0.1) (Fig. 13). The only differences in karyotype between these two species appear to be the following:

a)   *V. ramuliflora* has only one satellite chromosome whereas *V. unijuga* has two,

b)   the long arm of chromosome 6 in *V. ramuliflora* is larger than the long arm of chromosome 6 in *V. unijuga* by 0.2 units of DNA.

As indicated in Fig. 13, a reciprocal translocation between the long arm of chromosome 6 and the short arm of chromosome 2 in *V. ramuliflora* is one hypothesis to account for the difference between the two species.

V. ramuliflora.

One of the most interesting features about *V. ramuliflora* is the difference in size between the chromosomes of the diploid species (Fig. 13, Plate 1) and that of the tetraploid species.

TABLE 9.

Comparison of the chromosome sizes of three *Vicia* species
measured by Mettin and Hanelt (1968) and those reported here.

Species	Average chromosome length (µ)	
	Mettin and Hanelt (1968)	Those reported here.
V. hajastana	13.8	7.0
V. lathyroides	6.3	3.2
V. benghalensis	6.4	3.2
V. ramuliflora (tetraploid)	2.5	(1.3)
V. ramuliflora (diploid)		5.1

The species examined here[7] was a diploid (2n = 12) with an

average chromosome length of about 5.1 µ.   Mettin and Hanelt

(1968) reported a tetraploid with 29 chromosomes.   From Table 9

it can be readily seen that the lengths of the chromosomes of

other species reported by Mettin and Hanelt (1968) and those

reported here are not directly comparable.   This is possibly

due to different treatments with colchicine or different preferences

of cells for analysis.   Taking into account the relative difference

(approximately 2-fold) the average length of the tetraploid

chromosomes would be about 1.3 µ.   It appears that the

chromosome size in the diploid species is about 4 times that of the

tetraploid.   This implies that the ancestral form of the present

day diploid and tetraploid has undergone evolution in two ways;

firstly via polyploidy where the whole chromosome set was doubled,

and secondly, where part of each chromosome was multiplied giving

chromosomes that are about 4 times the size of those of the original

diploid species.   This example agrees with the earlier

observation (page 39   ) that tetraploids have small chromosomes.

---

[7] The diploid species of *V. ramuliflora* studied here was obtained
from the authors (Mettin and Hanelt) who described the
tetraploid species. These authors reconfirmed the identification
of the diploid species studied here.   The DNA content per cell
of the tetraploid species could not be measured because the
above two authors had only a small supply of those seeds and
were unable to spare any for this work.

3.3.3   Section Vicia.

Phylogenetically, the section Vicia is regarded as being an 'advanced' section of the genus *Vicia* (see page 35). It also contains the largest number of species. Although they have well-marked common characteristics, the species in this group are very diverse karyologically. Different species show considerable variability in arm ratios (3.1 to 16.5) although the majority of the chromosomes are acrocentric (Fig. 8) it should be noted that

a)   with one exception the average arm ratios in the section Ervum are all smaller than those in the section Vicia,

b)   many species (ten) in the section Vicia have average arm ratios that are similar to those in the section Cracca (1.8 to 5.2) while the rest have larger average arm ratios. In general, specialisation appears to be accompanied by the appearance of chromosomes with more subterminal centromeres.

Twelve species in the section Vicia can be divided into three karyotype groups. Group I consists of *V. sativa*, *V. angustifolia*, *V. macrocarpa*, *V. cordata* and *V. pilosa*. Group II consists of *V. hybrida*, *V. lutea*, *V. pannonica* and *V. hyrcanica* while Group III consists of *V. grandiflora*, *V. incisaeformis* and *V. sepium*.

Fig. 14. A comparison of the karyotypes of *V. pilosa,*
V. angustifolia, V. sativa, V. macrocarpa and *V. cordata.*

ar = arm ratio of chromosome.

aar = average arm ratio of the chromosomes of the

complement.

The number written above a chromosome denotes its

total area.

Chromosomes that are 'shared' are shaded.

Postulated pericentric inversion is arrowed.

Fig. 14

V. pilosa

RDC$/$cell $= 18.88 \pm 0.85$

$2n = 14$ aar $= 7.5$

0.42	0.28	0.13	0.13	0.13	0.35	0.55
0.95	1.63	1.30	1.30	1.30	0.78	
0.10						

V. angustifolia

RDC$/$cell $= 22.97 \pm 1.45$

$2n = 12$ aar $= 14.5$

0.43					
0.55	0.14	0.14	0.14	0.14	0.03
2.13	1.72	1.72	1.72	1.51	1.11
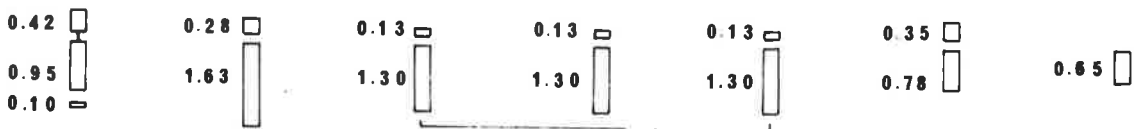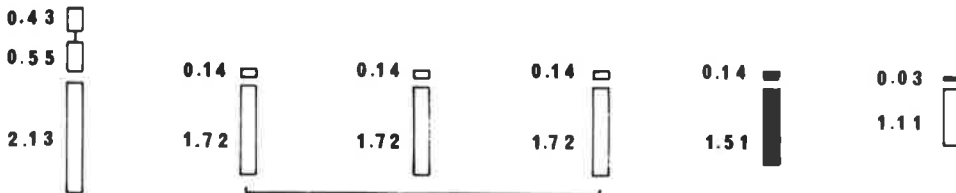
V. sativa

RDC$/$cell $= 19.80 \pm 0.98$

$2n = 12$ aar $= 10.6$

	1.67				
0.50	0.64	0.11	0.11	0.13	0.10
1.02	1.03	1.90	1.90	1.49	0.80
0.19					
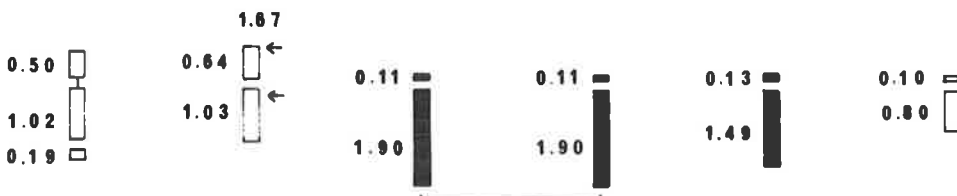
Fig. 14 contd.

V. macrocarpa

RDC/cell = 19.34 ± 0.52

2n = 12   aar = 7.3

0.17   0.18   0.39   0.39   0.18   0.18
0.81   1.33   1.65   1.65   1.33   1.33
0.08

V. cordata

RDC/cell = 17.22 ± 0.90

2n = 10   aar = 16.5

0.38   0.07 1.67   0.10   0.08   0.22
0.83   1.60   1.94   1.77   1.52
0.11
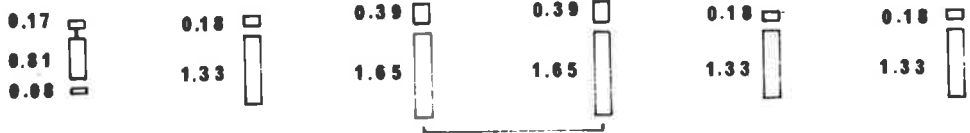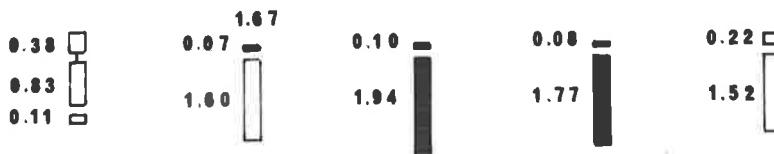
Group I.

V. sativa and its subspecies *V. pilosa, V. angustifolia,*
V. cordata and *V. macrocarpa.*

Taxonomically, *V. pilosa* (2n = 14), *V. angustifolia*
(2n = 12), *V. macrocarpa* (2n = 12) and *V. cordata* (2n = 10) are
regarded as subspecies of *V. sativa* (Hanelt and Mettin, 1966).
The diploid chromosome numbers 14, 12 and 10 are believed to form
a descending phylogenetic aneuploid series (Hanelt and Mettin,
1966). As shown on page 41, except for *V. angustifolia,*
all other subspecies have DNA contents per cell that are similar
to that found in *V. sativa.* This would indicate that the difference
in karyotype between this group of species (except *V. angustifolia*)
lie in structural changes. As shown in Fig. 14 this group of
species is karyologically very diverse; the chromosomes have
large variability in arm ratios.

Although taxonomically *V. pilosa* is regarded as the
most 'primitive' in this group of species (Hanelt and Mettin, 1966),
its chromosomes do not appear to bear much resemblance to those
found in the rest of the group. Except for chromosomes 1, 2 and 6,
all its chromosomes are smaller than those found in the other
species. This cannot be entirely accounted for by the fact that
it has an extra pair of chromosomes which could have been derived
from the breakage of a previously existing pair. It is obvious
from its karyotype that more complicated structural changes have

probably taken place.   Without more evidence the processes whereby structural changes could have taken place cannot be ascertained.

V. angustifolia has an exceptionally large satellite chromosome.   It is about twice the size of that found in *V. sativa*. Without further evidence, however, it is difficult to ascertain the origin of the additional amount of DNA.   One possible explanation is that it has been derived from the duplication of an existing chromosome followed by breakage and fusion involving two pairs of chromosomes.   This, however, is only speculative.

Except for *V. cordata* and *V. sativa* (which will be discussed in the next paragraph) the chromosomes in this group of species do not appear to bear much resemblance to each other.

The closest similarity in karyotype are observed between *V. sativa* and *V. cordata*.   Chromosomes 3 and 4 in *V. sativa* are 'shared'[8] with chromosomes 3 and 4 respectively in *V. cordata*.   Chromosome 2 in *V. sativa* is similar in total area but not in arm ratio to chromosome 2 in *V. cordata*.   It is probable that the difference between these two chromosomes lies in a pericentric inversion (as indicated, Fig. 14).   The long arm of chromosome 5 in *V. cordata* is also 'shared' with the long arm of chromosome 5 in *V. sativa*.

---

[8] shared - i.e. not significantly different at P = 0.05 (see pages 31 and 126.)

Fig. 15.   A comparison of the karyotypes of *V. pannonica*
and *V. hyrcanica*.

        aar =   average arm ratio of the chromosomes
of the complement.

Chromosomes that are 'shared' are shaded.

Postulated pericentric inversion in

chromosome 1 of *V. hyrcanica* is arrowed.

V. pannonica

RDC $/$ cell $= 50.87 \pm 0.97$

$2n = 12$ a ar $= 3.2$

1.44
1.10
1.39

1.0 1
0.49
2.60

1.16
4.42

0.79
3.43

0.84
3.05

0.78
2.95

V. hyrcanica

RDC $/$ cell $= 50.50 \pm 0.30$

$2n = 12$ a ar $= 3.8$

1.07 ←
0.55
←
2.42

1.24
4.26

0.85
3.45

0.74
3.07

0.74
3.07

0.74
3.07

It is clear from the examination of the karyotypes of this group of species that evolution of chromosomes in this group of species involved mainly structural changes such as pericentric inversions and translocations. The close similarity in morphology between these species is not paralleled by close similarity in their karyotypes.

Group II.

The four species in this group (*V. hybrida*, *V. pannonica*, *V. lutea* and *V. hyrcanica*) do not differ much in relative DNA content per cell and they 'share' a large number of chromosome arms and chromosomes between them (see pages 58-59). For clarity, *V. pannonica* is compared to each of the other three species rather than comparing each species with each other.

V. pannonica and *V. hyrcanica*.

Apart from the fact that these two species belong to the same section (Vicia), little else is known about the morphological degree of relateness between them. Karyologically, they are very similar. Chromosomes 3, 4, 5 and 6 in *V. pannonica* are 'shared' with chromosomes 2, 3, 4 and 5 respectively in *V. hyrcanica*. The satellite chromosome in *V. hyrcanica* is very similar in total area to the second satellite chromosome (chromosome 2) in *V. pannonica*. It is postulated that the difference between the two chromosomes lies in a pericentric

Fig. 16. A comparison of the karyotypes of *V. pannonica*, *V. hybrida* and *V. hajastana*.

  ar = arm ratio of chromosome.

  aar = average arm ratio of the chromosomes of the complement.

  The number written above a chromosome denotes its total area.

  Chromosomes that are 'shared' are shaded.

V. pannonica

RDC/cell = 50.87 ± 0.97

2n = 12   aar = 3.2

ar = 1.8

ar = 1.7

ar = 3.8

ar = 4.3

ar = 3.6

V. hybrida

RDC/cell = 51.10 ± 1.30

2n = 12   aar = 3.5

ar = 1.9

ar = 2.1

ar = 4.0

ar = 4.3
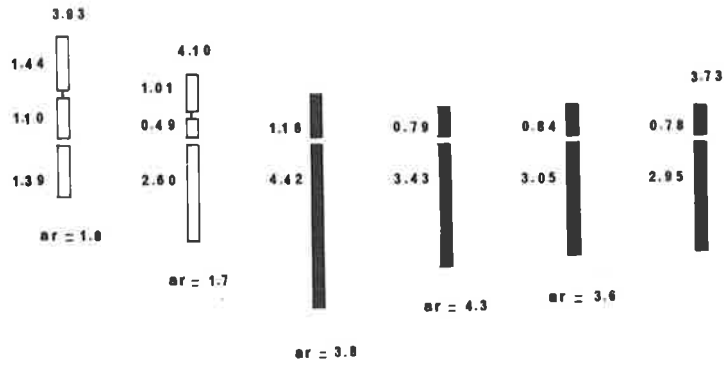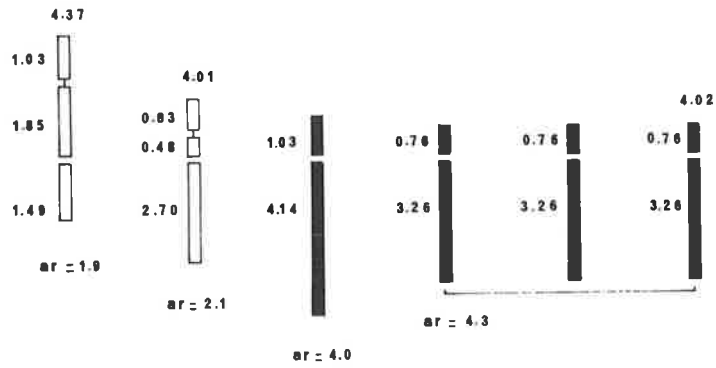
V. hajastana

RDC/cell = 56.22 ± 1.70

2n = 10   aar = 3.3

ar = 1.2

ar = 3.5

ar = 4.5

ar = 3.9

ar = 3.4
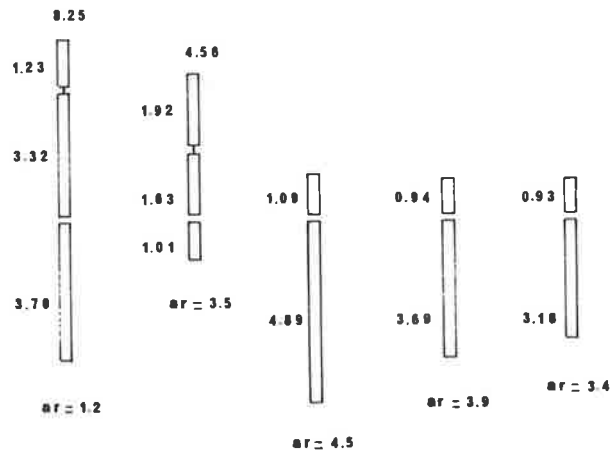
inversion in the satellite chromosome in *V. hyrcanica* (as
indicated, Fig. 15).

V. pannonica and *V. hybrida*.

V. pannonica (RDC/cell = 50.9 ± 1.0) and
V. hybrida (RDC/cell = 51.1 ± 1.3) are very closely related
taxonomically (Mettin and Hanelt, 1968).   Karyologically, however,
they are more similar than any other species pair in the genus
(Fig. 16).   Besides having similar DNA contents per cell,
chromosomes 3, 4, 5 and 6 in *V. pannonica* are 'shared' with
chromosomes 3, 4, 5 and 6 respectively in *V. hybrida*.   They also
'share' the proximal and distal arms of chromosomes 2 but the
satellite is larger in *V. pannonica*.   The main difference in
karyotype between the two species lies mainly in the first
satellite chromosome (chromosome 1).   Although they have similar
arm ratios, they have different total areas.   The changes that
have brought about this difference are unknown.

It appears that in this species pair, close similarity
in karyotype also indicates close taxonomic relationships.

V. pannonica and *V. lutea*.

V. pannonica (RDC/cell = 50.9 ± 1.0, 2n = 12) differs from
V. lutea (RDC/cell = 55.6 ± 1.3, 2n = 14) in

a)   having 4.8 units of DNA per cell less,

Fig. 17.   A comparison of the karyotypes of *V. pannonica* and *V. lutea*.

ar = average arm ratio of chromosome.

aar = average arm ratio of the chromosomes of the complement.

The number written above a chromosome denotes its total area.
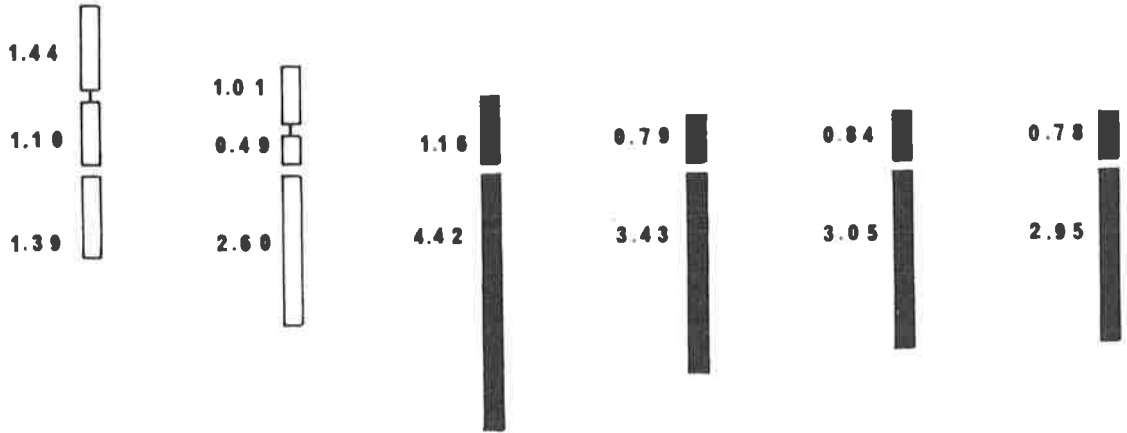
Chromosomes that are 'shared' are shaded.

Postulated pericentric inversion in chromosome 2 in *V. lutea* is arrowed.

V. pannonica

RDC / cell = 50.87 ± 0.97

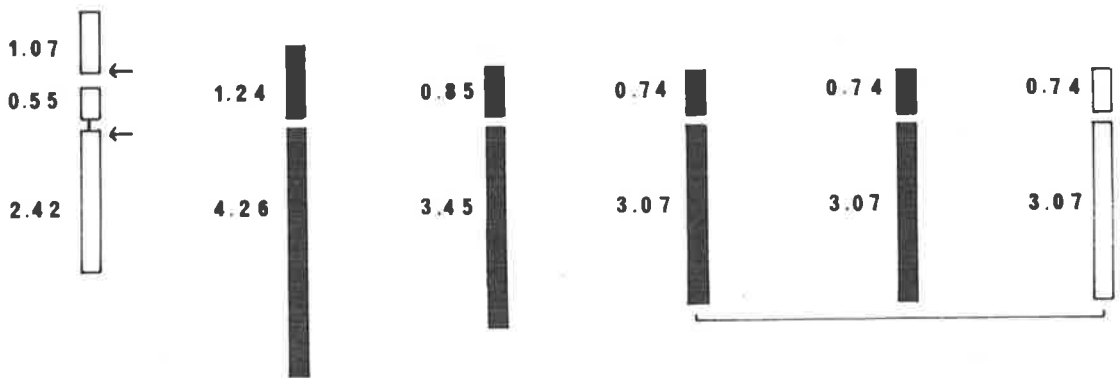2n = 12   aar = 3.2

3.93

1.44

1.10

1.39

4.10

1.01

0.49

2.60

5.57

1.16

4.42

4.22

0.79

3.43

0.84

3.05

0.78

2.95

ar = 1.8   ar = 1.7

ar = 4.3   ar = 3.6   ar = 3.8

ar = 3.8

V. lutea

RDC / cell = 55.64 ± 1.27

2n = 14   aar = 3.2

3.49

1.32

0.28

1.89

4.67

1.57

3.10

0.81

3.12

0.81

3.12

0.81

3.12

3.93

0.81

3.12

3.93

0.81

3.12

ar = 1.2   ar = 2.0   ar = 3.9
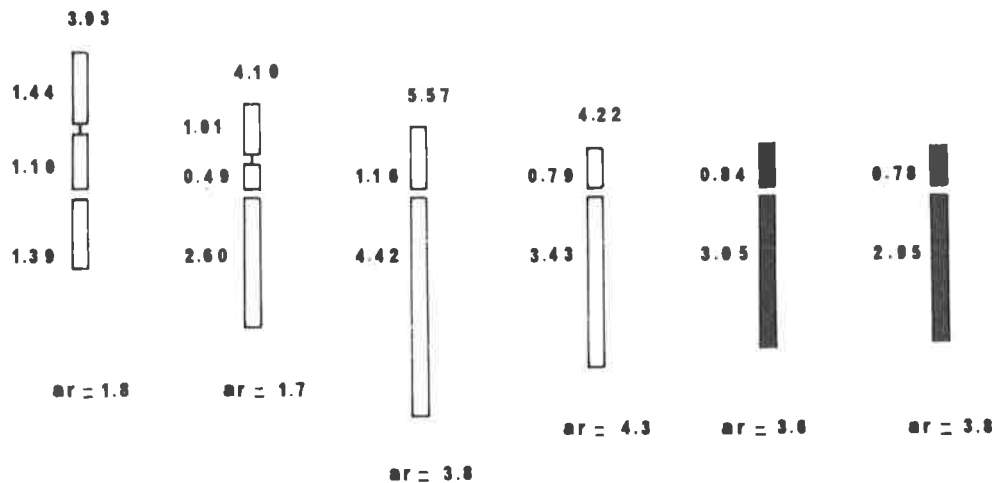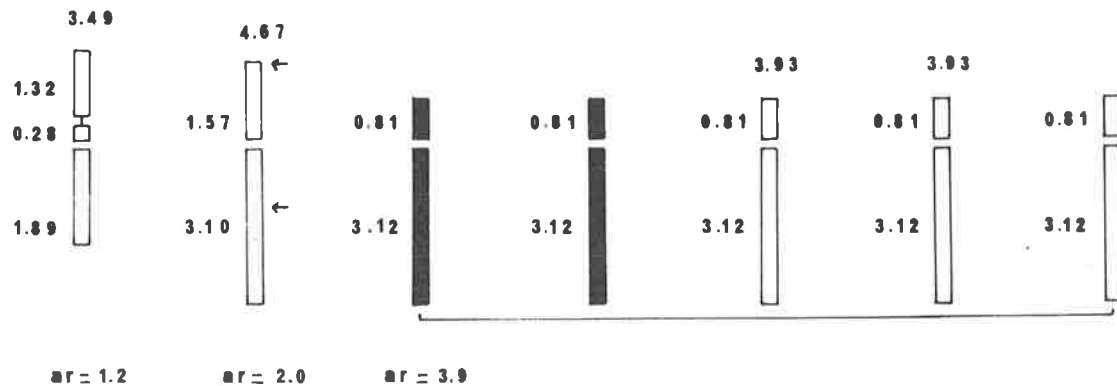
Fig. 18.   A comparison of the karyotypes of *V. pannonica*, *V. hyrcanica*, *V. hybrida* and *V. lutea*.

The chromosomes that are common to the four species are boxed.

ar = arm ratio of chromosome.

aar = average arm ratio of the chromosomes of the complement.

The number written above a chromosome denotes its total area.

Postulated pericentric inversions are arrowed.

Fig. 18

V. pannonica

RDC / cell : 50.87 ± 0.97

2n = 12   aar = 3.2

1.44
1.10
1.30

1.01
0.49
2.00

1.16
4.42

0.79
3.43

0.84
3.05

0.78
2.95

V. hyrcanica

RDC / cell : 50.50 ± 0.30

2n = 12   aar = 3.8

2.42
0.55
1.07

0.85
3.45

1.24
4.26

0.74
3.07

0.74
3.07

0.74
3.07
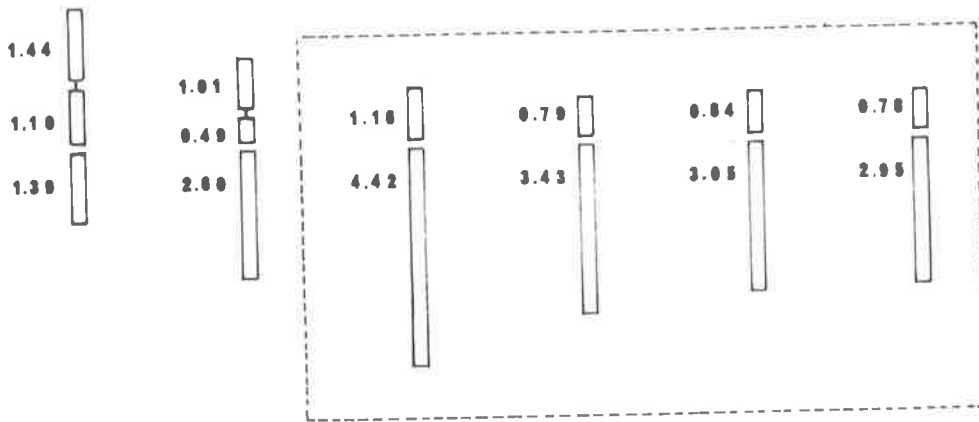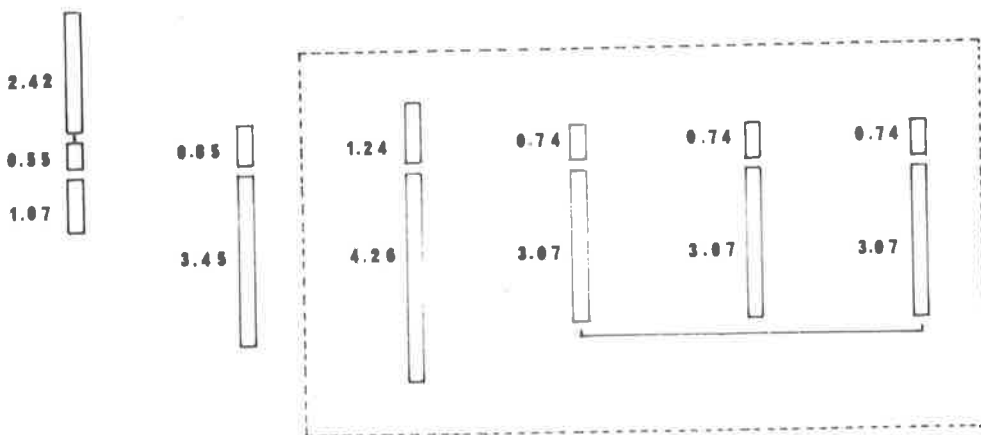
Fig. 18 contd.

V. hybrida

RDC/cell = 51.10 ± 1.30

2n = 12   aar = 3.5

1.03

1.85   0.83
        0.48

1.40   2.70   1.03   0.76   0.76   0.76

                4.14   3.26   3.26   3.26

V. lutea

RDC/cell = 55.64 ± 1.27

2n = 14   aar = 3.2

1.32
0.28   1.57   0.81   0.81   0.81   0.81   0.81
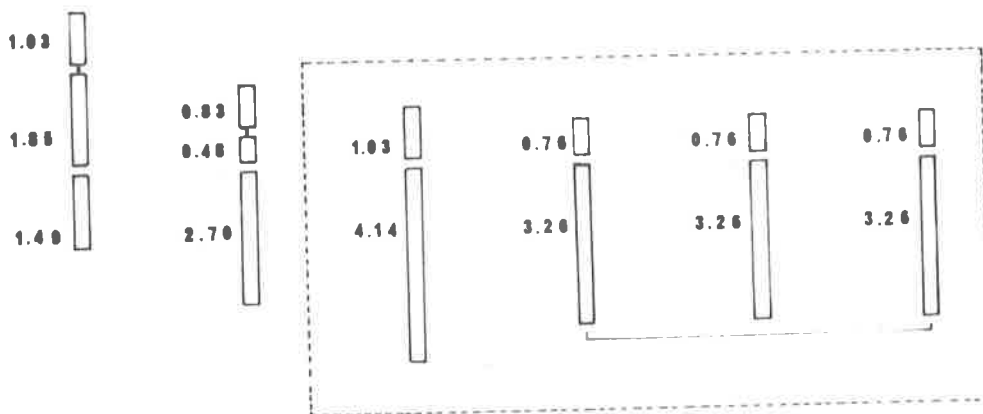
1.89   3.10   3.12   3.12   3.12   3.12   3.12
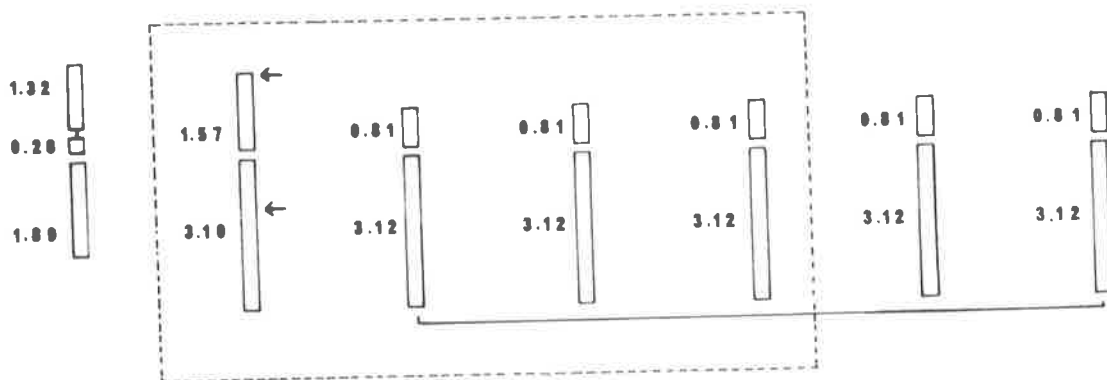
b)   having one chromosome pair less.   The additional

chromosome pair (chromosome 7) in *V. lutea* probably arose

from the duplication of an existing pair (chromosome 4, 5

or 6),

c)   having two satellite chromosomes which are of different

morphologies to the one in *V. lutea*.

Despite these differences, *V. pannonica* 'shares' two pairs of

chromosomes with *V. lutea* (Fig. 17).   Chromosome 1 in

V. pannonica is identical in total area with chromosome 6 in

V. lutea but it has a smaller arm ratio.   Chromosomes 2, 3 and 4

in *V. pannonica* have larger arm ratios and larger total areas than

chromosomes 1, 2 and 5 in *V. lutea*.   It is probable that

chromosome 3 in *V. pannonica* is equivalent to chromosome 2 in

V. lutea (see boxed chromosomes, Fig. 18) except that chromosome 2

in *V. lutea* has probably undergone a pericentric inversion (as

indicated in Fig. 17) accompanied by the loss of some chromosomal

DNA from the long arm since the long arm in *V. pannonica*

seems to be smaller.

Considering the karyotypes of the four species

(*V. pannonica*, *V. hybrida*, *V. hyrcanica* and *V. lutea*) as a whole,

it appears that four pairs of chromosomes are common among them

(boxed, Fig. 18).   Although some of them are significantly

different, they are, nevertheless, similar and they seem to have

undergone little change in spite of changes in the rest of

the karyotype.   Most of the differences in karyotype between

these species lie in structural differences rather than

differences in relative DNA content per cell.

V. pannonica, V. hybrida and *V. hajastana*.

Taxonomically, *V. hajastana* is very closely related to

V. hybrida and *V. pannonica* (Mettin and Hanelt, 1968).

V. hajastana (RDC/cell = 56.2 ± 7) has 5.4 units of DNA per cell

more than *V. pannonica* (RDC/cell = 50.9 ± 1.0) and also one

chromosome pair less.   Both species have satellite chromosomes

but the morphologies of these are different.   The first

satellite chromosome (chromosome 1) in *V. hajastana*,

besides being different in morphology, is also almost twice the

total area of chromosome 1 in *V. pannonica* (Fig. 16).   In view

of

a)   the close taxonomic relationship between *V. pannonica*,

V. hybrida and *V. hajastana*,

b)   the fact that *V. hajastana* has larger average DNA

content per chromosome than expected from its DNA content

per cell (see page 39),

it is postulated that chromosome 1 in *V. hajastana* is derived

from the fusion of chromosome 1 and 6 in *V. pannonica* or

Fig. 19.   A comparison of the karyotypes of *V. sepium* and *V. grandiflora*.

ar = arm ratio of chromosome.

aar = average arm ratio of the chromosomes of the complement.

The 'additional' DNA in *V. sepium* is shaded.

V. sepium

RDC / cell = 35.44 ± 0.65

2n = 14   aar = 3.6

0.35	0.56	0.66	0.66	0.39	0.39	0.72
0.38						
2.15	2.36	1.98	1.98	1.99	1.99	1.18

ar = 3.0   ar = 4.2   ar = 3.0   ar = 5.1   ar = 1.6
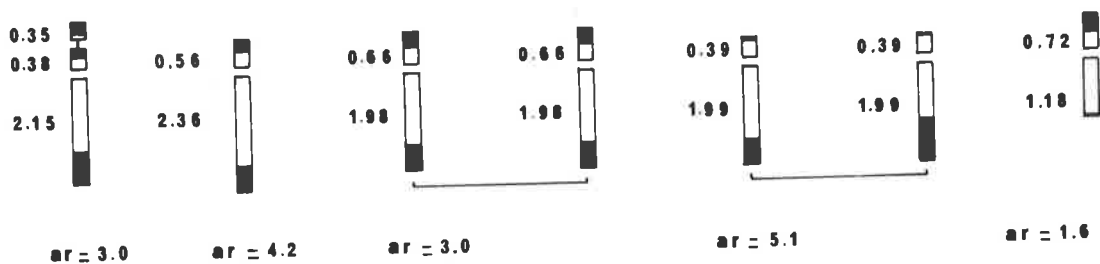
V. grandiflora

RDC / cell = 24.91 ± 0.81

2n = 14   aar = 4.4

0.11	0.30	0.31	0.31	0.31	0.37	0.37
0.25						
1.47	1.84	1.50	1.50	1.50	1.14	1.14

ar = 4.1   ar = 6.1   ar = 4.8   ar = 3.1
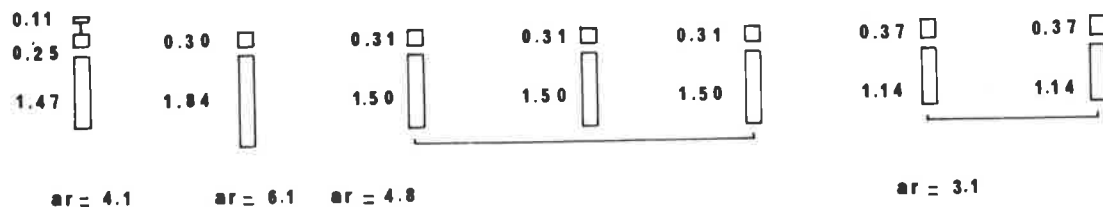
V. hybrida. Chromosome 2 in *V. hajastana*, besides having a different morphology is also larger in total area than chromosome 2 in *V. pannonica*. Chromosomes 3, 4 and 5 in *V. hajastana* (arm ratios = 4.5, 3.9 and 3.4, respectively) are similar in arm ratio to chromosomes 4, 3 and 5 (arm ratio = 4.3, 3.8 and 3.6, respectively) respectively, in *V. pannonica*. The similarities and differences found between *V. hajastana* and *V. pannonica* are similar to those found between *V. hajastana* and *V. hybrida*.

Group III.

V. sepium and *V. grandiflora*.

It was indicated (page 43) that the evolution (postulated by Hanelt and Mettin, 1970) from a perennial habit in *V. sepium* to an annual habit in *V. grandiflora* was accompanied by a loss of 10.5 units of DNA content per cell. Although the loss of DNA was derived from all the chromosomes of the genome of *V. sepium*, it was not contributed to evenly or proportionally by both the long and short arms[9] (Fig. 19): more asymmetrical chromosomes in *V. grandiflora* was the result. While there is no evidence that all these changes are causally related, the results are consistent

[9] The numbering of chromosomes is arbitrary but the decrease in DNA is still found to be derived unevenly and disproportionately from the chromosomes in *V. sepium* no matter how the chromosomes are arranged.

Fig. 20.   A comparison of the karyotypes of *V. sepium*
and *V. incisaeformis*.

ar =   arm ratio of chromosome.

aar =   average arm ratio of the chromosomes
of the complement.

Postulated interchange units are arrowed.

Chromosomes that are 'shared' are shaded.

V. sepium

RDC/cell = 35.44 ± 0.05

2n = 14   aar = 3.0

0.35		0.56		0.66		0.66		0.39		0.39		0.72
0.38													
2.15		2.36		1.98		1.98		1.99		1.99		1.18

ar = 3.0   ar = 4.2   ar = 3.0   ar = 5.1   ar = 1.6

V. incisaeformis

RDC/cell = 35.48 ± 0.29

2n = 14   aar = 4.0

reciprocal translocation

0.14		0.91 ←0.35		0.56		0.37 ←		0.37		0.37		0.78
0.34													
1.95		2.39		2.04		2.01		2.01		2.01		1.42 ← 0.2

ar = 4.1   ar = 2.6   ar = 3.1   ar = 5.4   ar = 1.6

reciprocal translocation
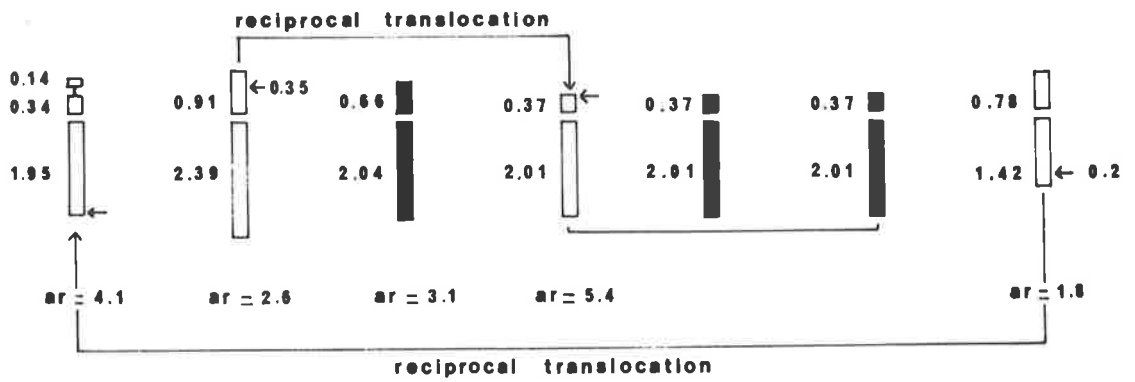
with the hypothesis that a shortening of the life cycle (from a
perennial habit to an annual habit) is accompanied by the loss of
DNA from all the chromosomes of the genome concerned.

V. sepium and *V. incisaeformis*.

Besides having similar DNA contents per cell,
V. sepium (RDC/cell = 35.4 ± 0.7) and *V. incisaeformis*
(RDC/cell = 35.5 ± 0.3) have similar karyotypes (Fig. 20).
Chromosomes 4, 5 and 6 in *V. sepium* are 'shared' with chromosomes
3, 5 and 6 respectively in *V. incisaeformis*.   The differences
in karyotype between the two species lie in

a)   the satellite in chromosome 1 in *V. incisaeformis*
which is smaller than the satellite in chromosome 1 in
V. sepium by 0.24 units of DNA,

b)   the short arm of chromosome 2 in *V. incisaeformis*
which is larger than the short arm of chromosome 2 in
V. sepium by 0.35 units of DNA and the short arm of
chromosome 4 in *V. incisaeformis* which is smaller than
the short arm of chromosome 3 in *V. sepium* by 0.29 units
of DNA.   The difference between these chromosomes could
be accounted for by a reciprocal translocation involving
the transfer of 0.35 units of DNA between the short arm of
chromosome 2 to the short arm of chromosome 4 in
V. incisaeformis,

c)   The long arm of chromosome 7 in *V. incisaeformis*

is larger than the long arm of chromosome 7 in *V. sepium*

by 0.24 units of DNA, while the distal arm in chromosome 1

in *V. incisaeformis* is smaller than the distal arm in

chromosome 1 in *V. sepium* by 0.20 units of DNA.

These differences would be minimised if a reciprocal translocation

is postulated to involve the transfer of 0.2 units of DNA to the

distal arm of chromosome 1 in *V. incisaeformis* (Fig. 20).

3.3.4   Section Faba.

Taxonomically, the section Faba is considered as an

'advanced' section of the genus *Vicia* (see page 35).

It is represented by only three species, *V. bithynica*,

V. narbonensis and *V. faba* (Ball, 1968).   *V. faba* and

V. narbonensis are placed in one karyotype group in view of their

close morphological relationship.   It is difficult to trace the

evolutionary trend in the karyotypes of the three species in this

section.   The main evolutionary trend appears to be an increase

in DNA content per cell and most of this is located in the long

arm of the chromosomes (see below).

V. narbonensis and *V. faba*.

Ball (1968) suggested that *V. faba* may have

developed under cultivation from *V. narbonensis* which it closely

resembles in morphological characteristics.   Chromosomally, however,

Fig. 21.   A comparison of the karyotypes of *V. narbonensis*
and *V. faba*.

aar =   average arm ratio of the chromosomes

of the complement.

V. narbonensis

RDC / cell = 54.50 ± 1.82

2n = 14   aar = 2.0

0.83
0.41
1.45
1.11
2.55
1.11
2.55
1.49
2.87
1.49
2.87
1.35
2.92
1.35
2.92

V. faba

RDC / cell = 100

2n = 12   aar = 13.0

2.43
4.53
8.60
0.57
7.60
0.41
6.38
0.41
6.38
0.41
6.38
0.37
5.55

Attempted crosses between twelve species of *Vicia*.

	V. angustifolia	V. atropurpurea	V. benghalensis	V. dasycarpa	V. faba	V. grandiflora	V. hybrida	V. lathyroides	V. lutea	V. melanops	V. narbonensis	V. pannonica	V. sativa
V. angustifolia					x						x		
V. atropurpurea			x	x							x		
V. benghalensis		x		x							x		
V. dasycarpa		x	x								x		
V. faba	x					x			x	x	x		
V. grandiflora					x				x		x		
V. hybrida										x			
V. lutea						x							
V. melanops						x	x					x	
V. narbonensis	x				x	x		x	x				x
V. pannonica													
V. sativa													

V. narbonensis (RDC/cell = 54.5 ± 1.8) has only about half as much DNA per cell as *V. faba* as well as having a very different karyotype (Fig. 21).

3.4   Interspecific crosses.

Staining with aceto-carmine indicated that the percentage viability of pollen grains of all the species used in crosses was 90 to 98%.   Intraspecific crosses showed that the method of pollination used was successful (out of 53 crosses carried out between *V. narbonensis* plants, 25 were successful).

Interspecific crosses between 12 species of *Vicia* were attempted (Table 10).   Pods with immature seeds were produced with some crosses.   The immature shrivelled seeds did not germinate so that no hybrids were obtained.   The formation of shrivelled seeds (from reciprocal crosses between *V. faba* and *V. angustifolia*; *V. faba* and *V. narbonensis*;   *V. narbonensis* and *V. angustifolia*; *V. narbonensis* and *V. faba*;   *V. narbonensis* and *V. grandiflora*; and *V. narbonensis* and *V. sativa*) suggested that cross-fertilisation had been effected and that the embryos aborted.   The failure to produce hybrids prevented examination of meiotic synapsis which would have allowed testing of many of the chromosomal relationships postulated from chromosomal morphology.

3.5   CHAPTER DISCUSSION.

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 species have not been
placed in a phylogenetic sequence within sections of the genus.
However, there is a larger range of DNA contents per cell in the
'more advanced' sections (Fig. 6).   Thus it can be said that
evolutionary increase in DNA content per cell has accompanied
morphological advancement of the species.   It should be noted,
however, that there is some evidence to indicate that the
evolution from a perennial habit to an annual habit in the section
Cracca is accompanied by a loss in DNA per cell.

From the study of the relationship between
morphological evolution, karyotype evolution and evolutionary
changes in DNA content per cell, it appears that there is some
correlation beween increasing asymmetry of chromosomes and
morphological advancement (Fig. 8).   The section Ervum which is
morphologically the 'most primitive' has a predominance of
symmetrical (average arm ratios = 1.9 to 3.8) chromosomes in the
karyotypes of its species.   The 'more advanced' sections Vicia and
Faba have the most asymmetrical chromosomes (average arm ratios =
2.0 to 16.5).   The section Cracca which is considered as the

connecting link between these two sections is intermediate in this respect (average arm ratio = 1.8 to 5.2). It should be noted, however, that some species in the section Vicia have average arm ratios that are similar to some of those in the section Cracca and to one in the section Ervum.

Although, in general, increase in DNA content per cell appears to accompany morphological advancement, increase in DNA content per cell does not necessarily accompany the evolution of the karyotype since although some of the more 'advanced' species have more asymmetrical chromosomes, they do not necessarily always have larger ones.

From the nine comparisons of the karyotypes of pairs of taxonomically very closely related species and groups of species, it appears that with two exceptions (viz. between *V. benghalensis* and *V. atropurpurea* and between *V. angustifolia* and *V. sativa*) the 'additional' DNA per cell is distributed to all the chromosomes of a genome. All species pairs and groups examined show, however, that the increase in DNA content per cell is not <u>evenly</u> distributed to all the chromosomes of a genome and is also not proportionally distributed to the chromosome arms. There is therefore a change in karyotype.

3.6   CONCLUSION.

Apart from some structural changes brought about by

a)   pericentric inversions and translocations which have

not been accompanied by significant changes in DNA content

per cell,

b)   gain of chromosomes which have been accompanied by a

corresponding increase in DNA content per cell (viz. *V. lutea*),

the major changes in karyotype in the genus *Vicia* have been brought

about by change in average DNA content per chromosome.   With the

failure of interspecific crosses, no evidence could be obtained to

determine if segmental duplications could account for some of the

increase in DNA per chromosome.   The evidence presented shows,

however, that either segmental duplications or local multiplicity

can account for all the increase in average DNA per chromosome in

the genus *Vicia*.   Evidence in favour of this is derived from

karyotype analyses between pairs of taxonomically very closely related

species or groups of species.   Without exception, it is found that

the increase in DNA content per cell is not <u>evenly</u> distributed

throughout all the chromosomes of a genome.   Neither is the

increase in DNA content per cell distributed proportionally to the

two arms of a chromosome.   This has resulted in changes in the

arm ratios of chromosomes and, hence, changes in karyotypes.   If

lateral multiplicity were the only explanation, the increase in DNA content per cell would be expected to be evenly distributed to all the chromosomes and chromosome arms. Nor do the distribution of average DNA per chromosome (Fig. 5) give much support to lateral multiplicity. A disjunct distribution forming a geometric series should be obtained if lateral multiplicity were involved and if it followed the same pattern as in insect development. Continuous distribution occur in the sections Ervum and Cracca. However, in the large section Vicia there appear to be three disjunct groups with means 4, 9.5 and 17.2; the three species in section Faba have values 4.9, 7.8 and 16.7. If it is assumed that these two series approximate to 1:2:4 ratios then these data would be consistent with change through lateral multiplicity. Supporting this is the evidence (page 40 and Table 7) that chromosomes constituting a genome are remarkably uniform in size.

An alternative explanation, however, is that the change is due to local multiplicity and/or segmental duplications. For this to be true, however, the number of sites of change involved would have to be large and scattered throughout all the chromosome arms. Additionally, in the sections Vicia and Faba, natural selection must have acted to produce adaptive peaks at or near multiples in a geometric series.

In brief, it seems certain that much variation in DNA content is the result of local multiplicity and/or segmental duplications but there is no evidence to distinguish between these. The possibility still remains, however, that the variation between groups in the sections Vicia and Faba may involve an additional mechanism like lateral multiplicity.

PART B.

CHAPTER 4.

INTRODUCTION.

In Chapter 1, it was suggested that most of the evolutionary increase in DNA content per cell lies in repetitious DNA. Repetitious DNA occurs in most organisms, from the simple (e.g. protozoans) to the complex (e.g. higher plants and animals) (see review by Britten and Kohne, 1969).

In his recent review, Walker (1969) divided the DNA in higher organisms into three broad fractions based on the rate of reassociation of denatured DNA

a) a fast fraction, i.e. a highly repetitious fraction which contains nucleotide sequences which are repeated from approximately 10^5 to 5×10^6 times per genome and may comprise up to 15% of the genome. It may not be present in the DNA of all species. Sometimes it can be isolated as a satellite band on caesium chloride gradients, e.g. mouse satellite DNA (Flamm, McCallum and Walker, 1967) (hence, it has been named satellite DNA),

b) an intermediate fraction, i.e. a repetitious fraction which contains nucleotide sequences which are repeated from approximately 10^2 to 10^5 times per genome and may represent

10 to 70% of the genome.   According to Britten and

Kohne, (1968) it is usually organised into families of

repeated nucleotide sequences[10],

c)   a slow fraction, i.e. a fraction which is repeated

once or a few times per genome.   This represents the rest

of the genome.

In recent years, the unique properties of satellite

DNA have excited a great deal of interest.   Satellite DNA has

been reported in some rodents (Waring and Britten, 1966;

Walker, 1968), cattle (Cheng and Sueoka, 1963; Polli, Corneo,

Ginelli and Bianchi, 1965), guinea-pig (Kit, 1961), plants

(Matsuda and Siegel, 1967) and crabs (Sueoka, 1961; Sueoka and

Cheng, 1962; Smith, 1963, 1964; Skinner, 1967).   The amount

of satellite DNA is found to vary between species.   It varies

from 2 to 12% of the total DNA content per cell in different

species of rodents (Hennig and Walker, 1970) and from 10 to 30% of

the total DNA content per cell in different species of crabs

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[10]   a family of repeated nucleotide sequences is a set of
related nucleotide sequences which differ by relatively few
base changes and which will reassociate with one another
after denaturation (after Bolton *et al*., 1967).
Theoretically, i) base substitutions of up to 14% can take
place before DNA hybridisation methods can distinguish
between the DNAs from two species,   ii) no homology can
be shown between the DNAs of two species by DNA
hybridisation methods after 42% of the bases have changed
(Walker, 1969).

(Sueoka, 1961; Sueoka and Cheng, 1962; Smith, 1963). Most of the satellite DNA sequences investigated also show gross differences from each other in base composition (Walker, 1968; Skinner, 1967; Smith and Quayle, 1963; Sueoka and Cheng, 1962; Smith, 1964; Hennig and Walker, 1970). The satellite DNA in mouse contains 32% GC[11] (Waring and Britten, 1966) while that in guinea-pig contains 43% GC (Kit, 1961). The GC content varies from 3 to 5% between crab species (Smith, 1964; Skinner, 1967).

Of all the satellite DNAs known, mouse satellite DNA has been most extensively investigated (Walker, 1968; Maio and Schildkraut, 1969; Jones, 1970; Hennig and Walker, 1970). Its properties can briefly be summarised as follows:

a) It is located in the condensed chromatin of interphase nuclei and is close to the centromere of chromosomes in mitotic cells,

b) It does not appear to code for proteins or ribosomal RNA,

c) It consists of tandem repeats of identical sequences about 300 to 400 base pairs long,

d) It comprises 10% of the genome,

e) It is more intimately associated with chromosomal proteins than the rest of the DNA.

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[11] Guanine + Cystosine (molar percentage).

Although so much is known about the properties of satellite DNA its function, at present, is only speculative (see pages 79-80).

Rates of reassociation of denatured DNA measured by hydroxyapatite fractionation and/or measurement of optical hypochromicity as a function of time have shown that most of the repetitious DNA in higher organisms falls into the 'intermediate' fraction. Britten and Kohne (1968) reported that this fraction of DNA is usually organised into families of repeated nucleotide sequences. The proportion of the genome which is organised into families of repeated nucleotide sequences varies in different organisms. In the mouse, it is about 30% (including the satellite DNA) (Walker, 1968), in the calf, 40%, and in the salmon and onion, greater than 50% (Britten and Kohne, 1968). The evidence indicates, therefore, that there are considerable differences in the way in which total DNA can be apportioned between 'fast' nucleotide sequences, families of nucleotide sequences with intermediate degrees of repetition and nucleotide sequences which are present in a few or single copies.

Comparison of the nucleotide sequences of the total DNA complement between species by the method of DNA-DNA hybridisation has been used as a measure of the magnitude of relationship or divergence between organisms (McCarthy and Bolton, 1963; Hoyer, McCarthy and Bolton, 1963, 1964; Bendich and Bolton,

1967; Laird and McCarthy, 1968). This method has the advantage of overcoming the barrier of reproductive isolation inherent in the biology of different living forms. It has, therefore been applied to the comparison of nucleotide sequences of the DNA of very distantly related groups of higher organisms (Hoyer *et al*., 1964; Gibson, 1968; Bendich and Bolton, 1967) as well as to more closely related groups such as members of the same family (McLaren and Walker, 1965) genus or species (McLaren and Walker, 1968, 1970; Laird and McCarthy, 1968). McCarthy and Hoyer (1964) showed that nucleotide sequence homologies are detectable among mammals, birds and many fishes. One special feature of some of these results is that approximately one-quarter of the total DNA nucleotide sequences appear to be conserved in mice, cattle, guinea-pig and humans (Hoyer, *et al*., 1964). The results demonstrate the variability of the rate of fixation of nucleotide substitutions in various parts of the genome. A surprising feature that emerged from the comparison of the DNA of various organisms is the magnitude of the differences found between individual species within a genus. In the genera *Bacillus* (Dubnau, Smith, Morell and Marmur, 1965) and *Neisseria* (Kingsbury, 1967), for example, only 10–15% nucleotide sequence homologies may be measured. Similarly, among the fungi, the genus *Saccharomyces* contains species that show only about 10% nucleotide sequence homology (Bicknell, 1967). Among

the insects, in the genus *Drosophila*, large differences in nucleotide sequence homologies (up to 75%) exist between DNAs from different species (Laird and McCarthy, 1968). Although the data at present are limited, such diversity has not yet been shown to exist within a genus among higher plants (see Chapter 7). The next major taxon (family) in plants, however, does seem to encompass this diversity. Thus, the garden pea (*Pisum sativum*) has 48% nucleotide sequence homology in common with the hairy vetch (*Vicia villosa*) and 19% in common with beans (*Phaseolus vulgaris*), all of which are members of the Leguminosae family (Bendich and Bolton, 1967). Among mammals, however, this extent of diversity has been found within an order and a family. Guinea-pig and mouse in the order *Rodentia* show only about 30% nucleotide sequence homology (Hoyer *et al.*, 1964). *Apodemus sylvaticus* and *Rattus norvegicus* have 8% homology in common with *Mus musculus* although all three are members of the rodent family *Muridae*. In general, the extent of nucleotide sequence homology is not in good agreement with the accepted taxonomic phylogenetic relationships.

The above findings have raised the following important questions:

a) Why are there such large differences in DNA homology between closely related species?

b) To what extent are the repeated nucleotide sequences expressed?

c)   How do repeated nucleotide sequences arise?

d)   What roles do repeated nucleotide sequences play in
evolution?

As yet, none of these questions has been answered
completely.   There is, however, some evidence providing tentative
answers to the first three questions, and stimulating provocative
speculation about the fourth.

With regard to the first question, large differences in
nucleotide sequence homology between closely related species have been
attributed to the degeneracy in the code (Walker, 1968). Walker
argued that if the wobble hypothesis (Crick, 1966) is true, there may
be little or no selective advantage in using some of the alternative
third code letters in synonymous codons. The absence of selective
advantage for specific codons could result in two related organisms
having DNA without detectable homology. Homology will only be shown
if two related organisms use at least some of the alternative codons
in common. The hypothesis that the 'slow' fraction is that part of
the genome for which there has been no selective advantage in using
specific codons accounts rather well for

a)   how closely related organisms can have demonstrably
different DNAs (Walker, 1968; Robertson and Chipchase, 1968),

b)   for the difference in the proportion of the intermediate
fraction in various organisms (Britten and Kohne, 1967b), and

c)   of how the intermediate fraction changes so rapidly in
evolution in related species.

Evidence regarding the extent to which repeated nucleotide sequences are expressed shows that members of families of repeated nucleotide sequences have genetic functions. McCarthy and Hoyer (1964) showed that hybridizable RNA of mouse differs in different tissues. Marked changes occur in the hybridisable RNA during liver regeneration as well as during mouse embryonic development (Church and McCarthy, 1967a and b). Changes in hybridisable RNA also occur during the development of *Xenopus laevis* (Denis, 1966). These results suggest that certain families of repeated nucleotide sequences, or members of these families, have to perform only a particular genetic function which is called into play at certain times in development or in certain tissues. The role of repeated nucleotide sequences in such a function could be regulatory or involve coded structural information. At this stage, it is not possible to say how many members of a family of repeated nucleotide sequences are expressed as RNA.

With regard to the third question (How do repeated nucleotide sequences arise?), there are difficulties in explaining the formation and the continued presence of repeated nucleotide sequences. Added to this difficulty are the properties of satellite DNA which are different from the familes of nucleotide sequences repeated 10^3 to 10^4 times (Walker, 1969; E. Southern, 1970).

Britten and Kohne (1968) postulated that families of repeated nucleotide sequences probably originated in rather sudden events of excessive replication of particular nucleotide sequences (saltation[12]), followed by the divergence of these nucleotide sequences with the consequent formation of the families of nucleotide sequences not so highly repeated. The mechanisms which initiate and halt these events are not known.

With regard to the fourth question (What roles do repeated nucleotide sequences play in evolution?), repetitious DNA must surely have a functional importance, especially when so many copies of nucleotide sequences have been maintained during evolution in competition with the tendency to accumulate random mutations. Furthermore, some of the members of families of repeated nucleotide sequences find expression as RNA. It is believed that the organisation of DNA into families of related nucleotide sequences will ultimately be found important to the phenotype (Britten and Kohne, 1968). Contrary to this, however, Walker (1969) postulated that repeated nucleotide sequences have a functional importance which has ensured their integrity and preserved their sequences. At present, the role of repeated nucleotide sequences is only speculative. Some of these speculations are:

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12   The hypothetical events by which families of hundreds of thousands of similar nucleotide sequences are produced in the DNA of an organism (after Bolton *et al*., 1967).

a) Multiple, nearly exact copies of a gene could provide higher rates of synthesis (Britten and Kohne, 1968). This might be true for structural proteins required in large amounts and is very likely true for ribosomal RNA. Multiple similar copies could provide a class of similar protein chains as appear to occur in antibody proteins (Gray, Dreyer and Hood, 1967; Cohen and Milstein, 1967; Edelman and Gally, 1967). However, their role could not be limited to the immunity system since they occur in large quantities in plants and other organisms in which antibodies have not been observed.

b) Repeated nucleotide sequences may be translocated to different parts of the chromosomal complement to form new combinations of large copies of repeated nucleotide sequences, and yielding a whole range of potential genetic activity. This could also arise by mutation of repeated nucleotide sequences. Potentialities important in the long term would have an opportunity to survive and reach fruitful expression. Britten and Kohne (1969) argued that the repeated nucleotide sequences in mouse originated as a saltatory step and that the repeated nucleotide sequences are in a process of divergence which may eventually result in many of the nucleotide sequences being able to code for new proteins.

c)   Britten and Davidson (1969) postulated a model of gene regulation.   They suggested that the evolutionary increase in DNA content per cell could be devoted to regulatory functions with most of it occurring as integrator and receptor genes.   New families of repeated nucleotide sequences formed by 'saltation' may be utilised to form integrator and receptor gene sets specifying novel batteries of producer genes with the production of new functions.

d)   Walker, Flamm and McLaren (1969) presented evidence indicating that mouse satellite DNA are probably concerned with some or all of the following:

   i)   chromosomal housekeeping, i.e. mediating the complex folding patterns and structural reorganisations which occur in chromosomes at various stages of the life cycle of an organism,

   ii)   initiation regions for replication or transcription of DNA,

   iii)   recombinator regions,

   iv)   pairing sites in meiosis,

   v)   centromere regions.

Compatible with the housekeeping hypothesis is the demonstration that mouse satellite DNA is

a)   located close to the centromere of chromosomes in

mitotic cells (Jones, 1970; Pardue and Gall, 1970),

b)   not restricted to chromosomes of a limited size or

type but is distributed in nearly the same proportion

throughout all the chromosomes (Maio and Schildkraut, 1969),

and

c)   appears to be more intimately associated with the

chromosomal protein than with the rest of the DNA (Maio

and Schildkraut, 1969).

Although other repeated nucleotide sequences (repeated

10^2 to 10^5 times) could have a role in coding protein (as postulated

above in a and b), it is unlikely that satellite DNA could have the

same function.   It has recently been shown that guinea-pig satellite

DNA contains repeating sequences of only six base pairs long and the

basic repeating sequence is C-C-C-T-A-A in one strand and G-G-G-A-T-T

in its complement.   If this is correct, it is inconceivable that

the cell should need a protein that is simply a series of repeating

dipeptides.   It is also interesting to note that of the six possible

reading frames of the repeating sequence in guinea-pig satellite

DNA, two give alternating nonsense or chain terminating codons.

In the following chapters (6 and 7) the DNAs of six

Vicia species are compared by the method of DNA-DNA hybridisation

(competition and reassociation rates).   It was hoped that a

consideration of

a)   the degrees of repetition of the nucleotide sequences within the DNAs of these six *Vicia* species and

b)   the degrees of DNA homology between these six *Vicia* species

would provide further information about the mechanism (viz. local or lateral multiplicity) of increase in DNA content per cell in the genus *Vicia*.

CHAPTER 5.

MATERIALS AND METHODS.

5.1.1 Radioactive materials.

^{32}P (as the orthophosphate in dilute HCl) was obtained from the Australian Atomic Energy Establishment, Lucas Heights, New South Wales, Australia. ^{3}H-thymidine was obtained from the Radiochemical Centre, Amersham, England.

5.1.2 Reagents.

All reagents used were of the highest possible grade (Analytical Reagents). Biochemicals and enzymes (pancreatic ribonuclease and egg white lysozyme) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

5.2 Bacterial culture.

E.coli K$_{12}$ (JC 2918) was grown at 37° with aeration in 1.5 litres of tris-glycerol media (Nomura, Matsubara, Okamoto and Fujimura, 1962) containing 0.2% glucose and threonine, leucine, histidine, arginine, proline, thiamine (all 20 µg/ml) together with Streptomycin (100 µg/ml).

For ^{3}H-thymidine labelled _E. coli_ DNA, ^{3}H-thymidine (0.013 µc/ml) was added to the culture when the cell density reached an optical density of 0.4 at 650 mµ. The cells were allowed to grow

for another hour by which time 70% of the ^{3}H-thymidine in solution had been incorporated into the cells. When the cell density reached an optical density of 0.8 at 650 mμ, the cells were harvested by centrifugation at 7,000 rpm for 20 min. The cells were washed in a solution of 0.15M sodium chloride and 0.1M EDTA[13] (saline EDTA) at pH 8 and resuspended in the same solution (50 ml).

5.3   <u>Isolation of bacterial DNA</u>.

The procedure of Marmur (1961) was employed in the isolation of the first crude extract of bacterial DNA. Further purification of this extract was carried out using the method described by Bendich and Bolton (1967).

The bacterial cells, suspended in saline EDTA, pH 8, were lysed by the addition of lysozyme (20 mg) and incubation for 45 min. in a water-bath maintained at 37°C with occasional shaking. Lysis of the culture resulted in an increase in viscosity which accompanied the release of the nucleic acid components. After the cells had been lysed, 25% sodium lauryl sulphate (4 ml) was added and the mixture transferred to a water-bath maintained at 60°C for 5 min. and then cooled. Sodium perchlorate (8M) was added to the lysed suspension to a final concentration of 1M.

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[13]   EDTA = ethylenediaminetetraacetic acid.

The rest of the procedure is as described for the isolation of plant DNA (see below). 1.0 to 1.5 mg of DNA was obtained from 1.0 g of wet packed cells. The concentration of DNA was determined by measurement of the optical density at 260 mμ. For this purpose, the molar extinction coefficient ε(P), of *E. coli* DNA was assumed to be 6740 (Mahler, Kline and Mehrotra, 1964). The specific activity of the ^{3}H-thymidine labelled *E. coli* DNA obtained was 250-260 cpm/μg.

5.4.1 <u>Isolation of plant DNA.</u>

The procedure of Bendich and Bolton (1967) was used for the extraction of plant DNA.

Roots or young seedlings (20 g) were cut into pieces (ca. 0.5 cm) and ground with sand for 2 min. in a solution (20 ml) containing 1% sodium dodecyl sulphate, 0.1M disodium EDTA and 3XSSC[14] at pH 7 and at 0°C. The resulting thick paste was transferred to a stoppered tube containing an equal volume of chloroform:n-octanol (90:1). The mixture was shaken with a Vortex junior mixer for 30 sec. and then centrifuged briefly to separate the phases. The upper aqueous layer which contained the DNA was poured into a preheated tube and incubated for 5 min. in a water-bath maintained at 72°C. The extract was then quickly cooled in an ice-bath and made 1M with respect to sodium perchlorate by the addition

[14] SSC = 0.15M sodium chloride, 0.015M sodium citrate.

Fig. 22. (Top). Typical spectrum of DNA isolated from *Vicia* seedlings. DNA isolated from *V. faba* seedlings as described in Materials and Methods was suspended in 0.01M tris-HCl 2XSSC and scanned from 320 to 220 mμ. Concentration of DNA in solution was calculated from the absorbance at 260 mμ as described in Materials and Methods.

Fig. 23. (Bottom). Sedimentation profile in a sucrose gradient of *V. faba* (o-o) and *V. sativa* (●-●) DNA fragments. The procedure used was as described in Materials and Methods. The acid-insoluble radioactivity (cpm) is plotted against the fraction number. The positions of the 23S, 16S and 4S peaks of *E. coli* RNA are shown.
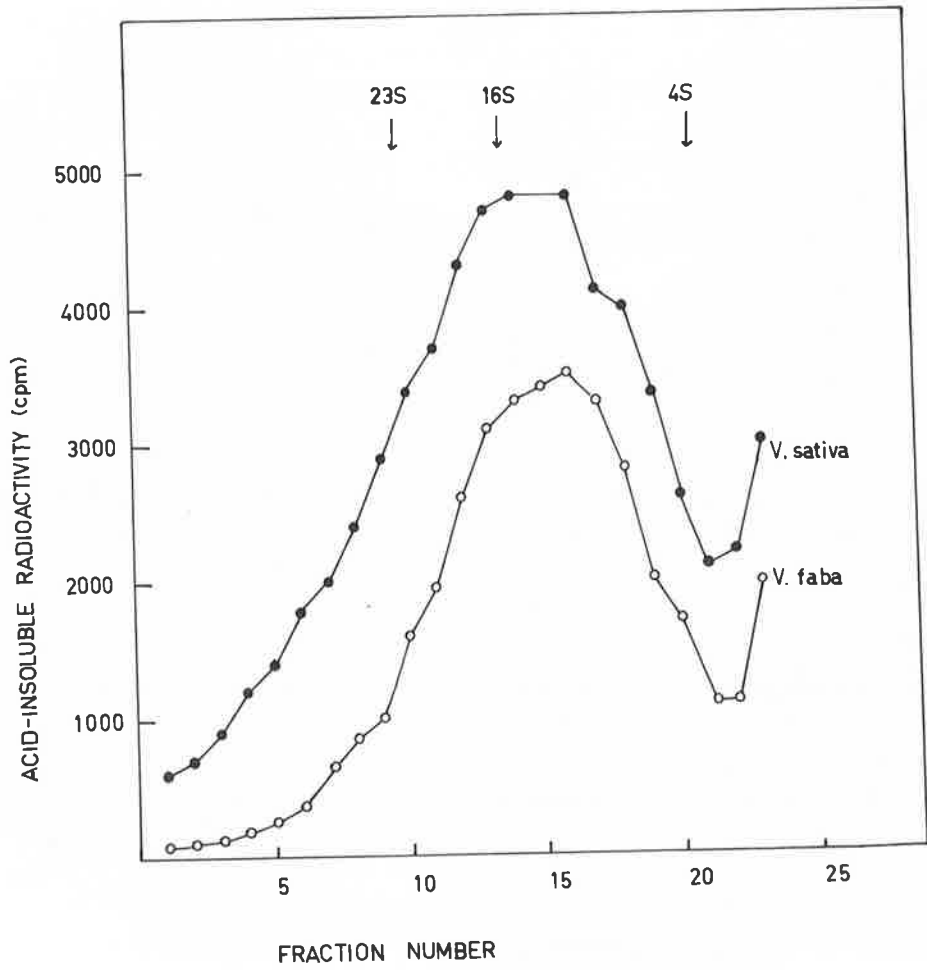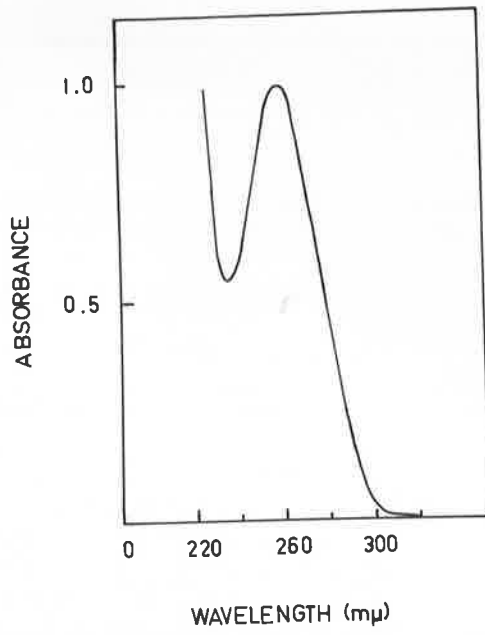
of 8M sodium perchlorate. It was then shaken with an equal volume

of chloroform:n-octanol and recentrifuged. The aqueous layer was

added dropwise to two volumes of 95% ethanol (redistilled,

benzene-free), the layers were slowly mixed, and then the DNA fibres

were removed with a glass rod. The fibrous DNA was transferred to

a vessel containing 0.1XSSC and the DNA was dissolved by gentle

shaking. The procedure to this point usually required about 0.5 hr.

DNA was further purified by treatment with pancreatic

ribonuclease (20-50 μg/ml for 0.5 hr. at 37°C) which had been

previously heated to 100°C for 5 min. to inactivate possible DNase[15]

contaminants, and by shaking with chloroform:n-octanol, and again

precipitating with 95% ethanol.

The yield, which varied considerably, depended on the

species and particularly upon the stage of development of the tissue.

Normally, 1-2 mg of DNA was extracted from 10 g of *Vicia* roots.

The concentration of DNA was determined by measuring the optical

density at 260 mμ(Fig. 22). The molar extinction coefficient

ϵ(P) of *Vicia* DNA was assumed to be 8000 (Markham, 1955).

5.4.2   Modification of the Bendich and Bolton (1967)

technique for extraction of plant DNA.

One of the difficulties of the method of Bendich and

Bolton (1967) of DNA isolation lay in the initial extraction where

the first aqueous layer of DNA had to be transferred to a preheated

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[15]DNase = deoxyribonuclease.

stoppered tube at 72°C within 4 min.   Rapid manual grinding of

Vicia roots to a smooth paste took at least 3 min.   Although the

time could be shortened to 30 sec. with a Servall Omni mixer, the

use of this instrument had the following disadvantage.   The DNA so

obtained had been sheared and could not satisfactorily be precipitated

in 95% ethanol at room temperature.   Sometimes it precipitated as

short fibres which could not be spooled but could be retrieved by

centrifugation for 5-10 min. at 5000 g.

The alternative method used in this work involved grinding

the roots with sand followed by extracting the DNA from them at 0°C.

The sand disrupted the cells without shearing the DNA, and DNase

activity was held at a minimum at 0°C.   The longer length of time

required for the extraction procedure did not result in excessive

loss of DNA.

According to the procedures of Marmur (1961) and Bendich

and Bolton (1967) deproteinisation by shaking with chloroform:n-octanol

was carried out manually.   This was found to be unsatisfactory since

much of the denatured protein remained attached to the DNA resulting

in a high loss of DNA, especially at the first deproteinisation.   In

the present work, the DNA was effectively separated into the aqueous

layer by shaking the extract for 30 sec. with a Vortex junior mixer.

The aqueous layer containing the DNA was added dropwise

to twice its volume of 95% ethanol (redistilled, benzene-free).   The

DNA precipitated as fibres which could be spooled and rapidly removed.

Bendich and Bolton (1967) precipitated their DNA by adding 95%
ethanol to the aqueous extract.   In the present work, this method
did not give much fibrous precipitate.

5.5   Preparation of ^{32}P-labelled DNA.

Seeds were surface sterilised with 1% sodium hypochlorite
for 20 min. and then their seed coats were removed.   They were then
germinated as described on page 28 .   Seedlings with lateral roots
were grown for 5 days in 500 ml distilled water containing ^{32}P-
orthophosphate (specific activity = 2.3 µc/ml) .   Sodium
bicarbonate was added to bring the pH of the solution to 7
(i.e. neutrality).   After the roots had been washed free of the
supporting medium, DNA was extracted from them using the method
described.

All the labelled plant DNA used was labelled with ^{32}P-
orthophosphate.   Only *E. coli* DNA was labelled with ^{3}H-thymidine.
The specific activity of the labelled plant DNA obtained was
10,000 to 60,000 cpm/µg.

5.6   Denaturation of DNA.

Native (double stranded)DNA preparations dissolved in
0.1XSSC were denatured (single stranded) by heating at 100°C for
5 min. followed by rapid cooling to 0°C.   A hyperchromicity of
29.5% indicated that the denaturation of DNA was complete (Denhardt,
1966).

DNA for use in the incubation mixture used for hybridisation was sonicated for 30 sec. at maximum output in an MSE 100 watt Ultrasonic Disintegrator (Denhardt, 1966). The probe was precooled to $0.5^{o}C$ and the vessel containing the DNA was placed in an ice-bath to minimise heating effects during disintegration. The average molecular weight of the sonicated DNA was determined by sucrose gradient analysis (see page 91).

5.7   Immobilisation of denatured DNA on nitrocellulose

           filters.

The procedure of Gillespie and Spiegelman (1965) was used for the immobilisation of denatured DNA on nitrocellulose filters but with one modification - that the filters were dried in a Petri dish at $80^{o}C$ at atmospheric pressure instead of under vacuum at $80^{o}C$.

The DNA preparation, dissolved in 0.1XSSC, was denatured and the concentration was adjusted to approximately 80 μg/ml in 6XSSC. It was then diluted 10 times with 6XSSC and passed through a filter (presoaked for 1 min. in 6XSSC and washed with 10 ml of the same solution), and then washed with 100 ml of 6XSSC. The filters were dried at room temperature for at least 4 hr. and at $80^{o}C$ at atmospheric pressure for an additional 2 hr. In all the experiments conducted, 20 μg of DNA was immobilised on each filter (Millipore 025 00, 25 mm, 0.45 μ).

5.8   Determination of the amount of denatured

           DNA on nitrocellulose filters.

A sample filter loaded with denatured DNA was immersed

in 5% perchloric acid (4 ml).   A blank was treated in the same

way.   Both filters were heated in a water-bath maintained at

90°C for 30 min. and then cooled to room temperature.   The amount

of denatured DNA from the sample filters was determined by the

difference in optical density between the sample from the filter

and the blank at 260 mμ.   The molar extinction coefficient, $\varepsilon(P)$,

of hydrolysed *Vicia* DNA was assumed to be 12,000 (Markham, 1955)

while that of denatured *E. coli* DNA was assumed to be 1136

(Mahler *et al.*, 1964).

5.9.1   DNA-DNA hybridisation - competition experiments.

Hybridisation of filter-bound DNA (20 μg) was carried

out in vials containing incubation mixture (1.0 ml) consisting of

sonicated denatured DNA (4 μg) and varying amounts (10 to 220 μg)

of homologous[16] or heterologous[16] unlabelled, sonicated, denatured

DNA in 2XSSC buffered with 0.01M tris-HCl of pH 7.   After

incubation at 60°C for 24 hr., the filters were removed, rinsed

briefly in 0.003M tris-HCl buffer 9.4 and washed on both sides

under suction with 0.003M tris-HCl pH 9.4 (100 ml) (Warnaar and

Cohen, 1966).

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[16] Homologous is used to describe DNAs of the same species and
      heterologous, to describe DNAs of a different species, when
      they are added to a system containing immobilised DNAs (after
      Bolton *et al.*, 1967).

The filters were dried at room temperature and the amount of radioactivity bound to the filters was counted in a Packard Tri-Carb liquid scintillation spectrometer. The scintillant used for ^{32}P counting was a toluene based scintillant consisting of PPO[17] (4 g), POPOP[18] (0.5 g) per litre of toluene. For ^{3}H-thymidine counting the scintillant used consisted of napthalein (60 g), PPO (4 g), POPOP (0.2 g), methanol (100 ml), ethylene glycol (20 ml) and finally 1,4 dioxane was added to give a volume of 1 litre. Samples labelled with ^{3}H-thymidine were counted to at least 30,000 counts while samples labelled with ^{32}P were counted to at least 50,000 counts. Preliminary experiments showed that filters had a quenching effect. The counting for ^{3}H-thymidine was corrected by the method of channel ratios (Bruno and Christian, 1961).

5.9.2   DNA-DNA hybridisation – reassociation rate

            experiments.

The reaction of sonicated labelled DNA with homologous filter-bound DNA was determined as a function of time. Each reaction consisted of the interaction of sonicated, labelled, denatured DNA (4 µg) with denatured homologous filter-bound DNA (20 µg) in 0.01M tris-HCl, 2XSSC, pH 7 (1.0 ml). Incubations were carried out at 60°C. At specified times (from 10 min. to 24 hr.), filters were removed, washed (as in competition

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[17]   PPO = 2,5 diphenyloxazole.

[18]   POPOP = 1,4-Bis-(5-phenyloxazole-2yl)benzene.

experiments)and filter-bound DNA was determined by liquid scintillation counting (as in competition experiments).

Unless otherwise stated, all DNA-DNA hybridisation experiments were conducted in triplicate.

5.10   Determination of molecular weight of

sonicated DNA.

The ^{32}P-labelled *V. faba* and *V. sativa* DNA fragments (0.2 ml) were each layered on to a linear 5-20% sucrose gradient (4.4 ml). The RNA of *E. coli* (23S, 16S and 4S, prepared by the method of Bolton, 1966), which was used as the marker, was layered on to a separate 5-20% sucrose gradient (4.4 ml). The samples were centrifuged in a Beckman SW 39 rotor at 33,000 rpm for 5 hr. Fractions (0.2 ml, 4 drops) were collected from the bottom of each tube and used for the estimation of acid-insoluble radioactivity. The optical density at 260 mµ of the marker RNA was determined and the positions of the 23S, 16S and 4S peaks were noted. The method of Studier (1965) was used in the calculation of molecular weight.

CHAPTER 6.

THE RATES OF REASSOCIATION OF THE DNAS OF SIX

VICIA SPECIES.

6.1   INTRODUCTION

The method of DNA-DNA hybridisation is based on the fact that complementary single strands of polynucleotides can form duplex structures *in vitro* under appropriate conditions of incubation (Marmur and Doty, 1961).   The extent of duplex formation depends upon the degree of complementarity among the polynucleotides (Britten, 1963;   Hoyer *et al.*, 1964;   Bolton and McCarthy, 1962).   In this work, two types of DNA-DNA hybridisation were carried out between six species of *Vicia*.   They were competition experiments (described in Chapter 7) and reassociation rate experiments (described below).

Reassociation rate experiments.

The reassociation of DNA is controlled by the collision of complementary nucleotide sequences.   The rate of reassociation is, therefore, determined by the concentration of each of the different nucleotide sequences present.   Theoretically, it is possible to determine the number of different nucleotide sequences present in the genome of a particular species by comparing its reassociation rate with that of an appropriate standard DNA.   If the repetitious fractions of DNA are neglected, the reassociation

rate is inversely proportional to the complexity of the DNA, i.e., to the size of the genome (Britten and Kohne, 1967b; Wetmur and Davidson, 1967; Dove and Davidson, 1962; Marmur and Lane, 1960; Subirana and Doty, 1966; Thrower and Peacocke, 1966).

In this work, a comparison of the rates of reassociation of the DNAs of six *Vicia* species (*V. faba, V. melanops, V. narbonensis, V. benghalensis, V. atropurpurea* and *V. sativa*) was made. Cytological studies (Chapter 3) have shown that the DNA contents per cell of these species range from 18.2 to 100 arbitrary units, i.e.,they have up to a 5.5-fold variation in DNA content per cell. In addition, *V. benghalensis* is regarded as being taxonomically synonymous with *V. atropurpurea* (Mettin and Hanelt, 1968) but it was shown (page 42) that they have significantly different DNA contents per cell. *E. coli* DNA which does not include repetitious DNA (Britten and Kohne, 1968) was used as a standard. Since only 4 µg of labelled DNA fragments were used in the hybridisation of 20 µg of homologous filter-bound DNA for 24 hours in the determination of the reassociation rates, it was assumed that hybridisation took place mainly between highly repeated nucleotide sequences. It was hoped that the reassociation rates would provide an insight into the degree of repetition of some of the highly repeated nucleotide sequences in the DNAs of the six *Vicia* species.

It was not possible, however, to determine quantitatively the degree of repetition of these highly repeated nucleotide sequences mainly because the real relationship between the amount of repetitious DNA and the rate of the reassociation reaction was complicated by the fact that one of the interacting components was immobilised on a filter while the other was in solution.

The method of fractionation of DNA by hydroxyapatite (Bernardi, 1965; Miyazawa and Thomas, 1965) followed by reassociation of the resulting fractions (Britten and Kohne, 1968; Laird, McConaughy and McCarthy, 1969) would have provided additional information on the nature of repeated nucleotide sequences. The author, however, was unable to get this technique to work satisfactorily, probably due to the fact that hydroxyapatite of the correct quality could not be obtained.

6.2 RESULTS AND DISCUSSION.

6.2.1 Molecular weight of sonicated DNA.

The molecular weights of the DNA fragments of *V. faba* and *V. sativa* were found to lie mainly between 170,000 (8S) and 900,000 (20S) (see Fig. 23). The average molecular weights of the DNA fragments of these two species were therefore assumed to be about 450,000 (13S). Since the DNA fragments of *E. coli* and other *Vicia* species which were used in DNA-DNA hybridisation studies in this

Fig. 24. The reassociation of the DNAs of six *Vicia* species
and *E. coli*. Filters with denatured DNA (20 µg) were each
immersed in incubation mixture (1.0 ml) containing labelled
homologous DNA fragments (4 µg). Incubations were carried
out at $60^{\circ}C$ for specified lengths of time (from 10 min. to
24 hr.). The procedure used was as described in Materials
and Methods. The percentage of labelled DNA fragments
hybridised to filter-bound DNA is plotted against time.
The DNA content per cell is shown against the name of the
species.
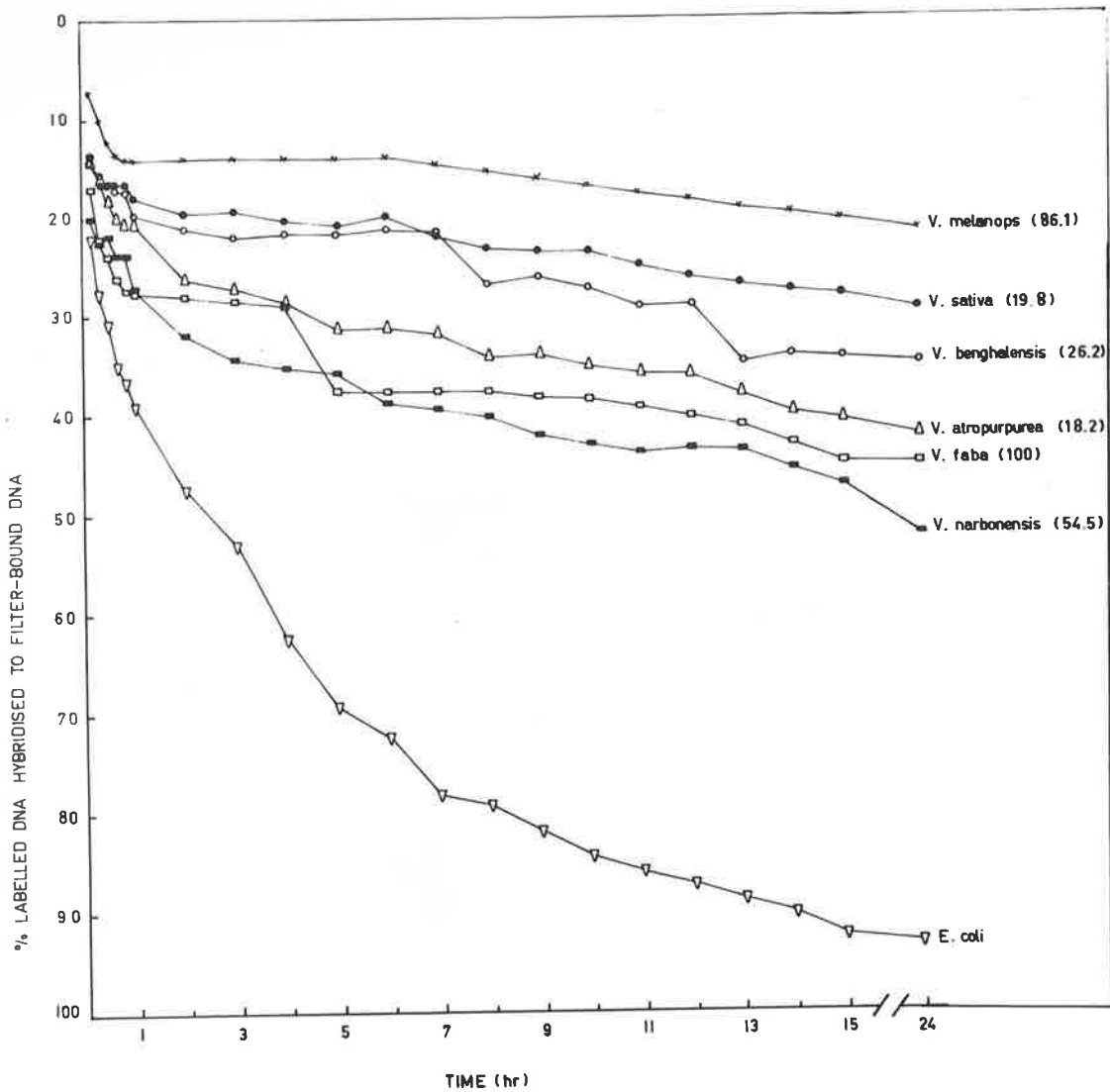
work were produced using the same method, their average molecular weights were also assumed to be approximately 450,000.

6.2.2   Reassociation rates of the DNAs of *E. coli*

and six *Vicia* species.

The percentages (the standard errors[19] of which have not been determined) of labelled DNA hybridised to homologous filter-bound DNA at different time intervals for six *Vicia* species and *E. coli* are shown in Fig. 24. For *E. coli*, the percentage of labelled DNA hybridised to filter-bound DNA increased rapidly at first and then, in comparison with DNA from *Vicia* species, continued to increase quite steadily until about 14 hours; between 14 and 24 hours the increase was only from 90 to 94%. For each of the six *Vicia* species

a)   the percentage of labelled DNA hybridised to filter-bound DNA increased rapidly with time until a more or less 'plateau level' was reached; this 'plateau level' was different for each species.

b)   after the 'plateau level' had been reached, the rates of reassociation were slower, but, as indicated by the fact that the graphs were approximately parallel, they were about the same for all six species.

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[19] Since the standard errors of the mean percentage homologies in the competition experiment between *V. faba* and *V. melanops* (see pages 105-106) range from 1.4% to 2.0%, there was no apparent reason to believe that the standard errors in reassociation rate experiments should be different.

In the interpretation of the reassociation rates shown in Fig. 24 it was assumed that the DNA that was hybridised before the 'plateau level' probably consisted mainly of DNA that was highly repetitious (fast[20]). The amount of DNA hybridised before the 'plateau level' gave, therefore, an approximation of the amount of fast DNA present in the genome. Between 8 and 24 hours, the rates of reassociation were similar for all six *Vicia* species and it is simplest to assume that, had the experiment been continued to completion, they would have remained similar. Britten and Kohne (1968) showed that in their experiments with higher organisms, non-repeated nucleotide sequences or nucleotide sequences repeated only a few times did not take a significant part in reassociation before 24 hours; it is probably this fraction that is involved at 'plateau level' in all six species.

6.2.3   Fast DNA.

The percentage of fast DNA in the genome of each of the six species of *Vicia* was calculated (see Table 11).

Fig. 24 shows that the DNA of *E. coli* had the fastest rate of reassociation (0.003 to 0.1 μg/min.) since it had no repetitious DNA (Britten and Kohne, 1968) and a very small genome

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[20] The relationship between the degree of repetition of nucleotide sequences and the reassociation rate is not known using the technique adopted here. The amount of 'fast' DNA is arbitrarily defined as the percentage of labelled DNA reassociated at 8 hours (see Fig. 24) multiplied by 94% (i.e. the percentage of labelled *E. coli* DNA hybridised in 24 hours). The reassociation rates of all *Vicia* DNAs appear to reach 'plateau levels' after 8 hours.

TABLE 11.

The proportions of 'fast' DNA in the genomes

of six *Vicia* species.

Species	Relative DNA content per cell ± standard error	'Fast' DNA		Remainder of DNA	
		% of genome	Amount*	% of genome	Amount*
V. atropurpurea	18.2 ± 0.9	32 (33.8 x 94)	5.8	68	12.4
V. sativa	19.8 ± 1.0	22 (23.3 x 94)	5.4	78	14.4
V. benghalensis	26.2 ± 0.2	25 (27.0 x 94)	6.6	75	19.6
V. narbonensis	54.5 ± 1.8	38 (40.3 x 94)	20.7	62	33.8
V. melanops'	86.1 ± 1.5	15 (15.5 x 94)	12.9	85	73.2
V. faba	100	35 (37.8 x 94)	35.0	65	65.0

* In arbitrary units; obtained by multiplying % of genome
by relative DNA content per cell.

($E.$ $coli$ consists of 0.004 x 10^{-9} mg of DNA per cell, Cairns,
1963). After 10 minutes, 22% of the labelled $E.$ $coli$ DNA was
hybridised to homologous filter-bound DNA. The amount hybridised
increased very rapidly and after 24 hours, 94% of the labelled
$E.$ $coli$ DNA was hybridised to homologous filter-bound DNA.

$V.$ $faba$ had approximately 150,000 times more DNA per cell
than $E.$ $coli$ ($V.$ $faba$ contains 6.02 x 10^{-9} mg of DNA per cell,
McLeish, 1963). The DNA of $V.$ $faba$ had a reassociation rate that
varied from 0.001 to 0.07 µg/min. While the amount of labelled
$V.$ $faba$ DNA hybridised between the first and fourth hour remained
approximately constant, a sharp increase of approximately 10%
between the fourth and the fifth hour was observed. This was a
consistent observation made on three occasions and is presumably
significant. The reason for this is uncertain. Table 11 shows
that $V.$ $faba$ had approximately 35% fast DNA in its genome.

Of all the DNAs of the six $Vicia$ species studied, that
of $V.$ $narbonensis$ had the fastest rate of reassociation (0.002 to
0.08 µg/min.). Although $V.$ $narbonensis$ (RDC/cell = 54.5 ± 1.8) had
only half as much DNA per cell as $V.$ $faba$ (RDC/cell = 100), its
DNA had reassociation rates that were faster at certain times and
similar at other times to those of $V.$ $faba$ DNA. This indicated
that some of its nucleotide sequences were multiplied to a
proportionally larger extent than those in $V.$ $faba$ DNA. Table 11
shows that $V.$ $narbonensis$ DNA consisted of 38% fast DNA while $V.$ $faba$
DNA consisted of 35% fast DNA.

V. atropurpurea DNA, had in general, a slower reassociation rate (0.001 to 0.06 µg/min.) than *V. faba* DNA. This suggested that some of the nucleotide sequences present in *V. atropurpurea* DNA (RDC/cell = 18.2 ± 0.9) were multiplied to a proportionally smaller extent than those in *V. faba*. Table 11 shows that *V. atropurpurea* DNA consisted of 32% fast DNA but *V. faba* consisted of 35% fast DNA.

In general, *V. benghalensis* DNA reassociated at a slower rate (0.001 to 0.06 µg/min.) than those of *V. faba* and *V. atropurpurea*. This suggested that some of the nucleotide sequences in *V. benghalensis* were multiplied to a proportionally smaller extent than those in *V. faba* and *V. atropurpurea*. Table 11 shows that *V. benghalensis* DNA consisted of 25% fast DNA, *V. atropurpurea* consisted of 32% fast DNA and *V. faba* consisted of 35% fast DNA.

During the hybridisation of labelled *V. benghalensis* DNA to filter-bound DNA, there were two 'sudden' increases of 5% in the amount of labelled DNA hybridised (between the seventh and the eighth and between the twelfth and thirteenth hours). It was uncertain if the two increases of 5% were significant.

Of the six *Vicia* species studied, the DNA of *V. sativa* had the second slowest rate of reassociation (0.001 to 0.05 µg/min.). Table 11 shows that *V. sativa* DNA consisted of 22% fast DNA.

Of all the DNAs of the six *Vicia* species studied, that of *V. melanops* (RDC/cell = 86.1 ± 1.5) had the slowest rate of

reassociation (0.001 to 0.03 µg/min.) despite the fact that its
DNA content per cell was almost as large as that of *V. faba*.
These results implied that unlike *V. faba*, the DNA of *V. melanops*
probably consisted of a larger number of nucleotide sequences
which were multiplied to a smaller extent.   Table 11 shows that
V. melanops DNA consisted of 15% fast DNA while *V. faba* consisted
of 35% fast DNA.

6.2.4   Remainder of the genome.

        Since the reassociation rates of the DNAs of the six
Vicia species were assumed to be approximately the same at the
'plateau levels', it was concluded that, other than the fast DNA,
the rest of the genome of each of the six *Vicia* species consisted of
nucleotide sequences that were multiplied to proportionally the same
degree.   Table 11 shows that approximately 62 to 85% of the genome
of the six species studied was either not multiplied or was evenly
multiplied.

6.3   CHAPTER DISCUSSION.

        As a result of a consideration of the reassociation rates
of the DNAs of the six species of *Vicia*, it is evident that increase
in DNA content per cell could result in an increase of either fast
DNA or the remainder of the genome or both.   The amount of DNA per
cell is no indication of the proportion of fast DNA in the genome.
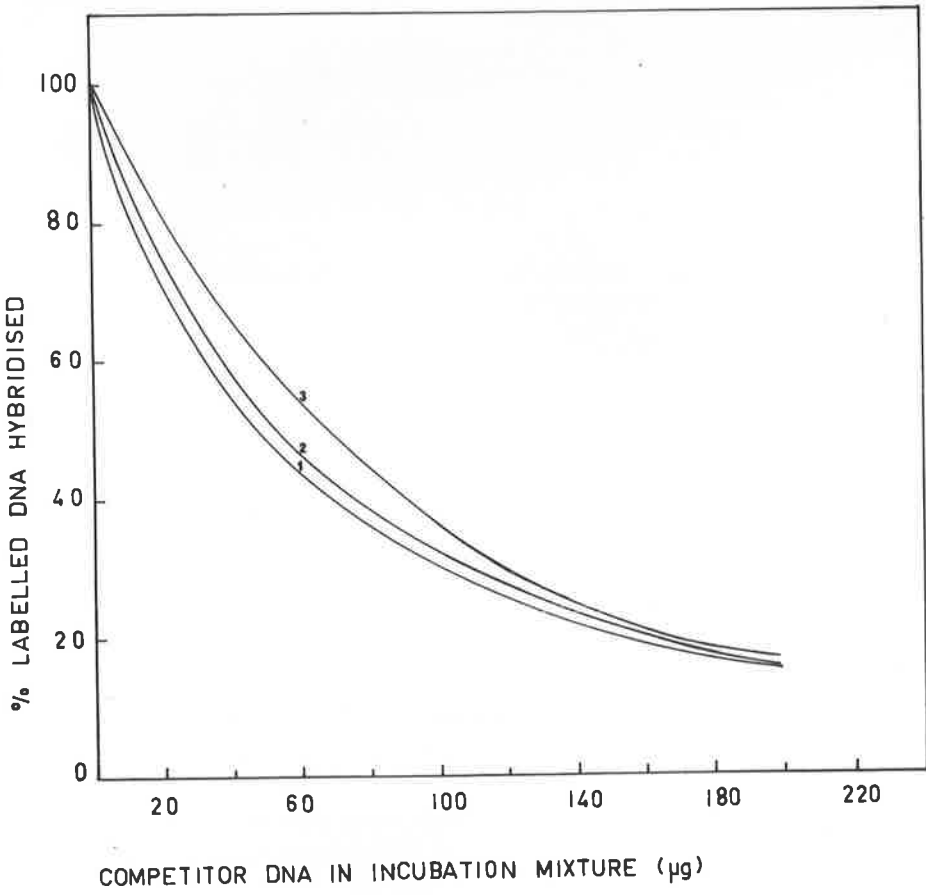In other words, species with small relative DNA contents per cell

(e.g. *V. atropurpurea*) could have relatively high percentages of fast DNA while species with relatively large DNA contents per cell (e.g. *V. melanops*) could have relatively low percentages of fast DNA. The explanation for this probably lies in the mechanism of increase in DNA per cell in *Vicia* species. The evidence presented above indicated that between 15 and 38% of the nucleotide sequences of the genomes involved uneven multiplication. The remaining 62 to 85% involved more or less even multiplication. The amounts of DNA (in arbitrary units) involved in even multiplication are shown in the last column of Table 11. Thus, in comparison with *V. atropurpurea*, *V. sativa* and *V. benghalensis* the degree of even multiplication is in the order of 2 to 3-fold for *V. narbonensis* and 4 to 5-fold for *V. melanops* and *V. faba*.

Fig. 25. (Top). Hybridisation of ^{32}P-labelled *V. faba* DNA fragments with filter-bound unlabelled *V. faba* DNA. Filters with unlabelled *V. faba* DNA (20 μg) were each incubated with varying amounts (10 to 220 μg) of ^{32}P-labelled *V. faba* DNA fragments. The amount of ^{32}P-labelled *V. faba* DNA hybridised to filter-bound DNA was determined as described in Materials and Methods. The amount of filter-bound *V. faba* DNA hybridised with the ^{32}P-labelled *V. faba* DNA fragments is plotted against the amount of ^{32}P-labelled *V. faba* DNA fragments in the incubation mixture.

Fig. 26. (Bottom). Hypothetical graphs showing

1) competition by unlabelled DNA fragments of species X in the reaction between labelled DNA fragments of species X and filter-bound DNA of species X (standard),

2) competition by unlabelled DNA fragments of species Y in the reaction between labelled DNA fragments of species X and filter-bound DNA of species X (difference in DNA content per cell between species X and Y reside in lateral multiplicity),

3) competition by unlabelled DNA fragments of species Z in the reaction between labelled DNA fragments of species X and filter-bound DNA of species X (difference in DNA content per cell between species X and Z reside in local multiplicity).

% FILTER-BOUND V. FABA DNA HYBRIDISED

^{32}P-LABELLED V. FABA DNA FRAGMENTS IN INCUBATION MIXTURE (μg)

% LABELLED DNA HYBRIDISED

COMPETITOR DNA IN INCUBATION MIXTURE (μg)
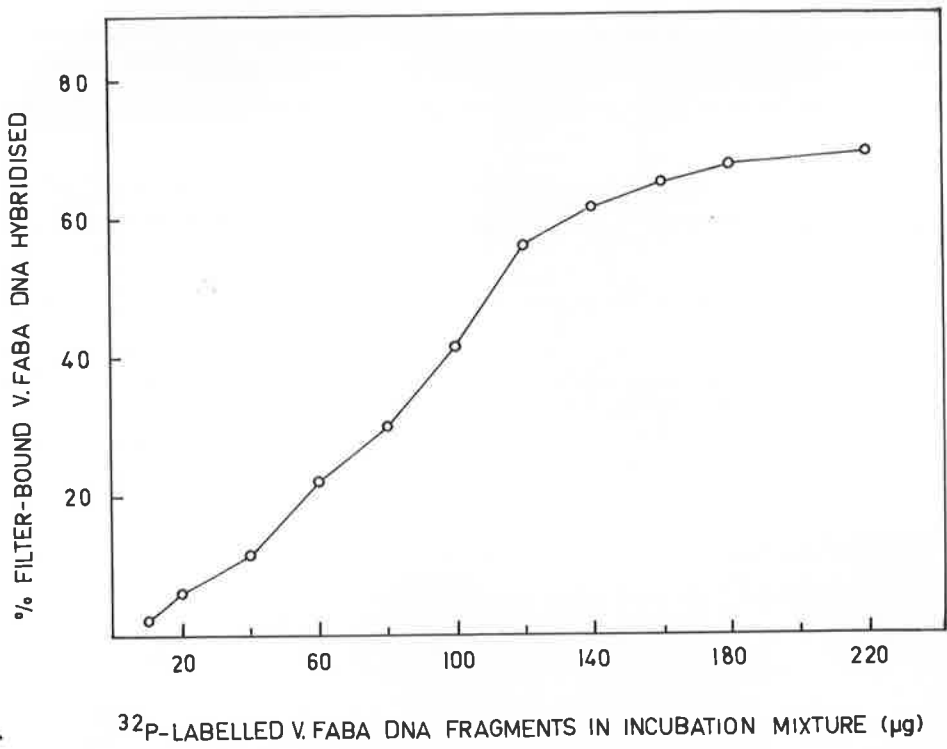
CHAPTER 7.

DNA-DNA HYBRIDISATION (COMPETITION) BETWEEN SIX

VICIA SPECIES.

7.1   INTRODUCTION

In the present work, the nucleotide sequences of *V. faba* DNA were used as the standard for the comparison of the nucleotide sequences of the DNAs of *V. melanops*, *V. narbonensis*, *V. benghalensis*, *V. atropurpurea* and *V. sativa* (see page 93).

In a typical DNA-DNA competition experiment conducted here, 20 μg of unlabelled *V. faba* DNA was immobilised on each filter. To each was added

a)   4 μg of denatured labelled *V. faba* DNA,

b)   *either* denatured unlabelled *V. faba* DNA (10 to 220 μg). The amount of labelled *V. faba* DNA hybridised at the various concentrations of competitor DNA served as a standard equivalent to 100% homology.   Fig. 25 shows that in the homologous *V. faba*-*V. faba* interaction, a maximum of 69% of filter-bound DNA could be hybridised in the time (24 hours) during which the experiment was performed.

or denatured unlabelled heterologous DNA (10 to 220 μg). The unlabelled heterologous DNA competed with the labelled *V. faba* DNA for 'sites' on the filter-bound DNA.   Hence, the amount of labelled *V. faba* DNA hybridised to filter-bound DNA was decreased.

The degree to which the hybridisation of labelled *V. faba* DNA
was decreased at the various concentrations of competitor DNA
gave a measure of homology.

Heterologous competition was monitored by the use of
Triticum vulgare and *E. coli* DNAs.   It was assumed that the DNA of
T. vulgare was not closely related to the DNAs of *Vicia* species
(Bendich and Bolton, 1967) and that the DNA of *E. coli* was not
related to that of plants.

Reciprocal experiments were also carried out to extend
the interpretation of nucleotide sequence homologies between the six
Vicia species.   When reciprocal experiments were done, the homologous
DNA-DNA interaction was also carried out simultaneously.   This was
necessary since variable results were obtained with different
preparations.

7.2 <u>Interpretation of results.</u>

The percentage homology[21] between different species
was calculated by the method of Bendich and Bolton (1967) where

percentage homology $= \dfrac{W - X}{W - Y} \times 100$

W - % hybridisation of ^{32}P-labelled DNA to filter-bound DNA.

X - % hybridisation of ^{32}P-labelled DNA to filter-bound DNA in the
    presence of n μg of heterologous unlabelled DNA.

Y - % hybridisation of ^{32}P-labelled DNA to filter-bound DNA in the
    presence of n μg of homologous unlabelled DNA.

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[21] Percentage homology is defined as the degree of similarity
    between the nucleotide sequences of the DNA of different
    species (after Bolton *et al.*, 1967).

Bendich and Bolton (1967) calculated the total percentage homology

shown by the DNAs of any two species at 'plateau level'. In the

results presented in this thesis, however, the percentage homology

shown by the DNAs of any two species was calculated at 20, 40, 80,

120 and 160 μg of competitor DNA in the incubation mixture. For

the sake of clarity in Figures 29, 32, 34, 37 and 40, the

percentage of homology was not indicated at all the concentrations

(10, 20, 30, 40, 50, 80, 120, 160 and 220 μg) of competitor DNA

in the incubation mixture. When the percentage homology shared

by the DNAs of the two species was calculated at the various

concentrations of competitor DNA, the differences in the degree of

multiplication of nucleotide sequences in the two DNAs compared

were more clearly defined.

In the interpretation of the results of competition

experiments, a high percentage homology obtained at low concentrations

(arbitrarily between approximately 1.0 and 40 μg) of competitor DNA

was assumed to be due mainly to hybridisation of the most highly

repeated DNA shared by both species. At high concentrations

of competitor DNA, both highly repeated and less highly repeated

DNA had more even chances of hybridising with filter-bound DNA.

The above two assumptions were based on the conclusions of Bolton

et al. (1967) that when the DNA of a higher organism reassociates,

most of the first part of the reaction is due to the collision of

nucleotide sequences that are present in perhaps a million (more or

less) similar copies. Non-repeated sequences collide with their

complements much less often and only very much later. It was
also assumed that nucleotide sequences that were not repeated
did not take part to a great extent in the hybridisation
experiments conducted in this work since the time of
hybridisation was limited to only 24 hours.

Theoretically, if the difference in DNA content per
cell between two species was due only to even multiplication of
DNA messages, the percentages of labelled DNA hybridised at the
various concentrations of heterologous competitor DNA should be
the same as those in the presence of the same concentrations of
homologous competitor DNA (Fig. 26). The same results should
be obtained in the reciprocal experiment.

If the difference in DNA content per cell between two
species was due to uneven multiplication of DNA messages, more
labelled DNA would be hybridised at lower concentrations of
heterologous competitor DNA if the heterologous DNA consisted of
lower multiplications of some nucleotide sequences (Fig. 26).
Less labelled DNA would, however, be hybridised at lower
concentrations of heterologous competitor DNA if the heterologous
competitor DNA consisted of higher multiplications of some
nucleotide sequences. In the case of difference due to local
multiplicity different results would be obtained in reciprocal
experiments.

7.3   <u>Determination of standard error.</u>

Preliminary experiments which were conducted to determine if there were variations in the amount of denatured DNA retained by filters and whether there were any inconsistencies in the amount of filter-bound DNA hybridised under similar conditions showed that:

a)   although the same DNA preparation was used, there were variations in the amount of DNA retained by a filter,

b)   the percentage retention of denatured DNA by filters varied between DNA preparations,

c)   there were small variations in the percentage of filter-bound DNA hybridised even though the same amount of filter-bound DNA was used in the hybridisation of the same concentration of DNA in the incubation mixture.

In view of these findings, it was, therefore necessary to determine the standard errors of the percentage homologies at various concentrations of competitor (homologous and heterologous) DNA added to the incubation mixture.   A heterologous DNA–DNA hybridisation experiment between *V. faba* and *V. melanops* DNAs and a control homologous DNA–DNA hybridisation experiment between the DNAs of *V. faba* were designed to determine the standard errors.   For these experiments, ten filters with the 'same' amount of denatured DNA were used in the hybridisation of each particular concentration

Fig. 27. (Top). Competition by unlabelled *V. melanops* DNA fragments in the reaction between ^{32}P-labelled *V. faba* DNA fragments and *V. faba* filter-bound DNA. Filters with *V. faba* DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous *V. melanops* DNA or homologous *V. faba* unlabelled DNA fragments. In this experiment, 10 filters containing the 'same' amount of DNA (20 μg) were used in the hybridisation at each concentration of competitor DNA. The rest of the procedure used was as described in Materials and Methods. The standard errors of the mean homologies at the various concentrations of competitor DNA were calculated. The percentage of ^{32}P-labelled *V. faba* DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    o-o   Competition of *V. melanops* DNA with *V. faba* DNA.

    ●-●   Competition of *V. faba* DNA with *V. faba* DNA.

Fig. 28. (Bottom). Competition by unlabelled DNA fragments in the reaction between ^{32}P-labelled *V. faba* (root) DNA fragments and *V. faba* filter-bound (root) DNA. Filters with *V. faba* (root) DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* (root) DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous or homologous unlabelled DNA fragments. The procedure used was as described in Material and Methods. The percentage of ^{32}P-labelled *V. faba* (root) DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    Δ-Δ   Competition of *V. faba* (root and shoot) DNA with *V. faba* (root) DNA.

    o-o   Competition of *E. coli* DNA with *V. faba* (root) DNA.

    ●-●   Competition of *V. faba* (root) DNA with *V. faba* (root) DNA.
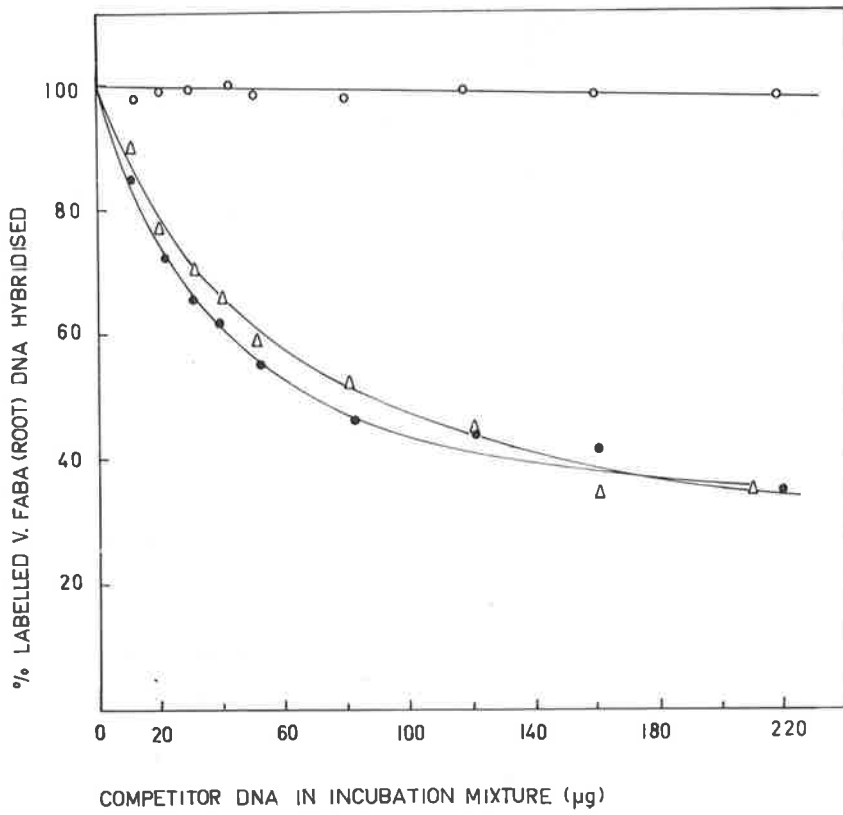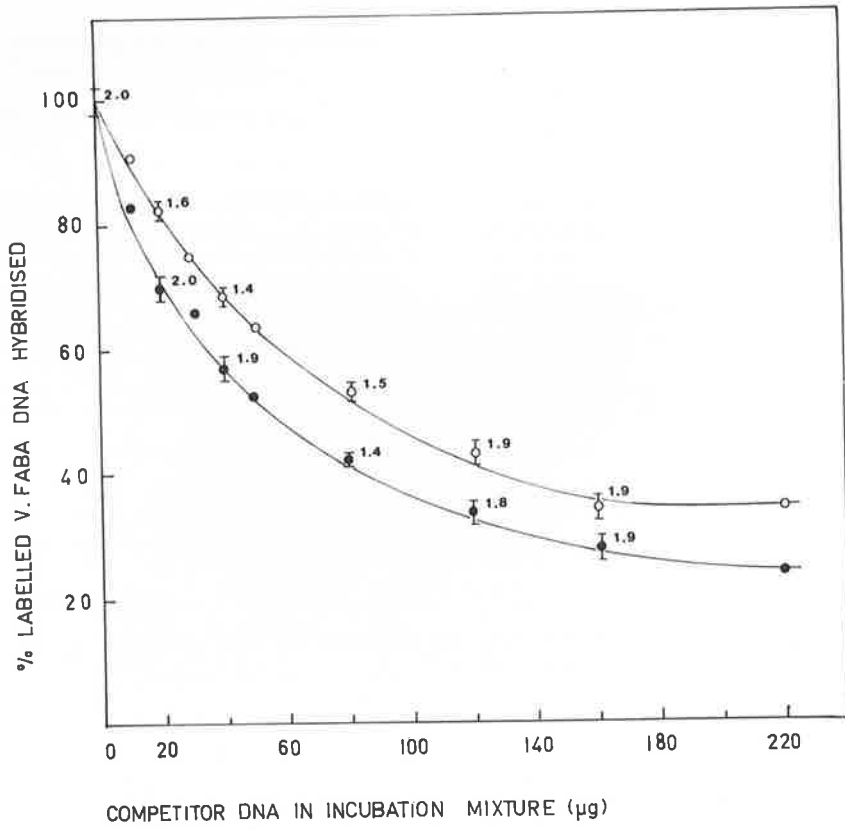
of competitor DNA.   The amount of labelled DNA hybridised by each

of the ten filters was calculated as in the other hybridisation

experiments and the standard error for these was calculated.   The

standard errors were determined at 0, 20, 40, 80, 120 and 160 µg of

competitor homologous and heterologous DNA.   These are shown in

Fig. 27.   It was assumed that errors in similar experiments were

comparable.

7.4   RESULTS AND DISCUSSION.

Interaction of *V. faba* (root and shoot) with

V. faba DNA (root).

Except for *V. faba*, all *Vicia* species have very small

amounts of root so that if only root tissue was used in DNA

extraction, a large number of plants would have to be used.   It

was decided to determine if there was any difference between the

competitive ability of root and shoot DNA as it is known that

plastids and mitochondria in shoot contain DNA (Shipp, Kieras and

Haselkorn, 1965;   Tewari, Vötsch, Mahler and Mackler, 1966).

In the experiment conducted, only root DNA was

immobilised on the filter.   Unlabelled root and unlabelled root

and shoot DNAs were used to compete with labelled root DNA.

The DNAs from root and shoot were indistinguishable

since unlabelled root and unlabelled root and shoot DNAs competed

equally well with the labelled DNA (Fig. 28).   Hence, root and

Fig. 29. (Top). The percentage homologies of the DNAs of five *Vicia* species, *T. vulgare* and *E. coli* with the DNA of *V. faba* are plotted against the amount of competitor DNA in the incubation mixture. The percentage homologies of the DNAs of the five *Vicia* species, *E. coli* and *T. vulgare* with the DNA of *V. faba* were calculated at various concentrations (20, 40, 80, 120 and 160 μg) of competitor DNA. The data for these calculations were obtained from Figs. 27, 28, 30, 35, 38 and 41.

Fig. 30. (Bottom). Competition by unlabelled DNA fragments in the reaction between ^{32}P-labelled *V. faba* DNA fragments and *V. faba* filter-bound DNA. Filters with *V. faba* DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous or homologous unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. faba* DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

   x-x Competition of *T. vulgare* DNA with *V. faba* DNA.

   o-o Competition of *V. sativa* DNA with *V. faba* DNA.

   ●-● Competition of *V. faba* DNA with *V. faba* DNA.
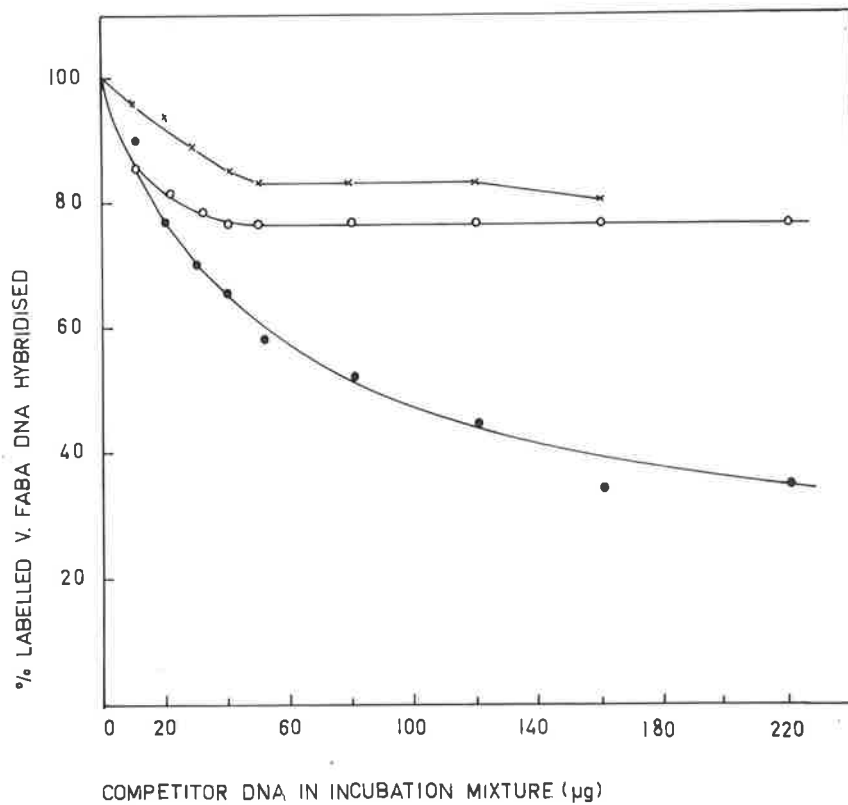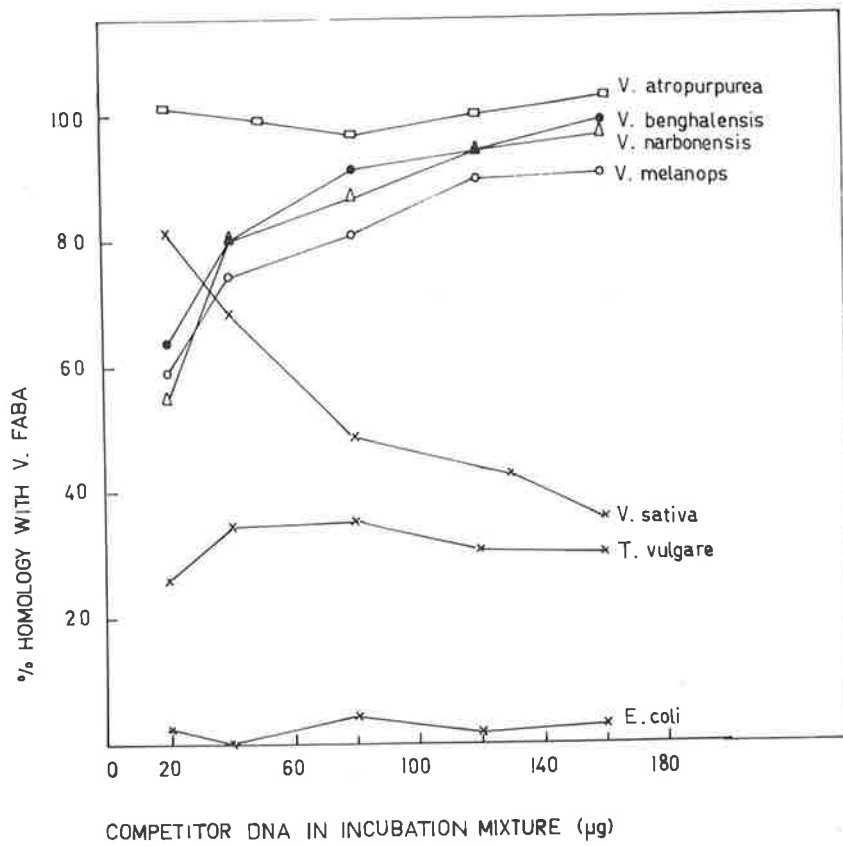
shoot DNA was used in all subsequent experiments.

Interaction of E. *coli* DNA and T. *vulgare* DNA with V. *faba* DNA.

As stated earlier, the homologous V. *faba*-V. *faba* interaction was used in all competition experiments as a standard for comparison.   The percentage of inhibition of the reaction of the ^{32}P-labelled V. *faba* DNA by homologous V. *faba* competitor DNA varied from 65% to 83% in different experiments.   The largest variation occurred at high (120 µg and above) concentrations of competitor DNA.   This was probably because at high concentrations of competitor DNA, some of the DNA fragments could hybridise partly with each other and partly with filter-bound DNA resulting in a considerable increase in the apparent length of the filter-bound DNA hybridised.

Fig. 28 shows that the presence of large quantities of competitor unlabelled E. *coli* DNA did not reduce the amount of ^{32}P-labelled V. *faba* DNA hybridised.   This indicated that E. *coli* DNA had no significant homology with V. *faba* (Fig. 29).

Some competition was, however, evident in the interaction of V. *faba* DNA with T. *vulgare* DNA (Fig. 30).   Although T. *vulgare* DNA showed 37% homology with V. *faba* DNA at 40 µg of heterologous competitor DNA, it did not increase further with increasing concentrations of competitor DNA (Fig. 29).   This suggested that the major portion of the DNA of T. *vulgare* was different from the DNA of V. *faba*.

The comparisons of the nucleotide sequences of the
DNAs of the six *Vicia* species will be discussed here in three
groups.   This grouping is based on the degree of homology shared
by the DNAs of the two species compared.

Group I.

In this group, the DNAs of the species compared
appeared to share only a small proportion of their basic
nucleotide sequences.   This group consists of the reciprocal
comparisons of the DNA of *V. sativa* with the DNA of *V. faba*.

Group II.

In this group, the nucleotide sequences of the DNAs
of the species compared appeared to be basically the same but
the relative degree of multiplication of some of the nucleotide
sequences was different.   This group consists of the reciprocal
comparisons of the DNA of *V. faba* with the DNAs of *V. melanops*,
V. narbonensis and *V. benghalensis*.
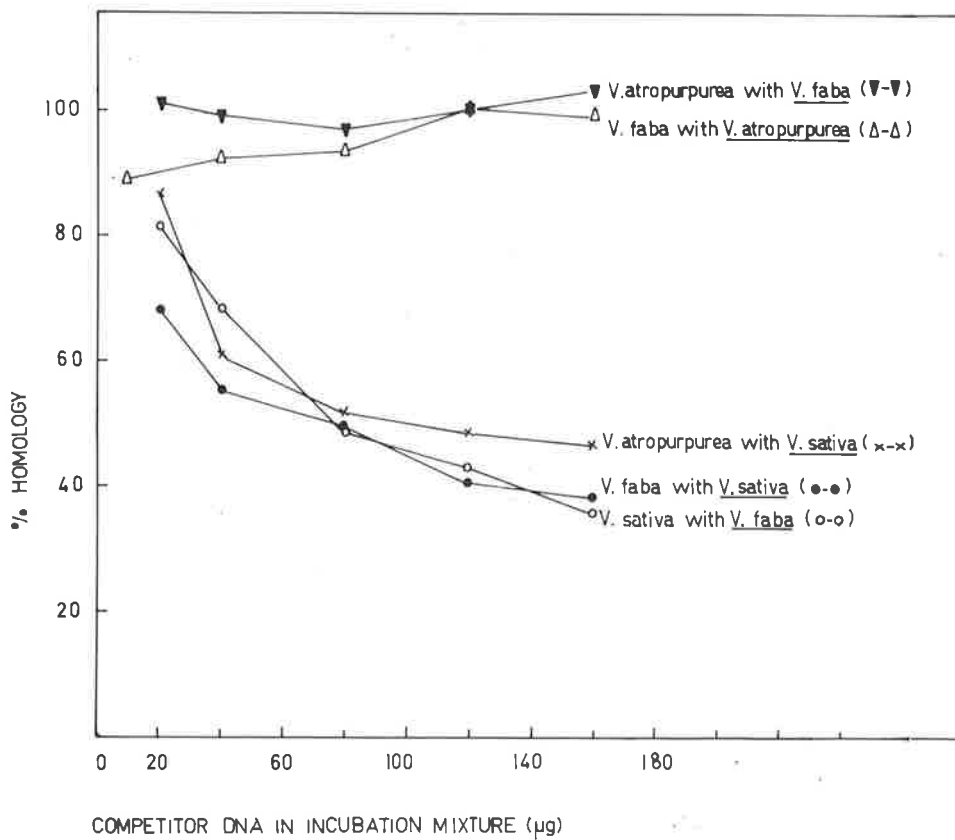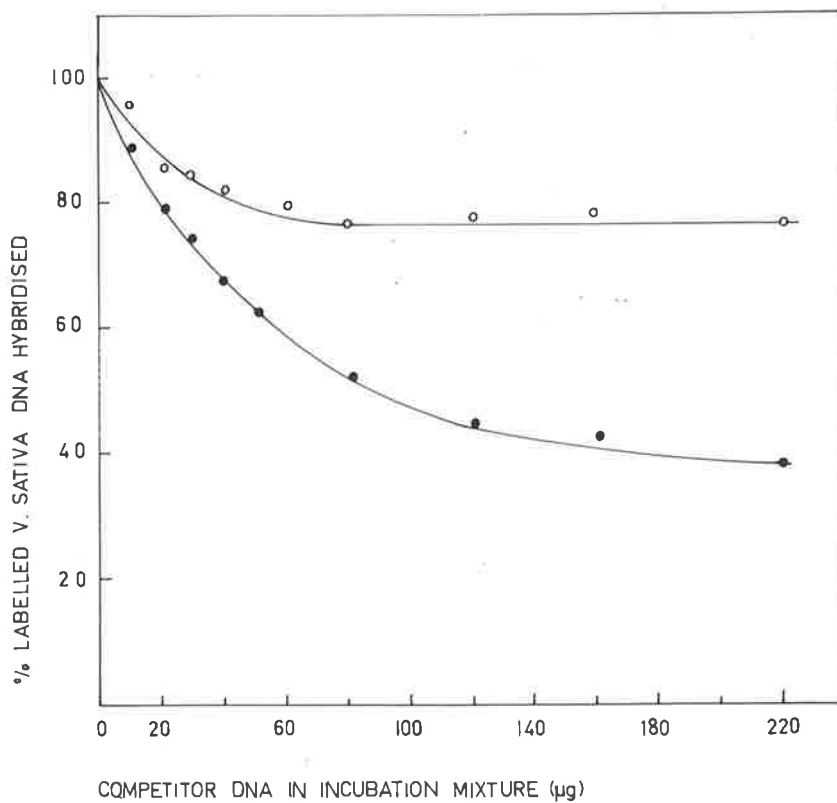
Group III.

In this group, the nucleotide sequences of the DNAs of
the species compared appeared to be basically the same and the
relative degree of multiplication of the majority of the
nucleotide sequences appeared to be similar.   This group consists
of the reciprocal comparisons of the DNA of *V. atropurpurea* with
the DNAs of *V. faba* and *V. benghalensis*.

Fig. 31., (Top). Competition by unlabelled *V. faba* DNA fragments in the reaction between ^{32}P-labelled *V. sativa* DNA fragments and *V. sativa* filter-bound DNA. Filters with *V. sativa* DNA (20 μg)` were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. sativa* DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous *V. faba* or homologous *V. sativa* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. sativa* DNA fragments hybridised to filter-bound *V. sativa* DNA is plotted against the amount of competitor DNA added to the incubation mixture.

    o-o   Competition of *V. faba* DNA with *V. sativa* DNA.

    ●-●   Competition of *V. sativa* DNA with *V. sativa* DNA.

Fig. 32. (Bottom). The percentage homologies of the DNA of *V. atropurpurea* with the DNA of *V. faba* (▼ - ▼),
V. faba with *V. atropurpurea* (△ - △),
V. atropurpurea with *V. sativa* (x-x),
V. faba with *V. sativa* (●-●) and
V. sativa with *V. faba* (o-o)
were calculated at various amounts (20, 40, 80, 120 and 160 μg) of competitor DNA added to the incubation mixture. These percentage homologies are plotted against the amount of competitor DNA in the incubation mixture. The data used for these calculations were obtained from Figs. 30, 31, 41, 42 and 43. The DNA that is filter-bound is underlined.

Group I.

Interaction of *V. sativa* DNA with *V. faba* DNA.

The interaction of *V. sativa* DNA with *V.faba* DNA
is shown in Fig. 30. The reciprocal experiment (unlabelled
V. faba DNA competing with labelled *V. sativa* DNA)is shown in
Fig. 31. Fig. 32 shows that at 20 µg of competitor *V. sativa*
DNA, *V. sativa* DNA showed 82% homology with the DNA of *V. faba*.
A maximum percentage homology of 68% was, however, obtained at
20 µg of heterologous *V. faba* competitor DNA. These results
suggested that, while a fraction of *V. sativa* DNA was similar to
that in *V. faba* DNA, a proportionally smaller fraction of *V. faba*
DNA was similar to that of *V. sativa* DNA. Increase in hybridisation
of competitor DNA with increasing concentrations of competitor DNA
took place, however, with larger concentrations of competitor DNA
when *V. faba* DNA was the competitor (up to 80 µg) (Fig. 31) than
when *V. sativa* DNA was the competitor (up to 40 µg) (Fig. 30).
This suggested that *V. sativa* DNA included higher multiplications
of fewer nucleotide sequences and that *V. faba* DNA included lower
multiplications of proportionally more nucleotide sequences. The
high percentage of nucleotide sequence homology shared at low
competitor DNA concentrations was probably due to the presence
of repeated nucleotide sequences. A large part of *V. sativa* DNA,
appeared, however, to have nucleotide sequences different from those
of *V. faba*.

Fig. 33. (Top). Competition by unlabelled *V. faba* DNA fragments in the reaction between ^{32}P-labelled *V. melanops* DNA fragments and *V. melanops* filter-bound DNA. Filters with *V. melanops* DNA (20 µg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. melanops* DNA fragments (4 µg) and varying amounts (10 to 220 µg) of heterologous *V. faba* or homologous *V. melanops* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. melanops* fragments hybridised to filter-bound *V. melanops* DNA is plotted against the amount of competitor DNA in the incubation mixture.

  o-o   Competition of *V. faba* DNA with *V. melanops* DNA

  •-•   Competition of *V. melanops* DNA with *V. melanops* DNA.

Fig. 34. (Bottom). The percentage homologies of *V. faba* DNA with *V. melanops* DNA (•-•) and those of *V. melanops* DNA with *V. faba* DNA (o-o) are plotted against the amount of competitor DNA in the incubation mixture. The percentage homologies at the various concentrations of competitor DNA were calculated from data in Figs. 27 and 33. The DNA that is filter-bound is underlined.
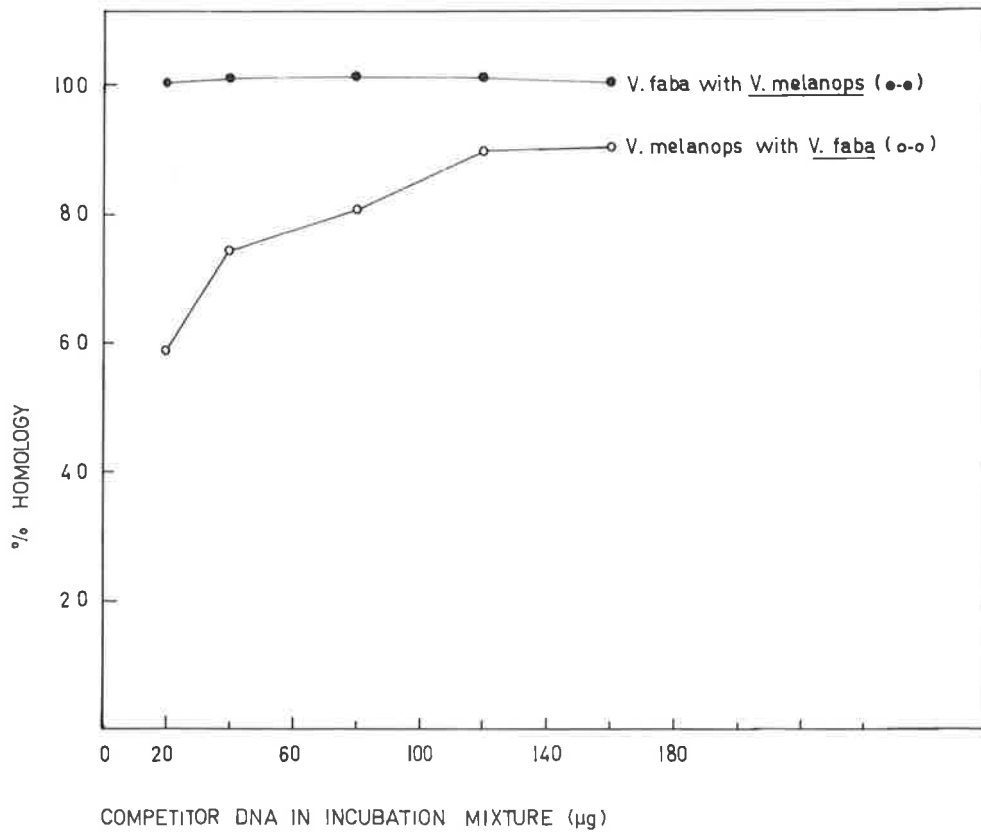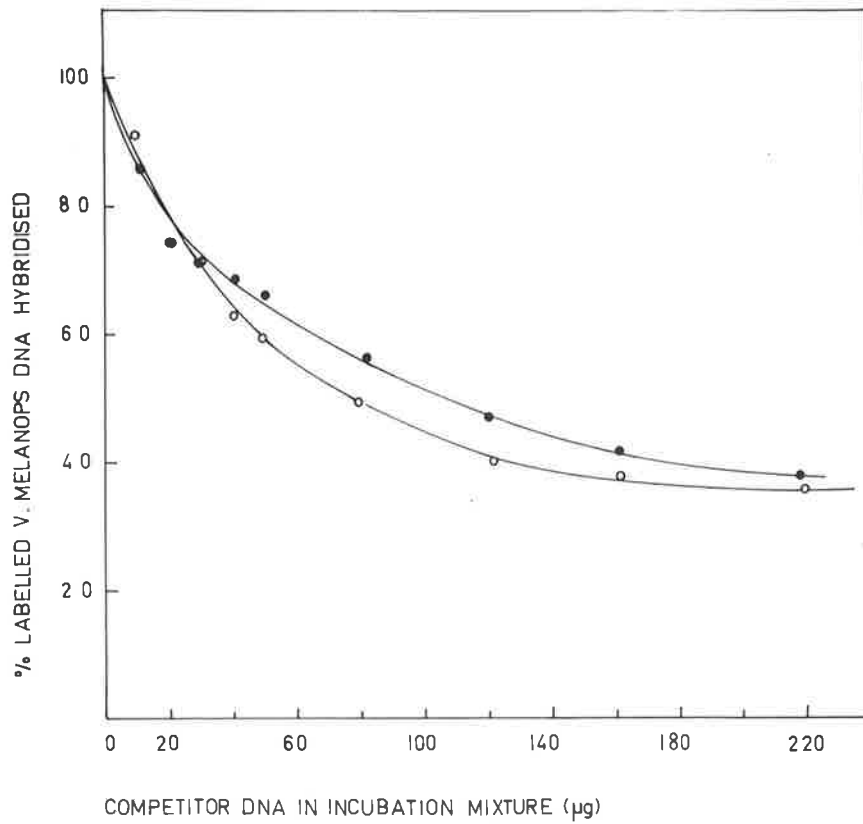
Why should such a large difference in DNA homology occur in such closely related species? Since the average amino-acid sequence of the proteins of the two species is probably similar, the large difference in homology could be explained by the divergence of the third base of many codons. This could take place without alterations in protein sequence if base substitutions resulted in synonymous codons. According to Walker (1968) and King and Jukes (1969), third position changes could be subject to less direct selection. It is uncertain if this hypothesis is correct. This hypothesis is in accord with two previous suggestions relating to closely related species of rodents (Walker, 1968) and *Drosophila* (Laird and McCarthy, 1968).

Group II.

Interaction of *V. melanops* DNA with *V. faba* DNA.

The interaction of *V. melanops* DNA with *V. faba* DNA and the reciprocal experiment (unlabelled *V. faba* DNA competing with labelled *V. melanops* DNA) are shown in Figs. 27 and 33, respectively. Fig. 34 shows that *V. faba* DNA had 100% homology with *V. melanops* DNA at every concentration of competitor *V. faba* DNA. The DNA of *V. melanops*, however, did not compete as efficiently as *V. faba* DNA for filter-bound *V. faba* DNA. The DNA of *V. melanops* showed increasing homology with *V. faba* DNA with increasing concentrations

Fig. 35. (Top). Competition by unlabelled *V. narbonensis* DNA fragments in the reaction between ^{32}P-labelled *V. faba* DNA fragments and *V. faba* filter-bound DNA. Filters with *V. faba* DNA (20 µg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* DNA fragments (4 µg) and varying amounts (10 to 220 µg) of heterologous *V. narbonensis* or homologous *V. faba* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. faba* DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

  o—o   Competition of *V. narbonensis* DNA with *V. faba* DNA.
  ●—●   Competition of *V. faba* DNA with *V. faba* DNA.

Fig. 36. (Bottom). Competition by unlabelled *V. faba* DNA fragments in the reaction between ^{32}P-labelled *V. narbonensis* DNA fragments and *V. narbonensis* filter-bound DNA. Filters with *V. narbonensis* DNA (20 µg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. narbonensis* DNA fragments (4 µg) and varying amounts (10 to 220 µg) of heterologous *V. faba* or homologous *V. narbonensis* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. narbonensis* DNA fragments hybridised to filter-bound *V. narbonensis* DNA is plotted against the amount of competitor DNA in the incubation mixture.

  o—o   Competition of *V. faba* DNA with *V. narbonensis* DNA.
  ●—●   Competition of *V. narbonensis* DNA with *V. narbonensis* DNA.

COMPETITOR DNA IN INCUBATION MIXTURE (µg)

COMPETITOR DNA IN INCUBATION MIXTURE (µg)
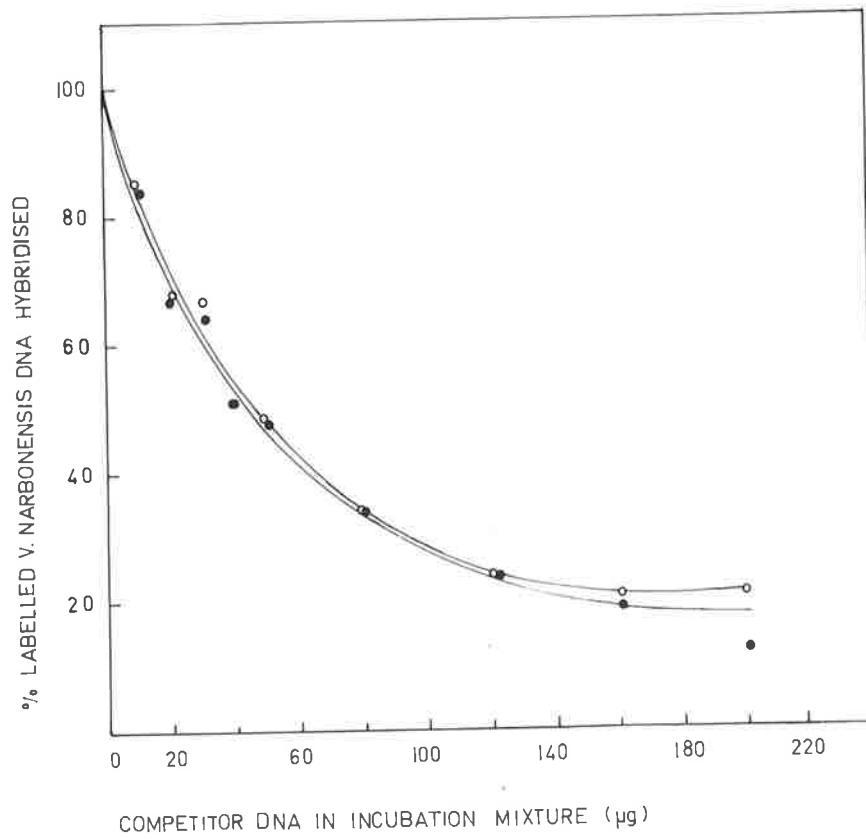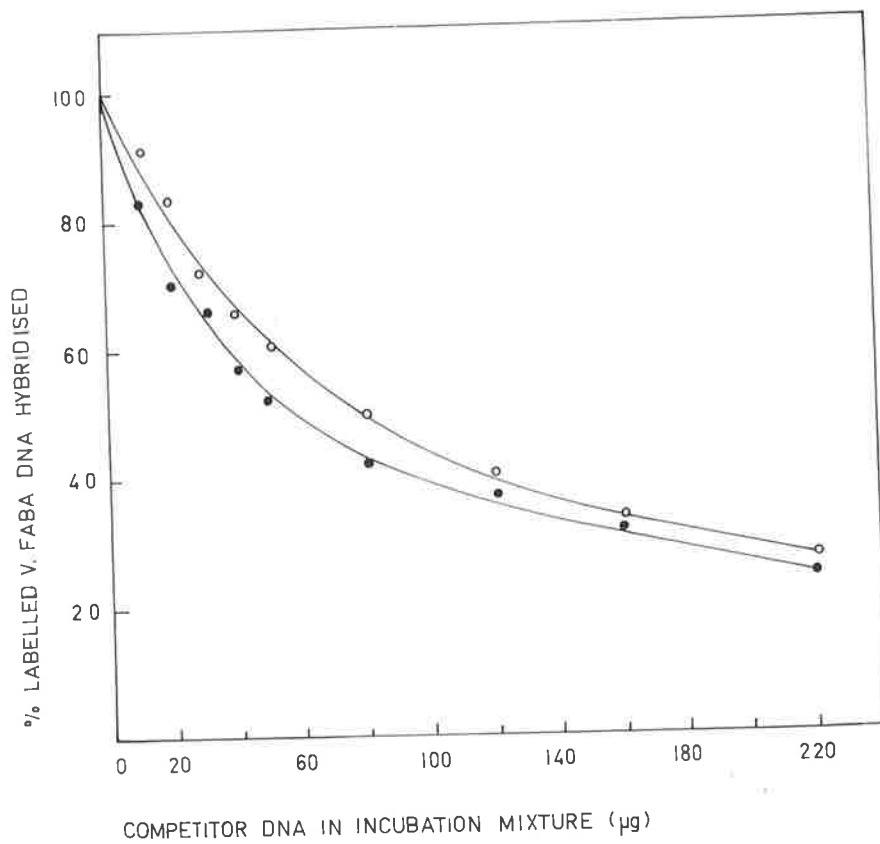
Fig. 37. (Top). The percentage homologies of *V. faba* DNA with *V. narbonensis* DNA (●-●) and those of *V. narbonensis* with *V. faba* DNA (o-o) are plotted against the amount of competitor DNA in the incubation mixture. The percentage homologies at the various concentrations of competitor DNA were calculated from data in Figs. 35 and 36. The DNA that is filter-bound is underlined.

Fig. 38. (Bottom). Competition by unlabelled *V. benghalensis* DNA fragments in the reaction between ^{32}P-labelled *V. faba* DNA fragments and *V. faba* filter-bound DNA. Filters with *V. faba* DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* DNA fragments (4 μg) and varying amounts (10 to 200 μg) of heterologous *V. benghalensis* or homologous *V. faba* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. faba* DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    o-o   Competition of *V. benghalensis* DNA with *V. faba* DNA.
    ●-●   Competition of *V. faba* DNA with *V. faba* DNA.

V. faba with V. narbonensis (●-●)

V. narbonensis with V. faba (o-o)

% HOMOLOGY

COMPETITOR DNA IN INCUBATION MIXTURE (μg)

% LABELLED V. FABA DNA HYBRIDISED

COMPETITOR DNA IN INCUBATION MIXTURE (μg)
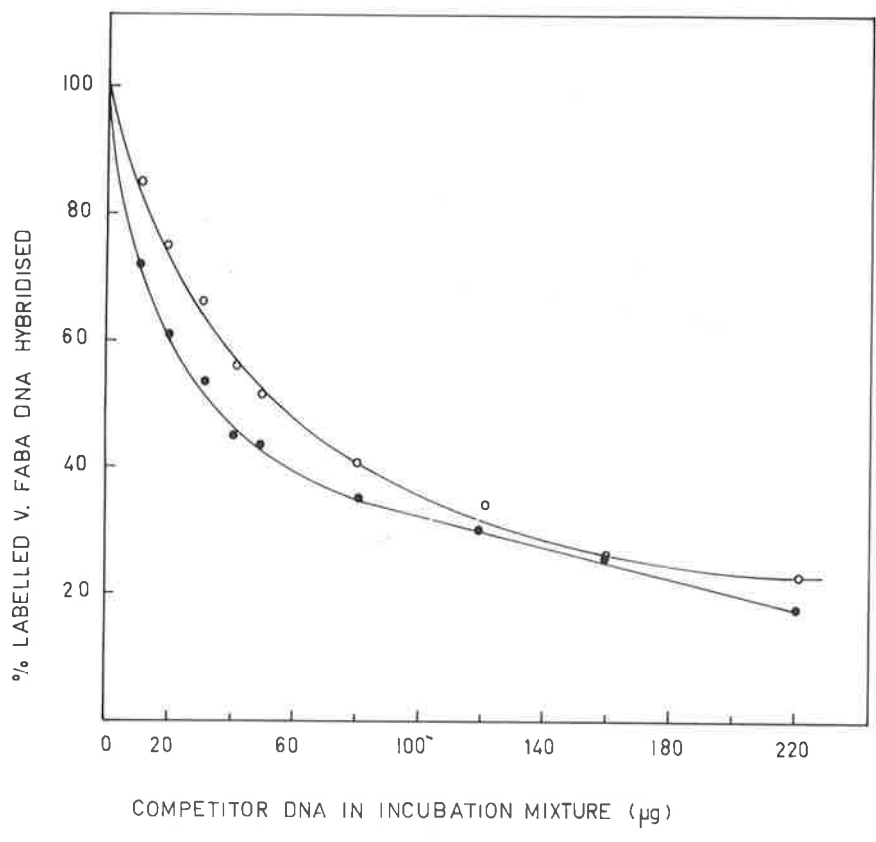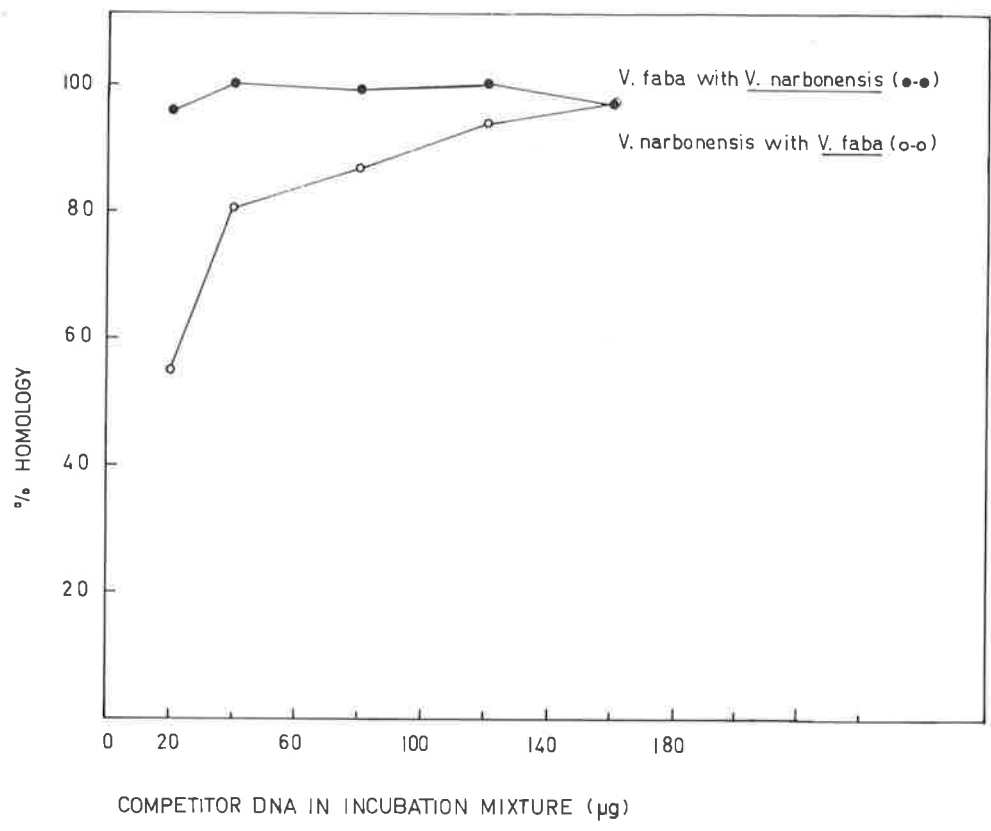
of competitor *V. melanops* DNA until 90% homology was reached.
The results suggested that the difference in DNA content per cell
between *V. faba* (RDC/cell = 100) and *V. melanops* (RDC/cell =
86.1 ± 1.5) resided in differential multiplication of some
nucleotide sequences. Many of the nucleotide sequences were
probably multiplied to a larger extent in *V. faba* DNA. This
was supported by the finding (page 97) that *V. faba* DNA included
35% fast DNA while *V. melanops* included only 15%.

Interaction of *V. narbonensis* DNA with *V. faba* DNA.

Fig. 35 shows that at most concentrations (10 to 120 µg)
of competitor DNA, *V. narbonensis* DNA did not compete as efficiently
as the homologous *V. faba* DNA. At higher concentrations
(ca. 120 µg and above), however, *V. narbonensis* DNA competed as
efficiently as *V. faba* DNA until approximately 97% homology with
V. faba DNA was reached. In the reciprocal experiment (unlabelled
V. faba DNA competing with labelled *V. narbonensis* DNA, see Fig. 36),
V. faba DNA showed 100% homology with *V. narbonensis* DNA at most
concentrations of competitor DNA (Fig. 37).

The above results suggested that most of the nucleotide
sequences in *V. faba* DNA were probably also represented in
V. narbonensis DNA (RDC/cell of *V. narbonensis* = 54.5 ± 1.8).
The majority of the nucleotide sequences were, however, multiplied
to a larger extent in *V. faba* DNA than could be accounted for by
the 1.9-fold difference in DNA content per cell between the two

Fig. 39. (Top). Competition of unlabelled *V. faba* DNA fragments in the reaction between ^{32}P-labelled *V. benghalensis* DNA fragments and *V. benghalensis* filter-bound DNA. Filters with *V. benghalensis* DNA (20 µg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. benghalensis* DNA fragments (4 µg) and varying amounts (10 to 220 µg) of heterologous *V. faba* or homologous *V. benghalensis* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. benghalensis* DNA fragments hybridised to filter-bound *V. benghalensis* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    o–o   Competition of *V. faba* DNA and *V. benghalensis* DNA.

    ●–●   Competition of *V. benghalensis* DNA and *V. benghalensis* DNA.

Fig. 40. (Bottom). The percentage homologies of *V. atropurpurea* DNA with *V. benghalensis* ($\triangle$ – $\triangle$), *V. benghalensis* DNA with *V. atropurpurea* ($\blacktriangle$ – $\blacktriangle$), *V. benghalensis* DNA with *V. faba* (o–o) and *V. faba* DNA with *V. benghalensis* (●–●) are plotted against the amount of competitor DNA in the incubation mixture. The percentage homologies at the various concentrations of competitor DNA were calculated from the data in Figs. 38, 39, 44 and 45. The DNA that is filter-bound is underlined.
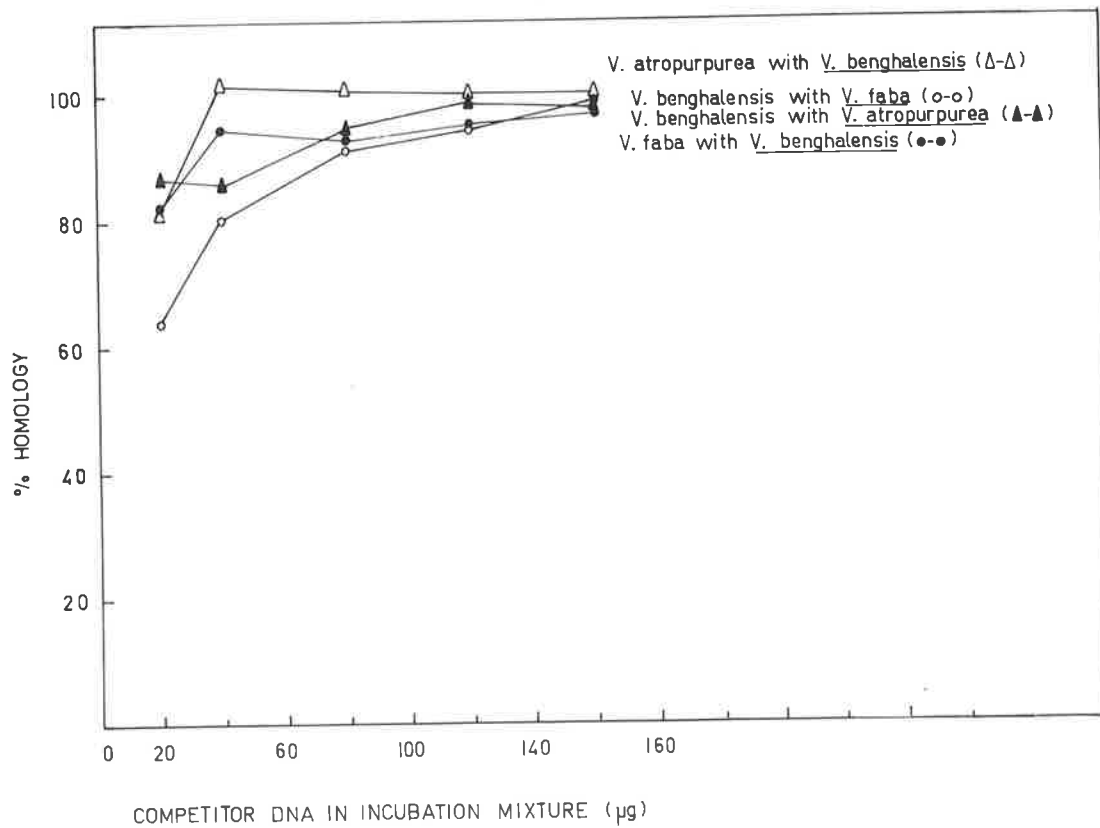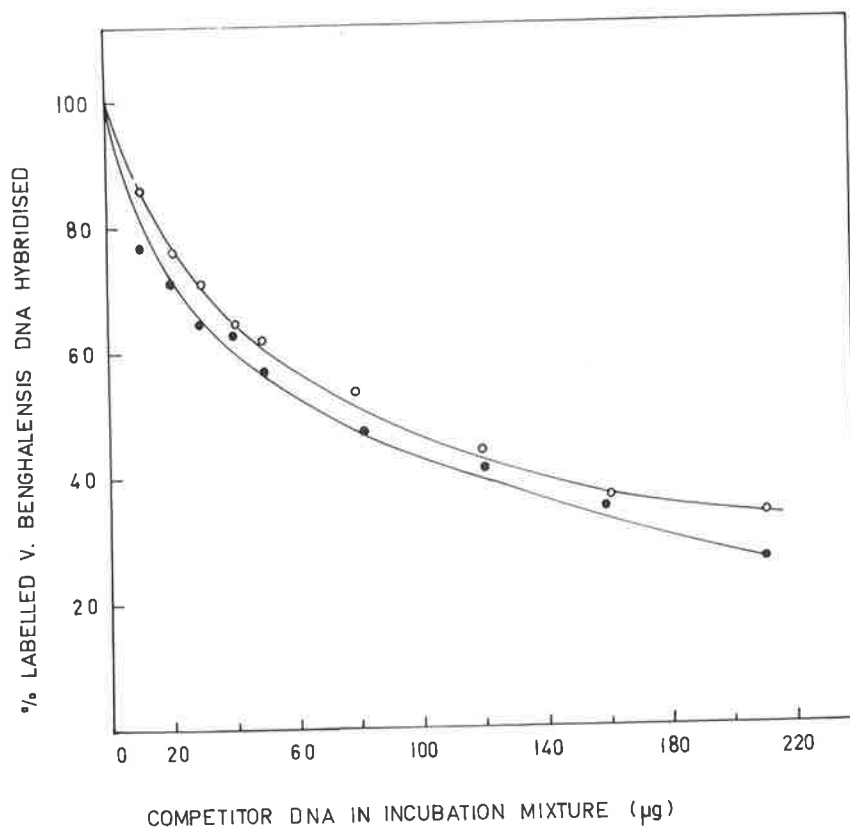
species. The results of reassociation rate experiments (page 97) showed, however, that *V. narbonensis* DNA included a larger proportion of fast DNA than that of *V. faba*. No satisfactory reason could be found to explain this inconsistency.

Interaction of *V. benghalensis* DNA with *V. faba* DNA.

The interaction of *V. benghalensis* DNA with *V. faba* DNA is shown in Fig. 38 while the reciprocal reaction is shown in Fig. 39. Fig. 40 shows that at most concentrations (10 to 80 µg) of competitor DNA, *V. benghalensis* DNA did not compete as efficiently as *V. faba* DNA. The percentage homology shared by the DNAs of the two species increased, however, with increasing amounts of competitor *V. benghalensis* DNA until it reached 99% at 160 µg of competitor DNA.

In the reciprocal experiment (unlabelled *V. faba* DNA competing with labelled *V. benghalensis* DNA), *V. faba* DNA showed almost 100% homology with *V. benghalensis* at most concentrations of competitor DNA.

These results showed that most of the nucleotide sequences in *V. benghalensis* (RDC/cell = 26.2 ± 0.2) were probably also represented in *V. faba* but the degree of repetition of nucleotide sequences was probably higher in *V. faba* DNA. The latter was supported by the finding that the DNA of *V. faba* consisted of a larger proportion of fast DNA than that of *V. benghalensis*. The evidence presented indicates, therefore, that

Fig. 41 (Top). Competition by unlabelled *V. atropurpurea* DNA fragments in the reaction between ^{32}P-labelled *V. faba* DNA fragments and *V. faba* filter-bound DNA. Filters with *V. faba* DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. faba* DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous *V. atropurpurea* or homologous *V. faba* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. faba* DNA fragments hybridised to filter-bound *V. faba* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    o-o   Competition of *V. atropurpurea* DNA with *V. faba* DNA.

    ●-●   Competition of *V. faba* DNA with *V. faba* DNA.

Fig. 42 (Bottom). Competition by unlabelled *V. faba* DNA fragments in the reaction between ^{32}P-labelled *V. atropurpurea* DNA fragments and *V. atropurpurea* filter-bound DNA. Filters with *V. atropurpurea* DNA (20 μg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. atropurpurea* DNA fragments (4 μg) and varying amounts (10 to 220 μg) of heterologous *V. faba* or homologous *V. atropurpurea* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. atropurpurea* DNA fragments hybridised to filter-bound *V. atropurpurea* DNA is plotted against the amount of competitor DNA in the incubation mixture.

    o-o   Competition of *V. faba* DNA with *V. atropurpurea* DNA.

    ●-●   Competition of *V. atropurpurea* DNA with *V. atropurpurea* DNA.

Standard errors shown are taken from Fig. 27.

COMPETITOR DNA IN INCUBATION MIXTURE (μg)

COMPETITOR DNA IN INCUBATION MIXTURE (μg)
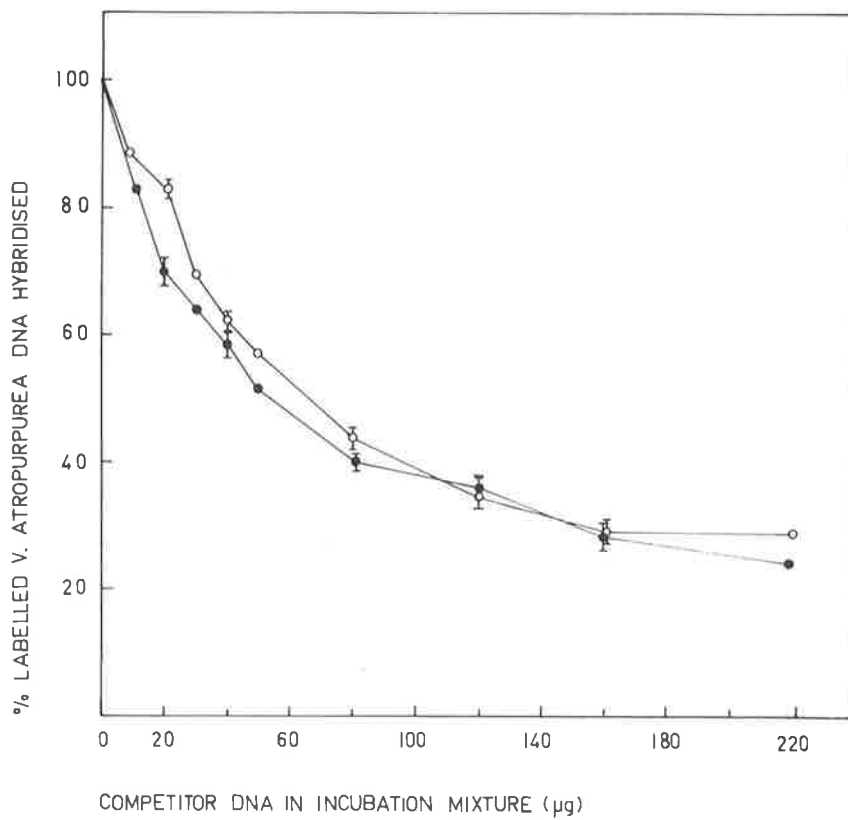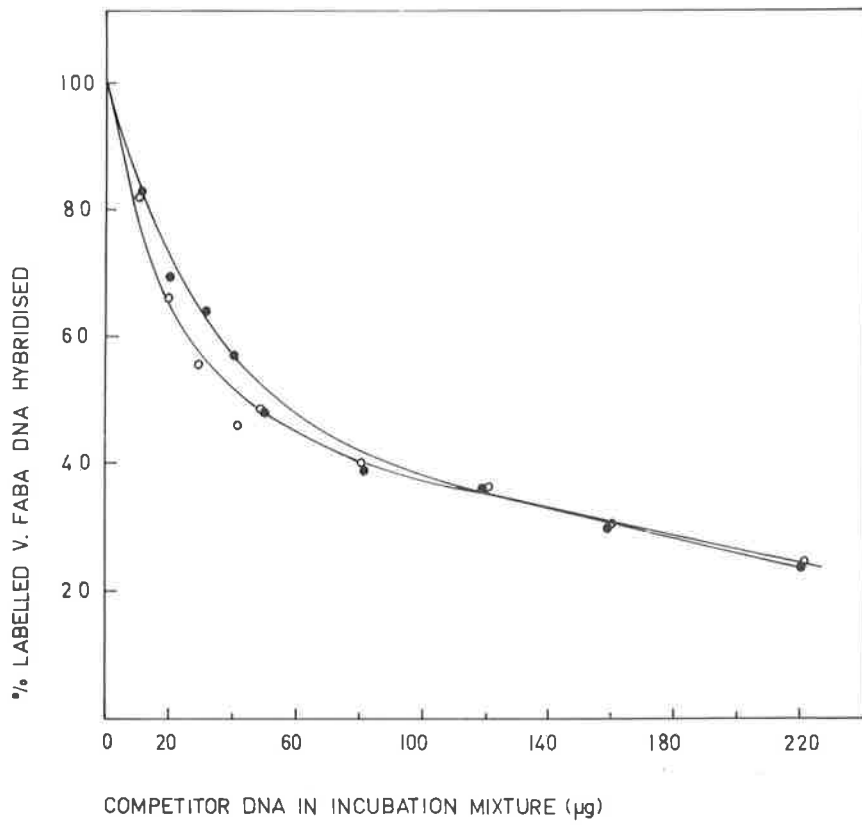
the 3.4-fold difference in DNA content per cell between the two

species resided in differential multiplication of some nucleotide

sequences.

Group III.

Interaction of *V. atropurpurea* DNA with *V. faba* DNA.

Figs. 32 and 41 show that *V. atropurpurea* DNA had 100%

homology with *V. faba* DNA at all concentrations of competitor

V. atropurpurea DNA. In the reciprocal experiment (unlabelled

V. faba DNA competing with labelled *V. atropurpurea* DNA, see

Fig. 42), the competitive ability of *V. faba* DNA was significantly

smaller than that of *V. atropurpurea* DNA at less than 40 μg of

competitor DNA if it was assumed that the standard errors of the

percentage homologies was not more than 2%.[22]

The above results showed that although the two species

had similar nucleotide sequences, the majority of which were

probably multiplied evenly to proportionally the same extent, a

few could have been multiplied to a smaller extent in *V. faba* DNA.

The results of reassociation rate experiments (page 98) showed,

however, that *V. faba* DNA had a higher proportion of fast DNA in its

genome than *V. atropurpurea* DNA. This inconsistency could be

attributed to the assumption that *V. atropurpurea* DNA included a

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[22] Since the standard errors of the mean percentage homologies
in the competition experiment between *V. faba* and *V. melanops*
(see pages 105-106) range from 1.4% to 2.0%, there was no
apparent reason to believe that the standard errors in other
competition experiments should be more than 2%.

Fig. 43. (Top).   Competition by unlabelled *V. atropurpurea*
DNA fragments in the reaction between ^{32}P-labelled *V. sativa*
DNA fragments and *V. sativa* filter-bound DNA.
Filters with *V. sativa* DNA (20 µg) were each immersed in
incubation mixture (1.0 ml) containing ^{32}P-labelled *V. sativa*
DNA fragments (4 µg) and varying amounts (10 to 200 µg)
of heterologous *V. atropurpurea* DNA or homologous *V. sativa*
unlabelled DNA fragments.   The procedure used was as
described in Materials and Methods.   The percentage of
^{32}P-labelled *V. sativa* DNA fragments hybridised to filter-
bound *V. sativa* DNA is plotted against the amount of
competitor DNA in the incubation mixture.

   o-o   Competition of *V. atropurpurea* DNA with *V. sativa*
          DNA.

   ●-●   Competition of *V. sativa* DNA with *V. sativa* DNA.

Fig. 44. (Bottom). Competition by unlabelled *V. atropurpurea*
DNA fragments in the reaction between ^{32}P-labelled
V. benghalensis DNA fragments and *V. benghalensis*
filter-bound DNA.   Filters with *V. benghalensis* DNA
(20 µg) were each immersed in incubation mixture (1.0 ml)
containing ^{32}P-labelled *V. benghalensis* DNA fragments (4 µg)
and varying amounts (10 to 220 µg) of heterologous
V. atropurpurea or homologous *V. benghalensis* unlabelled
DNA fragments.   The procedure used was as described in
Materials and Methods.   The percentage of ^{32}P-labelled
V. benghalensis DNA fragments hybridised to filter-bound
V. benghalensis DNA is plotted against the amount of
competitor DNA in the incubation mixture.

   o-o   Competition of *V. atropurpurea* DNA with
          V. benghalensis DNA.

   ●-●   Competition of *V. benghalensis* DNA with
          V. benghalensis DNA.

COMPETITOR DNA IN INCUBATION MIXTURE (μg)

COMPETITOR DNA IN INCUBATION MIXTURE (μg)
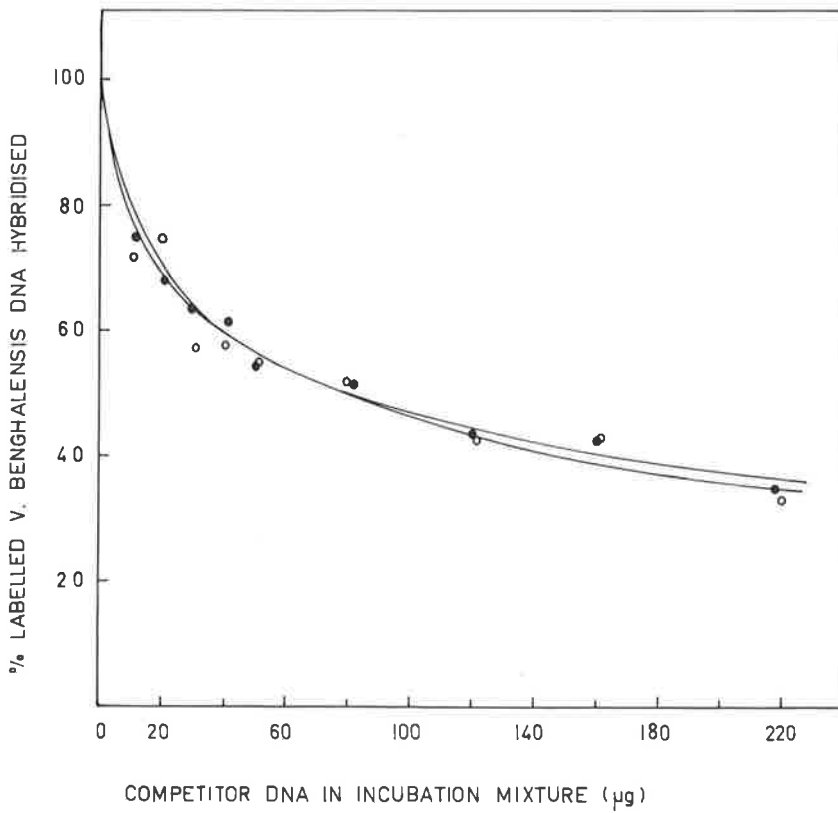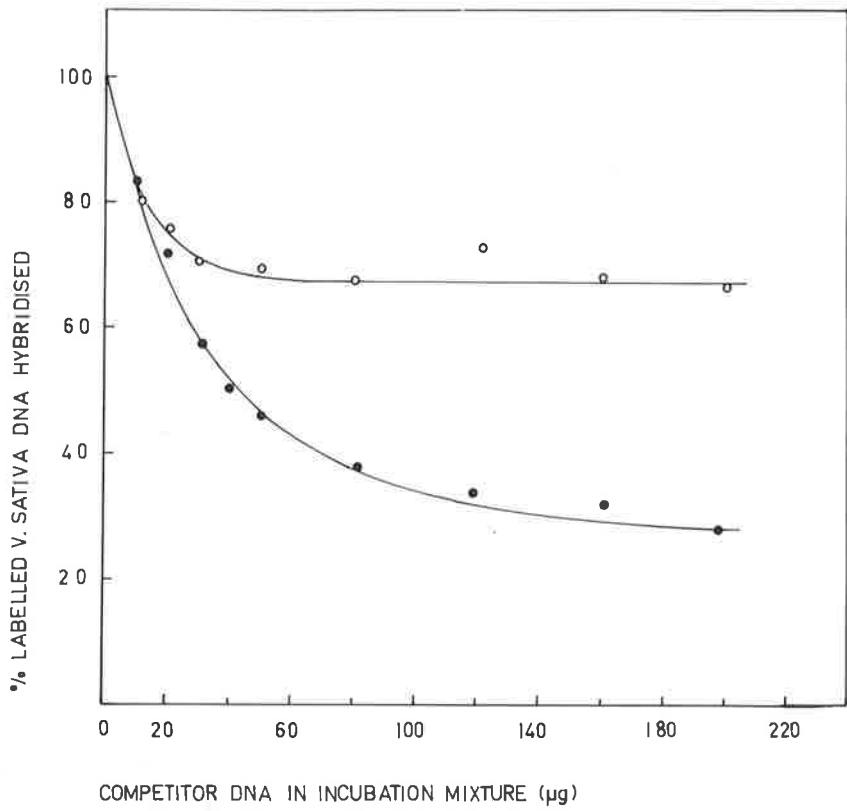
smaller number of nucleotide sequences that were multiplied to a larger extent than those in *V. faba* while *V. faba* DNA included a larger number of nucleotide sequences which were multiplied to a smaller degree. It is uncertain if this assumption is correct.

The evidence presented suggest, therefore, that the 5.5-fold difference in DNA content per cell between *V. faba* and *V. atropurpurea* resided

a) in uneven multiplication of a small number of nucleotide sequences,

b) in even multiplication of the majority of the nucleotide sequences.

The interaction of *V. atropurpurea* DNA with *V. sativa* DNA re-inforced the possibility that *V. faba* DNA is not an exact 5.5-fold multiple of *V. atropurpurea* DNA. This is because up to 20 µg of competitor DNA *V. atropurpurea* DNA competed with labelled *V. sativa* DNA significantly better than *V. faba* DNA (Figs. 32 and 43). At other concentrations of competitor DNA, *V. atropurpurea* DNA and *V. faba* DNA competed equally well with labelled *V. sativa* DNA.

Interaction of *V. benghalensis* DNA with *V. atropurpurea* DNA.

Figs. 40 and 44 show that *V. atropurpurea* DNA had 100% homology with *V. benghalensis* DNA at most concentrations of competitor DNA. In the reciprocal experiment (unlabelled

Fig. 45. Competition by unlabelled *V. benghalensis* DNA fragments in the reaction between ^{32}P-labelled *V. atropurpurea* DNA fragments and *V. atropurpurea* filter-bound DNA. Filters with *V. atropurpurea* DNA (20 µg) were each immersed in incubation mixture (1.0 ml) containing ^{32}P-labelled *V. atropurpurea* DNA (4 µg) and varying amounts (10 to 220 µg) of heterologous *V. benghalensis* DNA or homologous *V. atropurpurea* unlabelled DNA fragments. The procedure used was as described in Materials and Methods. The percentage of ^{32}P-labelled *V. atropurpurea* DNA fragments hybridised to filter-bound *V. atropurpurea* DNA is plotted against the amount of competitor DNA in the incubation mixture.

  o-o Competition of *V. benghalensis* DNA with
    V. atropurpurea DNA.
  ●-● Competition of *V. atropurpurea* DNA with
    V. atropurpurea DNA.
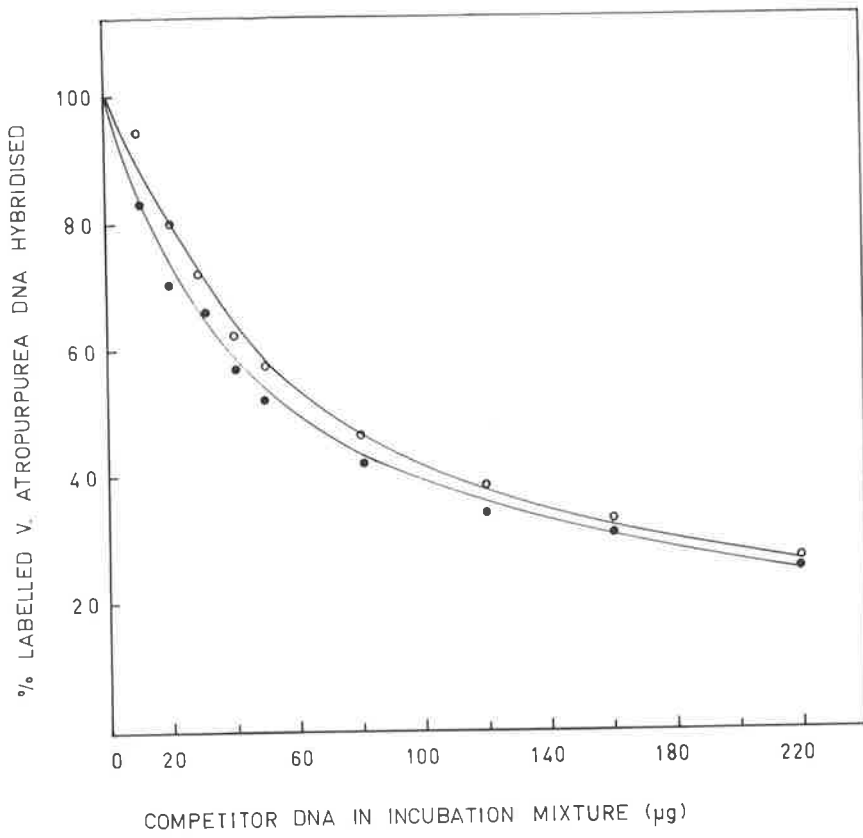
V. benghalensis DNA competing with labelled *V. atropurpurea* DNA),
V. benghalensis DNA had, however, 100% homology with
V. atropurpurea DNA only when the amount of competitor DNA was
120 μg or higher (Figs. 40 and 45).

The results presented are consistent with the suggestion
that the nucleotide sequences in the two species are probably similar.
The majority of the nucleotide sequences in *V. atropurpurea* was
probably represented to the same extent or evenly multiplied in *V. benghalensis* DNA. Some nucleotide sequences were probably multiplied,
however, to a higher degree in *V. atropurpurea* DNA.   This was
supported by the finding (page 98) that *V. atropurpurea* DNA had a
higher proportion of fast DNA.   In view of the findings that

a)   *V. benghalensis* (RDC/cell = 26.2 ± 0.2) had more DNA
per cell than *V. atropurpurea* (RDC/cell = 18.2 ± 0.9),

b)   *V. atropurpurea* had a higher proportion of fast DNA
in its genome than *V. benghalensis*

it is probable that *V. benghalensis* DNA had smaller multiplications
of a larger number of nucleotide sequences while *V. atropurpurea*
DNA had higher multiplications of fewer nucleotide sequences.

7.5   CHAPTER DISCUSSION.

The evidence presented above show that:

a)   except in the DNA of *V. sativa* where some of the
nucleotide sequences appear to have undergone nucleotide

sequence divergence,[23] all the DNAs of the other five *Vicia*
species studied probably have basically the same nucleotide
sequences.   In other words, the 'additional' DNA in *Vicia*
species with relatively high DNA contents per cell probably
occur as repetitious DNA,

b)   since each of the DNAs of the other five *Vicia* species
show different degrees of homology with *V. faba* at the same
concentrations (especially at low concentrations) of
competitor DNA (see Fig. 29), it is probable that some
nucleotide sequences in the DNAs of these six species are
unevenly multiplied,

c)   the number of nucleotide sequences that are unevenly
multiplied in each of the DNAs of these six species is
probably different.   For example, there is some evidence to
show that *V. atropurpurea* DNA may have a smaller number of
nucleotide sequences multiplied to a larger degree while
V. benghalensis DNA probably has a larger number of
nucleotide sequences multiplied to a smaller degree,

d)   while a portion of the DNA of the genome is capable of
being unevenly multiplied, the remainder is also capable of
being evenly multiplied.   The interaction of the DNA of
V. faba with that of *V. atropurpurea* showed that a large
portion of the DNA in *V. atropurpurea* is probably evenly

[23] nucleotide sequence divergence = the increasing degree of
mismatch among a set of nucleotide sequences that occur on an
evolutionary time scale (after Bolton *et al.*, 1967.)

multiplied in *V. faba* although a small portion is
probably not.

CHAPTER 8.

CONCLUSION

Cytological studies (Chapter 3) have shown that there is a 6-fold variation in DNA content per cell between 45 *Vicia* species. It has not been possible to make a detailed correlation between the direction of DNA increase or decrease 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. However, variations in DNA content per cell appear to increase with morphological advancement of the sections. In other words, larger variations in DNA content per cell are found in the 'more advanced' sections of the genus. These cytological findings have raised at least three interesting questions:

a)   What is the nature of the 'additional' DNA?

b)   Which mechanism (segmental duplication, local multiplicity or lateral multiplicity) was responsible for the increase in DNA content per cell?

c)   What is the evolutionary role of this 'additional' DNA?

a)   What is the nature of the 'additional' DNA?

      The results of a comparison between the nucleotide sequences of the DNAs of six species which have up to a 5.5-fold variation in DNA content per cell showed that, except in *V. sativa*, all the DNAs of the other five species studied have basically similar nucleotide sequences.   The differences in DNA content per cell between the other five species appear to lie, therefore, in repetitious DNA.   It is probable that some of the nucleotide sequences in *V. sativa* DNA have undergone a divergence.   The nature of this divergence is unknown.

b)   Which mechanism (segmental duplications, local multiplicity or lateral multiplicity) was responsible for the increase in DNA content per cell?

      Reassociation rate experiments (Table 11) indicated that

    i)   between 15 and 38% of the DNA of a genome (depending on species) is rapidly reassociating.   It is difficult to conceive that these fractions which represent highly repetitious DNA could have evolved by any mechanism other than local multiplicity,

    ii)   the remaining 62 to 85% of the DNA reassociates at approximately the same rate in all species studied despite the fact that these remainders varied 6-fold in actual amounts (Table 11).

This must mean that in those species with higher amounts of DNA (*V. faba, V. melanops* and *V. narbonensis*) all components of this remaining fraction must have been multiplied more or less to the same extent. This multiplicity could have been achieved through segmental duplications, lateral multiplicity or local multiplicity provided many of the genes are involved without high multiplicities. These conclusions agree with the cytological findings (see Chapter 3). Segmental duplication or local multiplicity can account for

a)   the small variations in DNA content per cell between species in the sections Cracca and Ervum,

b)   the differences in DNA content per cell between pairs or groups of taxonomically very closely related species.

For these mechanisms to be completely satisfactory, however, the number of 'sites' multiplied must be evenly spread throughout the genome since the chromosomes constituting a genome are found to be approximately uniform in size (page 40).

If it is assumed that the disjunct groups of average DNA content per chromosome in the sections Vicia and Faba approximate to 1:2:4 ratios (see page 67), then the disjunct groupings in these two sections would also be consistent with the lateral multiplicity hypothesis in which a geometric increase in the number of lateral strands has taken place.

c)   What is the evolutionary role of this 'additional' DNA?

Current hypotheses (see pages 77-80   ) regarding the role of repeated nucleotide sequences are probably not applicable to those in *Vicia* species.   This is due mainly to the magnitude of the difference in the amount of repeated nucleotide sequences and in the total DNA content per cell found between different species.   Walker (1968) believes that repeated nucleotide sequences in mouse are for 'housekeeping' purposes (page 79).   Since *V. faba* has six times more DNA than the species (*V. eriocarpa*) with the smallest amount of DNA per cell, it is probable that about five-sixth of *V. faba* DNA consists of repeated nucleotide sequences.   It seems unlikely that such a large quantity of repeated nucleotide sequences could have been evolved for 'housekeeping' purposes.   In addition, it seems unlikely that related *Vicia* species could have evolved up to a 6-fold difference in DNA content per cell for the same purposes. Britten and Kohne (1967b), however, have argued that repeated nucleotide sequences originated in 'saltatory steps' and are in the process of divergence.   This, according to them, may eventually result in many of the nucleotide sequences being able to code for new proteins.   Since similar proteins are probably synthesized by all *Vicia* species, it seems unlikely that all these *Vicia* species have multiplied their nucleotide sequences to such a large degree merely to provide for the evolution of new proteins.

It is very likely that the increase in DNA content per cell in *Vicia* species is adaptive. If it is assumed that increase in DNA content per cell acts as a potential for genetic adaptation to changing environments, it is conceivable that evolutionary increase in DNA content per cell in the genus *Vicia* has taken place via selective multiplication of certain parts of the genome. In other words, during speciation from a common ancestor, different short nucleotide sequences are selected for multiplication from the ancestral sequence pool. This is supported by the finding that the percentage of fast DNA in each of the genomes of the six *Vicia* species (see Table 11) is not proportional to the amount of DNA per cell. This selective multiplication may be adaptive since repeated genes are believed to be able to meet the requirements for an extreme rate of production of a given protein (Britten and Kohne, 1969). Multiplication of different nucleotide sequences probably take place in small steps during the evolutionary history of a species. This is supported by:

a)   the continuous distribution of DNA content per cell in the 'more primitive' sections Ervum and Cracca,

b)   the small variations in DNA content per cell between what are regarded as taxonomically synonymous species (viz. *V. atropurpurea* and *V. benghalensis*) and subspecies

(viz. between *V. sativa* and *V. angustifolia* and between

V. dasycarpa and *V. villosa*).

It is postulated here that although the increase in DNA content

per cell occurs in small steps at first, as distance from the

ancestral stock increases, so the size of the steps that can be

tolerated increases. This is supported by the finding that

variations in DNA content per cell appear to increase with

morphological advancement of the sections.

Although it is becoming increasingly clear that

multiplication of nucleotide sequences is not uncommon, the

significance of it is still obscure. There is some circumstantial

evidence, however, to support the possibility that it could be

adaptive since it is believed that

a) intraspecific variations in DNA content per cell in

Pinus glauca and *P. banksiana* (Miksche, 1967) and

b) variations in the base composition of the DNAs of

two populations of the grasshopper, *Myrmeleotettix maculatus*

(Gibson and Hewitt, 1970)

bear a strong correlation with environmental differences. It has

also been shown that changes in DNA content per cell in flax may

be induced by varying the nitrogen or phosphorus content of the

soil (Evans, 1968).

CHAPTER 9.

APPENDIX

9.1 Example showing procedure for calculation of

DNA value of species A relative to species B.

Microdensitometer readings in arbitrary values.

Slide 1		Slide 2		Slide 3		Slide 4		Slide 5	
A	B	A	B	A	B	A	B	A	B
33.3	85.5	31.7	85.2	35.8	81.7	33.7	82.3	30.8	83.2
32.2	81.7	35.0	80.7	31.8	82.0	32.0	83.0	34.7	84.7
35.5	83.7	34.2	83.3	35.5	82.8	32.0	81.0	34.2	81.0
31.5	83.0	33.5	84.3	35.5	82.0	34.3	84.8	33.2	82.0
34.5	80.0	34.3	81.7	35.3	85.2	33.3	80.5	33.7	80.3
30.2	83.2	36.3	85.8	33.5	84.7	30.5	85.2	33.7	84.8
30.8	84.7	36.7	85.2	31.7	81.8	31.7	81.8	34.0	83.3
35.8	85.0	33.0	82.2	34.2	84.5	35.0	82.3	30.2	81.2
34.2	85.3	35.2	85.5	35.3	82.0	32.2	84.3	33.0	86.8
33.7	83.2	33.7	81.5	32.3	82.5	34.7	81.2	31.7	81.2
Sum 331.7	835.3	343.6	835.4	340.9	829.2	329.4	826.4	329.2	828.5

$$\text{Mean value of species A} = \frac{331.7 + 343.6 + 340.9 + 329.4 + 329.2}{50} = 33.50.$$

Mean value of species B $= \dfrac{835.3 + 835.4 + 829.2 + 826.4 + 828.5}{50} = 83.10$

Analysis of variance

	Degrees of freedom	Sums of squares	Mean square	Variance ratio
Between slides	4	17.98540	4.49635	1.56738
Between species	1	61504.00000	61504.00000	21439.59347
Interaction	4	7.32900	1.83225	0.63870
Within samples	90	258.18400	2.86871	
	99	61787.49840		

Variance calculated from interaction + within samples.

Variance $= \dfrac{265.513}{90 + 4} = \dfrac{265.513}{94} = 2.825$

Variance of sample of 50 cells $= 2.825 \div 50$

Standard error $= \sqrt{\dfrac{2.825}{50}} = 0.2377$

Thus relative DNA value $= \dfrac{33.50 \pm 0.2377}{83.10} = \dfrac{33.50}{83.10} \pm \dfrac{0.2377}{83.10}$

Or expressed relative to species B = 100, this becomes 40.31 ± 0.2833

9.2   <u>Test of significance between chromosome arms</u>

        <u>of two species X and Y.</u>

The test of significance between chromosome arms of two species was based on the method of Martin and Hayman (1965). For each species, two quantities were involved, A (mean per cent area of chromosome arm) and D (relative DNA content per cell); both being converted to logarithms (base 10). The test was that A_X did not differ from $\dfrac{A_Y\,D_Y}{D_X}$

i.e. that $A_X \div \dfrac{A_Y\,D_Y}{D_X} = 1$

i.e. that $\log A_X + \log D_X - \log A_Y - \log D_Y = 0$

i.e. $\quad t = \dfrac{\log A_X + \log D_X - \log A_Y - \log D_Y}{\sqrt{s^2\left(\dfrac{1}{n_1} + \dfrac{1}{n_2} + \dfrac{1}{n_3} + \dfrac{1}{n_4}\right)}}$

with $n_1 + n_2 + n_3 + n_4 - 4$ degrees of freedom.

s^2 is the pooled estimate of the residual variance of the logs of A_X, B_Y, D_X and D_Y obtained from the analysis of variance.

(i.e. $s^2 = (\text{sslog}A_X + \text{sslog } A_Y + \text{DNA error ss}) \div (n_1 + n_2 + n_3 + n_4)$

where n_1, n_2, n_3, n_4 are number of measurements of A_X, A_Y, D_X, D_Y).

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