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

Chooi Wai Yean (B. Sc.).

Botany Department,
University of Adelaide.
November 1970.
TABLE OF CONTENTS.

PART A.

Chapter 1. INTRODUCTION.

1.1 Evolutionary change in DNA content per cell. 1.

1.2 Trends in evolutionary change in DNA content per cell within taxonomic groups. 4.

1.3 Mechanisms by which repeated DNA sequences might evolve. 6.

1.3.1 Evidence in support of local multiplicity. 12.

1.3.2 Evidence in support of segmental duplications. 15.

1.3.3 Evidence in support of lateral multiplicity. 16.

1.4 Parallel trends in evolution. 21.

1.5 Selection of study material. 23.

Chapter 2. MATERIALS AND METHODS.

2.1 Source of Vicia seeds. 25.

2.2 Measurement of DNA content per cell by Feulgen microspectrophotometry. 28.

2.3 Karyotype analysis, measurement of area and relative DNA content per chromosome arm. 30.

2.4 Species hybridisation. 31.
Chapter 3. DNA CONTENT PER CELL AND KARYOTYPES OF Vicia SPECIES.

3.1 Morphological evolution. 33
3.2 DNA content per cell of Vicia species. 35
3.2.1 Variation in DNA content per cell between Vicia species. 35
3.2.2 Variation in DNA content per cell between taxonomically synonymous and taxonomic subspecies. 41
3.2.3 Correlation between reduction in DNA content per cell and reduction in life cycle. 42
3.3 Karyotypes of diploid Vicia species. 44
3.3.1 Section Ervum. 45
3.3.2 Section Cracca. 47
3.3.3 Section Vicia. 53
3.3.4 Section Faba. 62
3.4 Interspecific crosses. 63
3.5 Chapter discussion. 64
3.6 Conclusion. 66

PART B.

Chapter 4. INTRODUCTION. 69

Chapter 5. MATERIALS AND METHODS.

5.1.1 Radioactive materials. 82
5.1.2 Reagents. 82
5.2 Bacterial culture. 82
5.3 Isolation of bacterial DNA. 83
5.4.1 Isolation of plant DNA. 84
5.4.2 Modification of the Bendich and Bolton technique (1967) for the extraction of plant DNA. 85
5.5 Preparation of $^{32}$P-labelled DNA. 87
5.6 Denaturation of DNA. 87
5.7 Immobilisation of denatured DNA on nitrocellulose filters. 88
5.8 Determination of the amount of denatured DNA on nitrocellulose filters. 89
5.9.1 DNA-DNA hybridisation – competition experiments. 89
5.9.2 DNA-DNA hybridisation – reassociation rate experiments. 90
5.10 Determination of molecular weight of sonicated DNA. 91

Chapter 6. THE RATES OF REASSOCIATION OF THE DNAs OF SIX Vicia SPECIES.

6.1 Introduction 92
6.2 Results and discussion. 94
6.2.1 Molecular weight of sonicated DNA. 94
6.2.2 Reassociation rates of the DNAs of E. coli and six Vicia species. 95
6.2.3 Fast DNA. 96
6.2.4 Remainder of the genome. 99
6.3 Chapter discussion 99

Chapter 7. DNA-DNA HYBRIDISATION (COMPETITION) BETWEEN SIX Vicia SPECIES.

7.1 Introduction. 101
7.2 Interpretation of results. 102
7.3 Determination of standard error. 105
7.4 Results and discussion. 106
7.5 Chapter discussion. 115

Chapter 8. CONCLUSION. 118

Chapter 9. APPENDIX.

9.1 Example showing procedure for calculation of DNA value of species A relative to species B. 124
9.2 Test of significance between chromosome arms of two species X and Y. 126

Bibliography. 127.
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 (Brvum 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.