

DNA VARIATION IN *FESTUCA*

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Received 9.xii.82

SUMMARY

There is substantial interspecific variation in nuclear DNA content in *Festuca* much of which cannot be accounted for by variation in chromosome number. The distribution of species DNA contents closely matches the taxonomic divisions within the genus. Polyploid taxa, especially high polyploids, tend to have less DNA than expected.

(a) There may be errors in the proposed modes of origin of polyploid forms. In particular, the distinction between European and Moroccan forms may have been underestimated, and/or

(b) polyploid species may have undergone a reduction in nuclear DNA content since their divergence, relative to their progenitor species of lower ploidy.

1. INTRODUCTION

The genus *Festuca* (Poaceae) comprises about 100 species of wide geographical distribution, some of which are important herbage crops in temperate regions (Malik and Thomas, 1966). Information contributing to an understanding of species relationships within the genus would be of use to breeders wishing to incorporate the desirable characteristics of many wild species into the agronomically important varieties (Chandrasekharan and Thomas, 1971*a*).

Cytologically the genus is diverse. Chromosome number ranges from diploid ($2n = 14$) to decaploid ($2n = 70$). About 74 per cent of species occur as polyploids (Malik and Thomas, 1966). Supernumerary chromosomes have been found in several species (Jones, 1975). Chromosome morphology varies between species and populations (Malik and Thomas, 1966). Heteromorphic bivalents are often found at meiosis in interspecific hybrids (Malik and Thomas, 1967). This suggests that, during or following speciation, homoeologous chromosomes have diverged in size and, therefore, in nuclear DNA content. Extensive interspecific variation in nuclear DNA content exists in the closely related genus, *Lolium* (Rees and Jones, 1967).

In this work, a survey of the genus *Festuca* was made in order to assess the extent and pattern of variation in nuclear DNA content among a wide range of taxa, both diploid and polyploid. The degree to which the observed variation coincided with taxonomic divisions based on other criteria was examined and the possibility of using DNA contents in tracing the ancestry of polyploids was considered.

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2. MATERIALS AND METHODS

The taxa used and their seed sources are shown in table 1. The DNA contents of Feulgen stained nuclei were estimated by microdensitometry (estimates of nuclear area were obtained simultaneously). The method is described in detail by Seal and Rees (1982). The 2C nuclear DNA content of *F. pratensis* cv. S215 was estimated to be 3.96 pg by comparison with *Allium cepa* (33.5 pg, Van't Hof, 1965) and was used as a standard with which all other estimates were compared.

TABLE 1
Sources of plant material

	Taxon	Source
1.	<i>F. polesica</i> Zapal.	W.P.B.S.*
2.	<i>F. scariosa</i>	W.P.B.S.
3.	<i>F. altissima</i> All.	W.P.B.S.
4.	<i>F. donax</i>	W.P.B.S.
5.	<i>F. drymeja</i>	W.P.B.S.
6.	<i>F. mairei</i> St. Yves Bs 3065	W.P.B.S.
7.	<i>F. pratensis</i> Huds. cv. S215	W.P.B.S.
8.	<i>F. pratensis</i> Huds. Induced autotetraploid cv. S215	W.P.B.S.
9.	<i>F. arundinacea</i> var. <i>glaucescens</i> Boiss. Bn 354	W.P.B.S.
10.	<i>F. arundinacea</i> cv. S170	W.P.B.S.
11.	<i>F. arundinacea</i> var. <i>atlantigena</i> forma <i>pseudo-mairei</i> B1 867	W.P.B.S.
12.	<i>F. arundinacea</i> var. <i>letourneauxiana</i>	W.P.B.S.
13.	<i>F. rubra</i> subsp. <i>rubra</i>	W.P.B.S.
14.	<i>F. alpina</i> Suter.	Botanischer Garten der Stadt Essen, W. Germany
15.	<i>F. rupicaprina</i> (Hack.) Kern.	Université Strasbourg, France
16.	<i>F. ovina</i> var. <i>firmula</i>	Jardin Botanique de la ville, Dijon, France
17.	<i>F. arundinacea</i> Schreb.	Jardin Botanique de la ville, Besançon, France
18.	<i>F. gigantea</i> C.L./Vill.	Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, DDR
19.	<i>F. pratensis</i> Huds.	Agricultural University of Norway

* W.P.B.S. = Welsh Plant Breeding Station, Aberystwyth, U.K.

3. RESULTS AND DISCUSSION

(i) *The extent of DNA variation in Festuca*

The mean 2C nuclear DNA contents of the 19 taxa (table 2) ranged from 2.88 pg in *F. alpina* to 13.40 pg in *F. arundinacea* var. *letourneauxiana*—a difference of more than 4-fold. Only some of this variation could be accounted for by differences in chromosome number which ranged from $2n = 14$ to $2n = 70$. Thus among diploids, the species with the highest DNA content (*F. drymeja*) had about two and a half times more DNA than that with the lowest (*F. alpina*). There was about a 1.4 fold variation in DNA content between the hexaploid taxa. Measurements of nuclear area

TABLE 2

Mean 2C nuclear DNA contents (with standard errors in brackets) and chromosome numbers of 19 *Festuca* taxa

Taxon	DNA content (pg)	No. of plants scored	Chromosome number (2n)
1. <i>F. alpina</i>	2.88 (0.07)	2	14
2. <i>F. rupicaprina</i>	2.96 (0.07)	6	14
3. <i>F. polesica</i>	3.40 (0.05)	9	14
4. <i>F. pratensis</i> (Norwegian popul.)	3.78 (0.12)	3	14
5. <i>F. pratensis</i> cv. S215	3.96 (0.06)	18	14
6. <i>F. scariosa</i>	4.73 (0.09)	9	14
7. <i>F. altissima</i>	5.79 (0.15)	5	14
8. <i>F. donax</i>	6.36 (0.14)	6	14
9. <i>F. drymeja</i>	7.17 (1.10)	6	14
10. <i>F. mairei</i>	6.93 (0.10)	6	28
11. <i>F. arundinacea</i> var. <i>glaucescens</i>	7.62 (0.04)	6	28
12. <i>F. pratensis</i> (autotetraploid S215)	7.94 (0.19)	6	28
13. <i>F. rubra</i> subsp. <i>rubra</i>	8.37 (0.06)	3	42
14. <i>F. ovina</i> var. <i>firmula</i>	9.23 (0.18)	2	42
15. <i>F. arundinacea</i> (nat. popul.)	10.53 (0.43)	3	42
16. <i>F. arundinacea</i> cv. S170	11.66 (0.20)	6	42
17. <i>F. arundinacea</i> var. <i>atlantigena</i> forma <i>pseudo-mairei</i>	11.19 (0.14)	6	56
18. <i>F. gigantea</i>	12.82 (0.19)	3	56
19. <i>F. arundinacea</i> var. <i>letourneauxiana</i>	13.40 (0.29)	6	70

showed that, in general, increases in nuclear DNA content were accompanied by increases in nuclear size.

Fig. 1 shows that nuclear DNA contents of diploids in *Festuca* are low when compared with those of diploid species from other genera of the Poaceae for which measurements for more than one species are available. The variation among species is similar to that in most other genera. DNA contents of polyploids in *Festuca* are also low compared with those of other genera in the Poaceae.

Variation within and between taxa of the same species and ploidy was generally insignificant. For example, two diploid samples of *F. pratensis* (the cultivar S215 and a natural population from Norway) showed no significant difference in DNA content. However, at the hexaploid level, a significant difference in DNA content was found between a cultivar and a natural population of *F. arundinacea*. Intraspecific variation in somatic chromosome morphology has also been found in hexaploid *F. arundinacea* (Malik and Thomas, 1966), including differences in chromosome size between populations which may suggest DNA variation.

(ii) *The pattern of DNA variation*

The nuclear DNA contents in table 2 show continuous variation throughout the range. There were no obvious gaps or clusters of taxa in

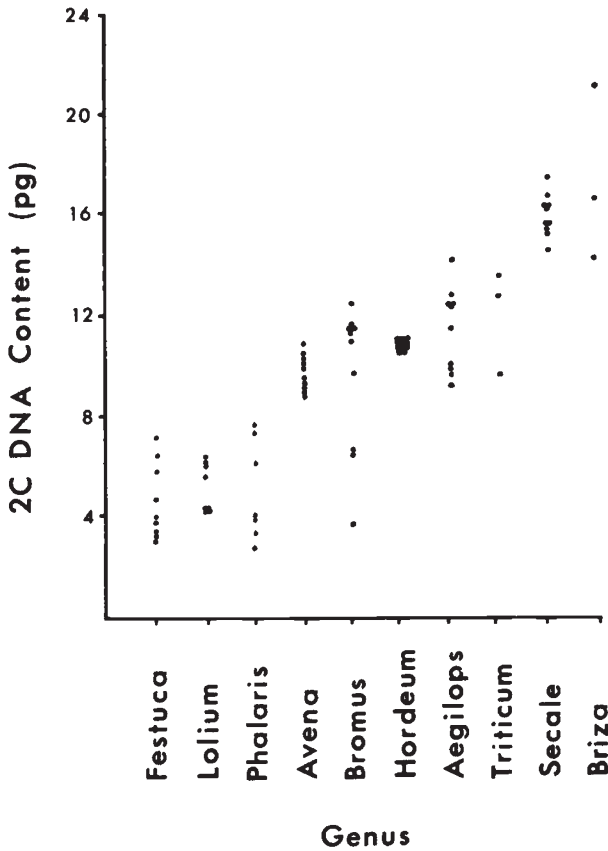


FIG. 1. Nuclear DNA contents of diploid species in the Poaceae. Data taken from Bennett and Smith, 1976 except for *Festuca* (data from present work) and *Lolium* (data from Hutchinson *et al.*, 1979).

any particular part of the range. This is remarkable in that taxa from five different ploidy levels are included. For example, the diploid *F. drymeja* has at least as much DNA as *F. mairei* which has twice as many chromosomes. Thus, a similar DNA content may be achieved in different species via very different evolutionary pathways. Another example of this phenomenon was previously found in the genus *Allium* (Jones and Rees, 1968).

The genus *Festuca* has been divided into sections on the basis of leaf morphology and the structure of the ovary (Hackel, 1882). Four of the six recognised sections were represented in this survey. The classification of the taxa into their respective sections shows a remarkable correspondence with their DNA contents (fig. 2). Within the diploids for example, the three taxa from the Montanae section have the highest mean DNA contents. The single diploid of the section Scariosae lies between the Montanae and Bovinae taxa while the three Ovinae taxa have the lowest DNA contents. Among the tetraploids, the Bovinae taxa each have more DNA than *F. mairei*—a tetraploid species of the Scariosae section. At the hexaploid level the two Ovinae fescues (as in the case of the diploids) have lower

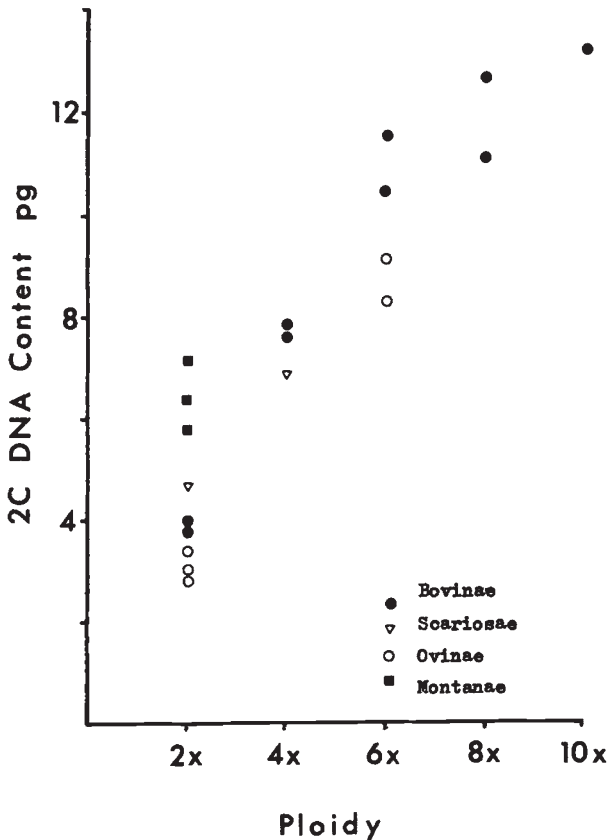


FIG. 2. The relationship between nuclear DNA content, ploidy and taxonomic divisions for the *Festuca* taxa listed in tables 1 and 2.

DNA contents than the two *arundinacea* taxa of the Bovinae section. The higher polyploids tested here have all been placed in the section Bovinae. Thus, within each ploidy level there are no overlaps in terms of DNA content between taxa of the different taxonomic sections and therefore, the taxonomic divisions are reinforced by the present data.

(iii) *Tracing the ancestry of polyploids*

Nuclear DNA contents have sometimes been used as evidence in proposing the possible origin of polyploid species (Rees and Walters, 1965). For example, if the sum of the DNA contents of two putative diploid ancestors matches the DNA content of the derived tetraploid then it is taken as evidence in favour of the origin of the tetraploid by doubling of the ancestral diploid hybrid. Rees and Walters (1965) using this approach, concluded that *Aegilops speltoides* was the most likely donor of the B genome in tetraploid wheat.

Malik and Thomas (1966) made the first detailed comparison of somatic chromosome morphology in *Festuca* species, all of which are outbreeders.

They concluded that most polyploids were allopolyploids, none originating as a result of true autopolyploidy alone. Within the section Bovinae, similarities were found between the karyotypes of *F. pratensis* (diploid) and the tetraploid, hexaploid and decaploid varieties of *F. arundinacea*. A link was also indicated between the karyotypes of *F. arundinacea* var. *glaucescens* and *F. mairei* of the sections Bovinae and Scariosae respectively.

Malik and Thomas (1967) found that despite karyotypic diversity many species formed viable hybrids. The meiotic behaviour of several intra- and interspecific hybrids was described and genomic formulae proposed. The results of subsequent studies are summarised in table 3. The genomic formulae were compiled from those of Chandrasekharan and Thomas (1971*b*) and Bowman and Thomas (1976).

F. arundinacea (6x)

Chandrasekharan and Thomas (1971*a*) proposed, on the basis of morphological data, that *F. arundinacea* var. *glaucescens* (4x) and *F. pratensis* (2x) were the progenitors of the hexaploid *F. arundinacea*. The sum of the DNA contents of the proposed ancestral species (11.58 pg) is very close to the DNA content of the hexaploid *F. arundinacea* cv. S170 (11.66 pg). Despite some intraspecific variation in DNA content among hexaploid varieties clearly hexaploid *F. arundinacea* could have arisen by chromosome doubling of the F₁ hybrid between *F. arundinacea* var. *glaucescens* and *F. pratensis*, without invoking subsequent changes in DNA content. However, the 2*n* = 42 colchipsoid of the *F. arundinacea* var. *glaucescens* × *F. pratensis* F₁ hybrid is morphologically unlike 6x *F. arundinacea* (Borrill, pers. comm.).

F. mairei (4x)

Malik and Thomas (1966) suggested, on the basis of similarity between some mitotic satellited chromosomes, that *F. mairei* (4x) and *F. arundinacea* var. *glaucescens* (4x) share one common genome. Meiosis in the tetraploid hybrid is characterised by a large number of bivalent associations (Malik and Thomas, 1967). This was confirmed by Bowman and Thomas (1976) who also found an appreciable frequency of quadrivalents in the colchicine derived 8x amphiploid. There is strong evidence that *F. arundinacea* var. *glaucescens* contains at least one genome from *F. pratensis* (Malik and Thomas, 1967) which is absent from *F. mairei*. Since the DNA contents of *F. arundinacea* var. *glaucescens* and *F. pratensis* are known, the DNA content of the genome common to both tetraploids would be, by subtraction, 3.66 pg. The remaining genome in *F. mairei* would then have a disomic DNA content of only 3.27 pg compared with 4.73 pg of *F. scariosa*, the proposed diploid donor species (Borrill, 1972).

F. arundinacea var. *atlantigena* forma *pseudo-mairei* (8x)

F. arundinacea var. *atlantigena* forma *pseudo-mairei* (8x) was thought to combine the genomes of *F. arundinacea* var. *glaucescens* and *F. mairei* (Chandrasekharan and Thomas, 1971*b*). Assuming no subsequent genomic

DNA changes, the expected DNA content of an octoploid derived in this way would be 14.55 pg which is 26 per cent more than the observed value (table 3).

TABLE 3

Comparison of observed DNA contents (Obs.) of *Festuca* taxa and those expected (Exp.) on the basis of proposed genomic formulae

Taxa	Ploidy	Proposed genomic formulae	2C nuclear DNA contents (pg)	
			Obs.	Exp.
<i>F. pratensis</i>	2x	PP	3.96	
<i>F. arundinacea</i> var. <i>glaucescens</i>	4x	AAGG	7.62	
<i>F. mairei</i>	4x	AAMM	6.93	
<i>F. arundinacea</i>	6x	PP AAGG	10.53/11.66	11.58
<i>F. arundinacea</i> var. <i>atlantigena</i> forma <i>pseudo-mairei</i>	8x	AAGG AAMM	11.59	14.55
<i>F. arundinacea</i> var. <i>letourneauxiana</i>	10x	PP AAGG AAMM	13.40	17.46/18.5

F. arundinacea var. *letourneauxiana* (10x)

The decaploid form was thought to combine the genomes of *F. arundinacea* (6x) and *F. mairei*, 4x (Borrill, 1972). Combining *F. mairei* with the *arundinacea* hexaploid taxon in table 1 having the lowest DNA content would produce a decaploid with 30 per cent more than the observed nuclear DNA content of *F. arundinacea* var. *letourneauxiana*. Even the smallest diploid genome among the taxa listed in table 2 has more than one fifth of the DNA content of the decaploid. *F. pratensis*, the only known diploid in the section Bovinae (to which the decaploid is assigned), has a DNA content of about one third of the corresponding decaploid value (table 2). Indeed, the highest possible DNA content of the smallest disomic genome in the decaploid is only 2.68 pg (13.40 divided by 5).

Thus, the DNA contents of polyploid species, especially high polyploids (8x and 10x), are in most cases substantially less than expected on the basis of their proposed modes of origin. There are at least three possible explanations:

1. The DNA contents of polyploid species may, for technical reasons, have been underestimated.

In some surveys a disproportionate change in DNA content with increasing chromosome number has been shown to result from technical bias (Verma and Rees, 1974). Fig. 3 shows that as DNA content increases so too does the DNA density (DNA per unit area) of the nuclei. In some studies nuclear DNA estimates have been "corrected" to account for differences in DNA density which otherwise would have led to the underestimation of the DNA content of nuclei having a high DNA density (Verma and Rees, 1974). Such corrections were not applicable to the present results for three reasons. Firstly, squashing nuclei in acetic acid rather than glycerol produces higher estimates of DNA content and reduces the DNA density

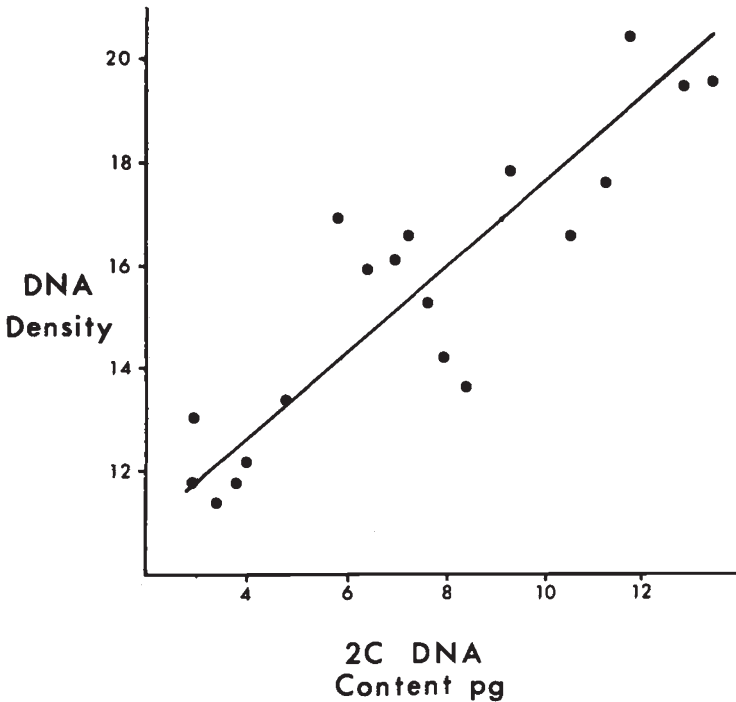


FIG. 3. The relationship between nuclear DNA density (DNA per unit area), expressed in arbitrary units, and nuclear DNA content of *Festuca* taxa.

of large nuclei (Seal—in preparation). Secondly, there was no significant correlation between DNA content and area (and, therefore, DNA density) for large nuclei squashed in acetic acid within individual slides. Only in the event of such a correlation is there any need for a “correction factor” (Verma and Rees, 1974). Thirdly, the inclusion of an induced tetraploid of *F. pratensis* cv. S215 in the present survey provided a direct test of the comparability of estimates of nuclear DNA content of two taxa differing in DNA content by a known factor (two-fold). A Chi-squared test showed no significant difference between the expected DNA content (calculated by doubling the diploid value) and the observed DNA content of the tetraploid despite a 17 per cent difference in DNA density.

From the above considerations it was assumed that all estimates in the present survey were comparable in their present form, with one possible reservation. The distribution of chromatin in nuclei of human fibroblast cells affects estimates of their DNA content made by Feulgen microdensitometry (Mittwoch, 1969). The DNA content of nuclei with dense chromocentres was underestimated when compared with similar nuclei showing a more uniform distribution of chromatin. In the present work it was noticed that the nuclei of *Festuca* species belonging to the section *Ovinae* consistently showed numerous dense chromocentres which were absent from the nuclei of other species. It is therefore possible that the nuclear DNA contents of species from this section were underestimated. However, since the estimated DNA contents of prophase nuclei which lack

such chromocentres were close to the expected 4C value in the Ovinae species, large discrepancies of this nature are unlikely.

2. The proposed genomic formulae may be incorrect. The true diploid ancestral species may not be represented in the survey. They may be undiscovered or extinct.

Borrill (1972) divided the taxa of the Bovinae and Scariosae sections into two groups on the basis of geographical distribution, morphology and crossability. The first group, found in Morocco, includes *F. mairei* (4x), *F. arundinacea* var. *atlantigena* forma *pseudomairei* (8x) and *F. arundinacea* varieties *letourneauxiana* and *cirtensis* (10x). The second group is predominantly European and includes *F. pratensis* (2x), *F. arundinacea* var. *glaucescens* (4x) and the hexaploid varieties of *F. arundinacea*. The results of Chandrasekharan *et al.*, (1972) support the view that the Moroccan fescues are more closely related among themselves than to fescues from other regions. This contradicts the traditional classification of the three Moroccan types in which *F. arundinacea* var. *atlantigena* forma *pseudomairei* and *F. arundinacea* var. *letourneauxiana* are placed in the Bovinae section while *F. mairei* is in the Scariosae section. Indeed, Borrill (1972) suggested that these species should either all be included in an enlarged Scariosae section or that this section should be merged with the Bovinae.

The DNA contents of the *Festuca* taxa (tables 2 and 4) provide further evidence in support of the distinctive nature of the Moroccan group. The

TABLE 4

A comparison of the 2C nuclear DNA contents of Festuca taxa from Morocco and Europe

Ploidy	Moroccan taxa	Nuclear DNA contents (pg)	
			European taxa
2x			<i>F. pratensis</i> 3.96
4x	<i>F. mairei</i>	6.93	<i>F. arundinacea</i> var. <i>glaucescens</i> 7.62
6x			<i>F. arundinacea</i> cv. S170 11.66
8x	<i>F. arundinacea</i> var. <i>atlantigena</i> forma <i>pseudomairei</i>	11.19	
10x	<i>F. arundinacea</i> var. <i>letourneauxiana</i>	13.40	

three Moroccan taxa are rather low in DNA content compared with European taxa. The tetraploid *F. mairei* has the lowest DNA content among the tetraploid taxa. the octoploid, *F. arundinacea* var. *atlantigena* forma *pseudomairei* has a DNA content which is not significantly different from that of the hexaploid *F. arundinacea* cv. S170. The DNA content of the decaploid is too low to have been formed by multiplication of even the smallest diploid genome in the survey. The three Moroccan species show the greatest deviations of observed from expected DNA contents on the basis of their proposed genomic formulae. If these deviations are to be explained by the existence of unknown diploid species of low DNA content then perhaps it is Moroccan diploids that are missing.

Table 2 shows that each step in the series *F. scariosa* (2x)—*F. mairei* (4x)—*F. arundinacea* var. *atlantigena* forma *pseudo-mairei* (8x)—*F. arundinacea* var. *letourneauxiana* (10x) involves the addition of about 1.1 pg of DNA per genome added. Whether this is coincidental or reflects the existence of optimal levels of DNA content or the addition of copies of a single genome is unknown. However, if such a genome did exist then the ancestral Moroccan diploid would have an expected DNA content of only 2.2 pg. No *Festuca* species has yet been found with such a low DNA content and the existence of such a genome would undermine the close affinities found between European and Moroccan species by Chandrasekharan and Thomas (1971a and 1971b).

Borrill (1972), on morphological grounds, suggested that the three Moroccan polyploids had each incorporated at least one genome from the diploid *F. scariosa*. This diploid is now found only in the Sierra Nevada region of Spain but opportunities for hybridisation are thought to have once existed (Borrill, 1972). Such a relationship is not supported, however, by the cytogenetic evidence from triploid *F. scariosa* (2x) × *F. mairei* (4x) or tetraploid *F. scariosa* (4x) × *F. mairei* (4x) hybrids in which few meiotic associations were found between chromosomes of the two species (Bowman and Thomas, 1976). Unlike the Moroccan fescues, *F. scariosa* has a DNA content somewhat intermediate among other taxa of the same chromosome number (table 2). It seems unlikely that such a large genome could have combined with others to form the Moroccan polyploids without subsequent changes in DNA content.

Thus, the separation of the Moroccan from the European taxa provides a possible explanation of some of the discrepancies between the DNA contents and the accepted evolutionary pathways. Interestingly, the two groups comply with the cline of increasing DNA content with increasing latitude known to exist among many crop species (Bennett, 1976).

3. The DNA contents of the ancestral species may have changed relative to those of their derived polyploids since their divergence—the diploids having gained DNA or the polyploids having lost it. Two of the three Moroccan fescues are representatives of the highest polyploids in the genus. Perhaps their high chromosome numbers rather than their geographical distribution are related to the discrepancy between observed and expected DNA amounts. Such a relationship could be tested by matching the DNA contents of high polyploids from Europe with their proposed genomic constitutions when these become available. If these polyploids also show unexpectedly low DNA contents then one would conclude that upward changes in ploidy level may be accompanied or followed by loss of DNA. DNA diminution in polyploids has been proposed before (Pai *et al.*, 1961; Yamaguchi and Tsunoda, 1969) but seldom confirmed (Rees and Walters, 1965; Verma and Rees, 1974). Alternatively, it is conceivable that the ancestral diploid genomes have differentially increased in DNA content since the formation of the polyploids.

If the "homoeologous" genomes of species of different ploidy levels differed in DNA content (and therefore size) one might expect to find heteromorphic bivalents in their F_1 hybrids at meiosis. Malik and Thomas (1967) noted the occurrence of such bivalents in hybrids of *F. pratensis* (2x) × *F. glaucescens* (4x), *F. glaucescens* × *F. arundinacea* (6x), and *F. arundinacea* (6x) × the decaploid. Heteromorphic bivalents could, of

course, arise without an accompanying change in genomic DNA content by translocation or by intragenomic pairing—a common feature in such hybrids (Malik and Thomas, 1967). However, changes in DNA content among polyploid species seem possible—even probable—when one considers the variation found among diploids. Changes in DNA amount between diploid species are undeniable. Moreover, they are of common occurrence and often of substantial size. *F. drymeja* and *F. scariosa*, for example, differ in nuclear DNA content by about 50 per cent and yet are sufficiently closely related to produce viable F_1 hybrids (Borrill *et al.*, 1977). Thus, massive differences in genomic DNA content do not necessarily indicate extensive evolutionary divergence of two species. Moreover, since large-scale DNA variation is often found between closely related species the changes can, in evolutionary terms, occur rapidly. There is no reason why the genomes of polyploids should be immune from such changes. Indeed in view of the inevitable duplication of many DNA sequences in polyploids, they may be more tolerant of and, therefore, more prone to variation in DNA content than diploids (Jackson and Casey, 1980).

It seems then that the DNA content of a genome is not immutable and so its value in tracing the ancestry of polyploids is somewhat diminished. However, the correspondence of taxonomic divisions with the distribution of nuclear DNA contents in fig. 2 shows that the extent of divergence in DNA content may be a useful indication of the extent of evolutionary divergence among diploid species.

Acknowledgements. Thanks are due to Professor H. Rees, Dr M. Borrill, and Dr H. Thomas for their helpful comments and advice. This work was funded by an SRC studentship.

4. REFERENCES

- BENNETT, M. D. 1976. DNA amount, latitude, and crop plant distribution. *Environ. and Exp. Bot.*, **16**, 93–108.
- BORRILL, M. 1972. Studies in *Festuca*. III. The contribution of *F. scariosa* to the evolution of polyploids in sections Bovinae and Scariosae. *New Phytol.*, **71**, 523–532.
- BORRILL, M., KIRBY, M. AND MORGAN, W. G. 1977. Studies in *Festuca* 11. Interrelationships of some putative diploid ancestors of the polyploid broad-leaved fescues. *New Phytol.*, **78**, 661–674.
- BOWMAN, J. G. AND THOMAS, H. 1976. Studies in *Festuca*. 8. Cytological relationships between *F. glaucescens* ($2n = 28$), *F. mairei* ($2n = 28$) and *F. scariosa* ($2n = 14$). *Z. Pflanzenzüchtg.*, **76**, 250–257.
- CHANDRASEKHARAN, P. AND THOMAS, H. 1971a. Studies in *Festuca*. 5. Cytogenetic relationships between species of Bovinae and Scariosae. *Z. Pflanzenzüchtg.*, **65**, 345–354.
- CHANDRASEKHARAN, P. AND THOMAS, H. 1971b. Studies in *Festuca*. 6. Chromosome relationships between Bovinae and Scariosae. *Z. Pflanzenzüchtg.*, **66**, 76–86.
- CHANDRASEKHARAN, P., LEWIS, E. J. AND BORRILL, M. 1972. Studies in *Festuca*. II. Fertility relationships between species of sections Bovinae and Scariosae, and their affinities with *Lolium*. *Genetica*, **43**, 375–386.
- HACKEL, E. 1882. *Monographia Festucarum europaeorum*. Fischer, Kassel and Berlin. Reprinted by Micro Methods Ltd and Johnson Reprint Corp. 1964, Wakefield and New York.
- HUTCHINSON, J., REES, H. AND SEAL, A. G. 1979. An assay of the activity of supplementary DNA in *Lolium*. *Heredity*, **43**, 411–421.
- JACKSON, R. C. AND CASEY, J. 1980. Cytogenetics of polyploids. In Lewis, W. H. (ed.) *Polyploidy: Biological Relevance*, Plenum Press, New York and London, pp. 17–44.
- JONES, R. N. 1975. B-chromosome systems in flowering plants and animal species. *Int. Rev. Cytol.*, **40**, 1–100.
- JONES, R. N. AND REES, H. 1968. Nuclear DNA variation in *Allium*. *Heredity*, **23**, 591–605.

- MALIK, C. P. AND THOMAS, P. T. 1966. Karyotypic studies in some *Lolium* and *Festuca* species. *Caryologia*, *19*, 167-196.
- MALIK, C. P. AND THOMAS, P. T. 1967. Cytological relationships and genome structure of some *Festuca* species. *Caryologia*, *20*, 1-39.
- MITTWOCH, U. 1969. Scope and limitations of Feulgen microdensitometry. *Chromosomes Today*, *2*, 90-98.
- PAI, R. A., UPADHYA, M. D., BHASKAVAN, S. AND SWAMINATHAN, M. S. 1961. Chromosome diminution and evolution of polyploid species in *Triticum*. *Chromosoma*, *12*, 398-409.
- REES, H. AND JONES, G. H. 1967. Chromosome evolution in *Lolium*. *Heredity*, *22*, 1-18.
- REES, H. AND WALTERS, M. R. 1965. Nuclear DNA and the evolution of wheat. *Heredity*, *20*, 73-82.
- SEAL, A. G. AND REES, H. 1982. The distribution of quantitative DNA changes associated with the evolution of diploid *Festuceae*. *Heredity*, *49*, 181-192.
- VANT HOF, J. 1965. Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. *Expl. Cell Res.*, *39*, 48-58.
- VERMA, S. C. AND REES, H. 1974. Nuclear DNA and the evolution of allotetraploid Brassicaceae. *Heredity*, *33*, 61-68.
- YAMAGUCHI, Y. AND TSUNODA, S. 1969. Nuclear volume, nuclear DNA content and radiosensitivity in *Brassica* and allied genera. *Japan J. P. Breeding*, *19*, 350-356.