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NUCLEAR DNA, MITOTIC PHASES, AND SPECIES RELATIONSHIPS IN AVENA

by

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Candidate

Supervisor

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ABSTRACT

Microspectrophotometric measurements of nuclear DNA of various species of oats revealed substantial differences among the diploid species. The karyotypes had been divided into two distinct groups, A and C, on the basis of cytogenetic studies, and this was confirmed by the microspectrophotometric data. The tetraploid Avena barbata, with the genome AABB, has a lower DNA content than does autotetraploid A. strigosa (AAAA). This suggests that tetraploid oats originated via segmental allotetraploidy rather than via autotetraploidy. The DNA content of the B genome was about 20% lower than that of the A genome. This may serve as a marker in the search for the potential donor of the B genome. Autoradiographic studies of the average durations of mitotic phases in the root tip cells of three oats species with different amounts of nuclear DNA showed that the DNA synthetic phase (S) of diploid and autotetraploid strigosa were similar, suggesting that homologous genomes in Avena species replicate in synchrony. It is proposed that the origin of certain genomes in polyploid oats might be resolved by comparing the replication patterns of chromosomes between the diploid and the polyploid species.

Résumé

Des mesures microspectrophotométriques de l'ADN de quelques espèces d'avoine ont révélé d'importantes différences entre les espèces diploides. Les caryotypes ont été divisés en deux groupes distincts, A et C, sur la base des études cytogénétiques et cette division fut confirmée par les données microspectrophotométriques. L'Avena barbata tétraploide, avec le génome AABB, contient moins d'ADN que l'Avena strigosa autotétraploide, AAAA. Cela suggère que les avoines tétraploides sont d'origine allotétraploide segmentale plutôt qu'autotétraploide. Le contenu du génome B en ADN était de vingt pour cent inférieur à celui du génome A. Cette différence peut servir dans la recherche du donateur potentiel du génome B. La durée moyenne des phases mitotiques dans les cellules des bouts de racines était mesurée par des méthodes autoradiographiques sur trois espèces d'avoines contenant des quantités différentes d'ADN. Ces études ont démontré que la phase de synthèse de l'ADN (S) en A. strigosa diploide et autotétraploide est semblable, suggérant que la reproduction des génomes homologues des espèces d'avoines soit synchrone. Il est proposé que l'origine de certains génomes dans les avoines polyploides peut être déterminée en comparant la reproduction des dessins de chromosomes des espèces diploides et polyploides.

PART ONE

RELATIVE AMOUNTS OF NUCLEAR DNA AND RELATIONSHIPS OF *AVENA* SPECIES

Introduction

The origin of cultivated oats, Avena sativa, the fourth most important crop in the world (Stanton 1953) is not as well understood as that of the other important cultivated crop species such as wheat, cotton, maize and tobacco. Many aspects of the evolutionary pathways in oats are still obscure. The search for the origins of the important crops, on which man depends for his food, is of great theoretical interest, and it can also facilitate higher efficiency in crop breeding. In order to accomplish this, it is necessary to study the relationships of species in the genus.

Cosson (1854) and Thellung (1911) were the earliest to classify the species of Avena according to their morphological characters. Chromosome number was applied in taxonomical studies in the twenties (Maltzew 1930). Nishiyama (1929, 1934 and 1936) analyzed genome relationships in different species in the genus on the basis of chromosome pairing in interspecific hybrids. Unfortunately this work was not continued for nearly two decades after 1940 apart from sporadic efforts. Concerted efforts were resumed when Rajhathy and Morrison published their paper on chromosome morphology in the genus Avena in 1959. This apparently has given new impetus to cytogenetic research in Avena.

The main tool of conventional genome analysis is the pairing behaviour of chromosome in interspecific hybrids.

However, this technique provides limited information, especially when fine structural changes are involved. Homology between genomes of related species is reflected by the pairing pattern of meiosis. This can only be carried out with closely related species which are cross compatible. Incompatibility amongst many species have made such studies inapplicable. In addition, the pairing pattern of the chromosomes may be under genetic control (Riley and Chapman 1958). Asynaptic and diploidizing genes as well as those controlling chiasma formation may further complicate genome analysis. Karyotype analysis may provide further information. However, it can only detect major structural changes of the chromosomes; usually it is not sufficiently sensitive for detecting small changes which may have important genetical and evolutionary consequences.

The rapid advance of molecular genetics and cytology provided new techniques facilitating refinements of genome analysis. One of these is the study of the relative amounts of deoxyribonucleic acid (DNA) in related species. Hughes-Schrader (1951, 1953 and 1957) was one of the pioneers who studied the nuclear DNA content of mantid species from an evolutionary point of view. John and Hewitt (1966) compared the karyotypes and quantities of nuclear DNA of two groups of species of grasshopper (Acrididae), in which one group has 17 chromosomes, the other 23, but the chromosome arms in both groups number 23. They have found that significant

differences in DNA content exist within some of the species of both groups as well as between the groups; in the majority of species belonging to the 17 chromosome group, the DNA was higher than that of the 23 chromosome group. This, coupled with the existence of both acro- and telocentric elements in the chromosomes of these species led them to refute the generally claimed karyotypic stability within the acridids.

Based on the comparative studies of DNA values and on karyotypes in different species of fish and of some mammals, Ohno and her colleagues (Ohno and Atkin 1966, Ohno et al. 1967, and Ohno et al. 1968) have claimed that gene duplication, including polyploidization, has played an important role in the early evolution of vertebrates. Examination on nuclear DNA in wheat species was carried out by Rees and Walters (1965) to trace their ancestors. They obtained further evidence that Aegilops speltoides was the probable donor of the B genome for the tetraploid (AABB) and hexaploid (AABBDD) wheat species. These studies suggest that a comparison of the DNA content may reveal evolutionary changes that may pass undetected in conventional cytogenetic studies.

The aims of this study are first, to determine whether differences in nuclear DNA amounts are associated with the divergence of the Avena species at different ploidy levels; and second, to obtain more information about the species relationship in oats.

In order to gain a clear picture of our present knowledge about the species relationships in Avena, it is essential to survey briefly the literature.

The cytogenetical architecture of *Avena* species

The *Avena* species form a polyploid series which consists of diploids (2n = 2X = 14), tetraploids (2n = 4X = 28) and hexaploids (2n = 6X = 42). The basic number of the genus is X = 7. The species by chromosome number and genomes are listed in Table I (from Rajhathy).

1. Karyotypes

Rajhathy and Morrison (1959), Rajhathy and Dyck (1963) and Rajhathy and Thomas (1967) have studied the karyotypes of several diploid species in *Avena*. Chromosomes were classified by their arm ratios and relative lengths. Based on the position of centromere, chromosomes were defined as median, submedian and subterminal. The nucleolar chromosomes, having a satellite, were called satellited chromosomes. Since all the diploid species contain 7 pairs of chromosomes with some basic similarities, their chromosomes were designated as the A genome. On the basis of the differences the diploids were classified into four karyotype groups:

A) Karyotype A_s --- *A. hirtula* Lag., *A. wiestii* Steud. Thell., *A. strigosa* Schreb., *A. brevis* Roth. This karyotype is characterized by two satellited chromosomes, one having subterminal the other submedian centromeres, one with small satellite and the other with a large one; two median chromosomes, one of them being the largest in the complement;

Table 1

Common species in *Avena*

Ploidy	Diploids 2n=2x=14				Tetraploids 2n=4x=28		Hexaploids 2n=6x=42
Genomes / Floret separation	A_sA_s	A_lA_l	A_pA_p (C_pC_p)	A_vA_v (C_vC_v)	AABB	A?A?MM	AACCDD
Wild	*hirtula* *wiestii*	*longiglumis*	*clauda*		*barbata* *vaviloviana*		*fatua*
Semiwild			*pilosa*	*ventricosa*		*magna*	*sterilis*
Cultivated	*strigosa*				*abyssinica*		*sativa* *byzantina*

two submedians and one subterminal with a small short arm. The average short arm/long arm ratio in this group is 0.64 with a range from 0.33 to 1.00.

B) Karyotype A_1 --- A. longiglumis Dur. is the only species with this karyotype. This karyotype is a modification of A_s type. One of the satellite chromosomes is slightly longer than the corresponding one in A_s. There is no subterminal chromosome in this set. There are two submedian and three median ones. Chromosomes in this group are more isobrachial than those of A_s. The average short arm/long arm ratio is 0.74. The total length of the chromosomes of A. strigosa is reported (Rajhathy 1961) to be 17 arbitrary units shorter than that of A. longiglumis. This length is approximately equal to the longest chromosome arm in the strigosa set.

C) Karyotype $A_p(C_p)$ --- This group consists of A. pilosa M. Bieb. and A. clauda Dur. There are two satellited chromosomes similar to those in A_s. The other chromosomes are strongly heterobrachial with an average short arm/long arm ratio of 0.37.

D) Karyotype $A_v(C_v)$ --- A. ventricosa Bal. is the only species with this karyotype. This karyotype is even more heterobrachial than that of A_p, average arm ratio being 0.30. A distinctive feature of this karyotype is that it has only one satellited chromosome. There is also a short chromosome which is the least heterobrachial with an arm ratio of 0.50.

Rajhathy and Morrison (1959) found that the A_s set of chromosomes (A genome) is present in three tetraploid species (A. barbata Pott., A. aviloviana M., A. abyssinica Hochst). There is no major cytogenetic difference among these three species. The second genome is somewhat differentiated from the A set by morphological differences, so it has been designated B. Thus, the karyotype of these tetraploid species is AABB.

The designation of the hexaploid karyotype of A. sativa L. is AACCDD (Rajhathy and Morrison 1959, Rajhathy 1963), since the A_s set is found in the complement but not the B set, and since the other 28 chromosomes do not appear to be duplicates of any other diploid set, they were designated as C and D.

2. Chromosome pairing

A) Diploid hybrids

The analysis of chromosome pairing in the F_1 hybrids of A. strigosa and A. hirtula confirmed that they have homologous genomes as indicated by previous karyotype studies. Holden (1966) observed 7 bivalents in the F_1 hybrid of A. strigosa and A. hirtula. Rajhathy (1961), who studied the F_1s of A. longiglumis x A. strigosa and A. longiglumis x A. hirtula found the same frequencies of synaptic configurations in these two hybrids. Slight structural differentiation between the chromosomes of strigosa and hirtula was reported by Ellison (1940) who found that 1% of the pollen mother cells of their F_1 hybrids had 6 bivalents and 2 univalents.

Basic homology between the chromosomes of A. longiglumis and those of A_s karyotype was shown by the high amount of pairing (very low frequency of univalents), but there were structural differences for large segments as shown by long chains (Thomas and Jones 1965, Rajhathy 1961, Holden 1966). For example, Holden (1966) found only 6.2% of the chromosomes were bivalents while 93% were trivalents or larger associations in the F_1 hybrids of A. longiglumis and five other diploid species. In each hybrid, the chromosome association ranged from univalents to associations of 8, and the number of associations of different sizes were uniformly distributed in each hybrid. This indicates that members of the A_s group of diploid species have a homologous genome, while the chromosomes of A. longiglumis are structurally differentiated from A_s. It is suggested that at least six chromosomes of A. longiglumis are involved in major translocations (Thomas and Jones 1965).

Structural differentiations were also found between the chromosomes of the A_p and A_v genomes as was shown in the hybrids of A. pilosa and A. ventricosa. In these hybrids, a high frequency of trivalents and univalents, with some quadrivalents, was observed (Rajhathy and Thomas 1967).

It has been impossible to compare directly the chromosomal structures of A. pilosa and A. ventricosa with those of A. strigosa and A. longiglumis by chromosomal pairing thus far, because of the apparent cross-incompatibility (Rajhathy and Thomas 1967).

B) Triploid hybrids

Nishiyama (1929) had studied chromosome pairing in the F_1 hybrids of A. barbata x A. strigosa and observed that 38% of the cells contained $7_{II} + 7_I$, 17% $8_{II} + 5_I$ and 2 cells with $9_{II} + 3_I$. In his later studies (Nishiyama 1936) in the F_1 of an induced autotetraploid strigosa x A. barbata, he frequently observed 12 bivalents and sometimes 13. The number of univalents in most cells was one or two. He concluded that the genome of strigosa was completely homologous to one of the genomes in A. barbata and partially homologous to the other.

In F_1 hybrids of A. barbata x A. hirtula, Holden (1966) found that only 6% of the cells contained $7_{II} + 7_I$, 4% $8_{II} + 5_I$, while the rest of the cells contained one or more trivalents or more complex configurations. A mean frequency of trivalents of 1.9% per cell, while a maximum of five trivalents was obtained.

Holden's results have been confirmed recently by Sadasivaiah and Rajhathy (1968) in a more detailed study. They found that in the triploid hybrid (A_sAB) between A_s genome diploid species (A. hirtula or A. strigosa) and tetraploid Avena (A. barbata or A. abyssinica) more than 90% of the cells contained trivalents, 12% having 4 or more, with a maximum of 6. The most complex association of chromosomes was a chain of 12. When the tetraploid hybrid of autotetraploid A. strigosa and A. abyssinica (A_sA_sAB) was compared

with the triploid hybrid (A_sAB), the mean frequency of trivalents in the former was lower, but the frequency of quadrivalents was doubled. This led Sadasivaiah and Rajhathy to conclude that A genomes are homologous and that a homoeologous relationship exists between the A and B genome chromosomes; following Huskins (1931) the term "homoeologous" meaning "similar" has been applied to the relationship between non-partner chromosomes in the same group.

These results are of great significance in regard to the origin of the B genome in tetraploid oats, because the homoeologous relationship between A and B indicates that they originated from a common genome or from closely related genomes. The recent study of Ladizinsky and Zohary (1968) suggested that tetraploid oats were of autotetraploid origin. Their evidences were based on (1) the great number of aneuploids present among the viable progeny of the triploid hybrids of A. barbata x A. strigosa and (2) a great morphological overlap between diploid and tetraploid species.

An autotetraploid origin would imply that tetraploid oats originated by chromosome doubling of a diploid species. However, Holden (1966) and Sadasivaiah and Rajhathy (1968) proposed a segmental allopoplyploid origin for tetraploid oats. A segmental allopolyploid as first defined by Stebbins (1947) was "an allopolyploid of which the component genomes bears the majority of their chromosomal segments in common". Thus, the first tetraploid oats may have originated from

the hybridization of two closely related forms followed by doubling of the chromosomes.

C) Tetraploid hybrids

The meiotic behaviour in the tetraploid F_1 hybrids of A. sativa x A. strigosa was reported by Kihara and Nishiyama (1932) and by Marshall and Myers (1961). The number of bivalents observed by the former authors ranged from 3 to 9 with the mode being 7. In the latter's studies they found that the majority of cells contained 5 to 7 bivalents. The number of trivalents was low. Ellison (1940) also reported that in the F_1 of A. sativa x A. longiglumis, there were at least 7 bivalents in each cell. These three reports support the conclusions made by karyotype analysis (Rajhathy and Morrison 1959, Rajhathy 1963), that genome A is present in A. sativa. The different frequencies of bivalents observed in these two combinations could be just a reflection of the structural differences between the chromosomes of strigosa and longiglumis.

The genome of A. pilosa was reported partially homologous to one genome in A. sativa, since in the F_1 hybrid of pilosa x sativa, a mean chromosome pairing of $19.76_{I} + 4.12_{II} + 0.02_{I}$ per cell was observed (Thomas and Rajhathy 1967). This average, however, was lower than those found in both F_1 hybrids of strigosa-sativa (Marshall and Myer 1961) and of hirtula-sativa (Swami and Thomas 1966). It is not known yet which genome in A. sativa is partially homologous to that

of pilosa. From the studies above, it was concluded that the genome of pilosa (A_p) is more differentiated from its corresponding genome, than the genome of strigosa or hirtula (A_s) from A genome in sativa.

D) Pentaploid hybrids

Pentaploid hybrids of A. barbata x A. fatua L. and A. barbata x A. sterilis L. were studied by Nishiyama (1929). In the F_1 of the former cross, 2 to 11 bivalents including 1 to 4 trivalents were found, while in the latter 7 to 13 bivalents with 0 to 4 trivalents were found. Emme (1932) found 7 to 9 trivalents in the F_1 of A. sativa x A. abyssinica and A. sativa x A. barbata. Similar results were reported in the F_1 of A. sterilis x A. abyssinica which showed 5 to 11 bivalents with 23 to 13 univalents per cell. Trivalents were also occasionally found. The fact that more than 7 bivalents were usually observed in pentaploid hybrid cells indicates that a completely homologous genome (A) is present in both tetraploid and hexaploid species. The presence of trivalents suggests a partial homology between A and B genomes.

3. The hypothesis for the origin of the C genome of hexaploid Avena.

The origins of C and D genomes in hexaploid Avena are still not known. However, Rajhathy (1966) has advanced the hypothesis that A. ventricosa could be the donor of the C genome in hexaploid oats. The evidences he provided were

mainly from karyotype analysis and chromosome pairing studies, substantiated by genetic, morphological, and geographical findings. From the chromosome pairing studies in the F_1 of A. pilosa x A. sativa (A_pACD) and A. hirtula-sativa x A. pilosa-sativa, Thomas and Rajhathy (1967) suggested that pilosa and strigosa genomes may not be homologous to the same genome in sativa. This speculation could gain support from the cross-compatibility results which show that ventricosa-pilosa are not cross-compatible with strigosa-longiglumis, nevertheless, the F_1s of pilosa x sativa and strigosa x sativa were easily obtainable. This may indicate that the two have different homologies in the sativa complement.

More evidence is from karyotype analysis. According to the standard idiogram of sativa (Rajhathy 1963), there are seven pairs of subterminal chromosomes in the sativa complement; one pair belongs to the A genome and the other six would be from C and D genomes. Since A. ventricosa and A. pilosa both have five pairs of subterminal chromosomes, they are both potential donors of one of the unknown genomes. However, the satellited chromosome (SAT) of A. ventricosa is very similar to the only SAT chromosome within the CD karyotypes of sativa; in addition, the short nearly submedian chromosome of ventricosa is considered to be the same as the second SAT chromosome of pilosa minus the satellite (Rajhathy and Thomas 1967) and this chromosome appears to be very similar to the SM_{15} (submedian chromosome) in the standard idio-

gram of sativa. Based on these similarities between the chromosomes of A. ventricosa and of CD genomes in A. sativa, A. ventricosa is considered to be the most probable donor of the C genome in hexaploid oats.

The assumption that SM_{15} in A. sativa was evolved from the presumed second satellited chromosomes of A. ventricosa by losing its satellite was supported by the findings of McGinnis (1966) who suggested that SAT_2 and SM_{15} in A. sativa might be homoeologous. These findings seem to indicate the possibility of A. ventricosa being the donor of the C genome in hexaploid oats. However, this still remain a hypothesis until proved by more direct evidence.

In summary, great structural differentiations exist among the different genomes of Avena. At least four karyotypes have been observed in the diploids, i.e. A_s ---- A. strigosa; A_l --- A. longiglumis; $A_p(C_p)$ --- A. pilosa and $A_v(C_v)$ --- A. ventricosa. Closer affinity was found within A_s --- A_l and A_v --- A_p than between them. One A genome is present in both tetraploid species (AABB) and hexaploid species (AACCDD). There are strong indications that the A genome is partially homologous to the B genome. The origin of tetraploid oats is more likely to be autotetraploidy or segmental allotetraploidy than amphidiploidy. It is not certain yet whether the tetraploid species evolved through one step, i.e. by doubling of the chromosome of one species or by two steps, i.e. hybridization of two related varieties

followed by chromosome doubling. Further investigations are needed to find the donor of B genome. The origin of C and D genomes in A. sativa are not known, however, indirect evidences suggest that A. ventricosa could be the donor of C.

Materials and Methods

1. Plant materials

The species used in this study are listed in Table II.

Table II

Species of *Avena* used in the studies of nuclear DNA content

	Chromosome No. 2n	Genomes	Origin
Diploids			
A. hirtula	14	A_sA_s	Aberystwyth, U.K.
A. strigosa	14	A_sA_s	La Estanzuela, Uruguay
A. longiglumis	14	A_lA_l	Israel
A. pilosa	14	A_pA_p	Cyprus
A. ventricosa	14	A_vA_v	Oran, Algeria
Tetraploids			
A. barbata	28	AABB	Cambridge, U.K.
A. strigosa	28	$A_sA_sA_sA_s$	Ottawa, Canada
Hexaploids			
A. sativa	42	AACCDD	Cv. Garry, Canada
Amphiploid			
A. hirtula-sativa	56	A_sA_sAACCDD	Ottawa, Canada

2. Preparation of slides

Nine seeds were germinated on a moist Whatman No. 1 filter paper in 9 cm Petri dishes in the dark at 25° ± 1°C. After 48 to 60 hours (depending on the species used), when the roots reached a length of about 15 mm, 10 mm segments of the tips were cut off and fixed in acetic alcohol (1:3) for 1 hour at room temperature. After fixation, the roots were washed for 15 minutes in three changes of 95% alcohol, passed through 80% alcohol for 5 minutes and stored in 70% alcohol for usually less than 2 days.

Before the roots were stained, they were moved through 50% and 30% alcohol to water, then hydrolyzed in 1N HCl at 60°C for twelve minutes, rinsed in distilled water and stained in Feulgen reagent at pH 1.4 for 2 hours at 20°C in the dark. The Feulgen reagent was prepared by the method of Johansen (1940). The stained root tips were washed for 15 minutes in three changes of SO_2 water at pH 1.7 and then rinsed in distilled water. The stained root tip was placed on a thoroughly cleaned slide. The root cap, which usually showed no cells in division, was cut off for about 0.5 mm in length, and the next 1 mm of meristematic tissue was used for the squash preparation. A drop of 45% acetic acid was added and covered by a clean cover glass. Gentle tapping by the eraser-tipped end of a pencil was applied to the coverglass to loosen the cells. The slide was then pressed by the thumb under a few layers of papers. Slides were made permanent by the quick freezing method (Conger and Fairchild 1953) and mounted with Permount.

3. The validity of Feulgen photometry for quantitative determination of DNA

The Feulgen nuclear reaction has been accepted for many years as a specific test for localizing DNA *in situ*. Nevertheless, from time to time its validity was questioned (Carr 1945, Stedman and Stedman 1950, Pearse 1960). However, these objections were refuted by later workers (Dodson 1946, Kasten 1956, and cf. Sharma and Sharma 1965). Convincing evidence for the specificity of the Feulgen test for DNA was shown by the work of Brachet (1947) who tested nuclei with thymonucleodepolymerase, which removed DNA, and found the Feulgen reaction became negative for those nuclei. Catcheside and Holmes (1947) confirmed these results by showing that the Feulgen positive bands in the salivary gland chromosome of *Drosophila* could be removed by deoxyribonuclease.

Ris and Mirsky (1949) have gone further to prove that the Feulgen nucleal reaction can be used as a quantitative cytochemical procedure for DNA. They found that under carefully controlled conditions the color intensity produced by the Feulgen nucleal reaction was proportional to the quantity of the DNA in the nuclei of several types of animal cells. The similar applicability of Feulgen photometry for plant material was shown by McLeish and Sunderland (1961). Hence, in recent years abundant results on the quantity of nuclear DNA have been reported using Feulgen photometry as a basic method.

4. Microspectrophotometry

The apparatus used for making microspectrophotometric measurements was designed by Dr. George Setterfield (Department of Biology, Carleton University, Ottawa) according to the general specifications of Swift and Rash (1956). A movable circular metal disc with fixed apertures was mounted beneath the photocell so that the diameter of the light beam reaching the photocell could be controlled accurately.

The two wavelength method (Patau 1953) was used for measurements throughout. This method has been shown to be the most effective in correcting distributional errors which are caused by nonuniform dye distributions in objects such as mitotic and meiotic nuclei. Other common errors caused by stray light and wavelength can also be controlled (Patau 1953, and Patau and Swift 1953).

The Feulgen-stained slides were immersed in xylol for a few hours to dissolve the Permount. In order to obtain a homogeneous background and to eliminate all light scattering in the specimen, a new mount medium (from Cargille) with the refractive index matching that of the cytoplasm of the cells was substituted. Thus only the stained nuclei could be seen through the microscope.

A homogeneously stained interphase nucleus was selected and an extinction curve was drawn throughout the range from 450 mμ to 570 mμ (Figure 1). The two wavelengths λ_1 and λ_2 were carefully chosen at the most linear section of the as-

Figure 1

Extinction curve of Feulgen stained nucleus of Avena strigosa 2x.

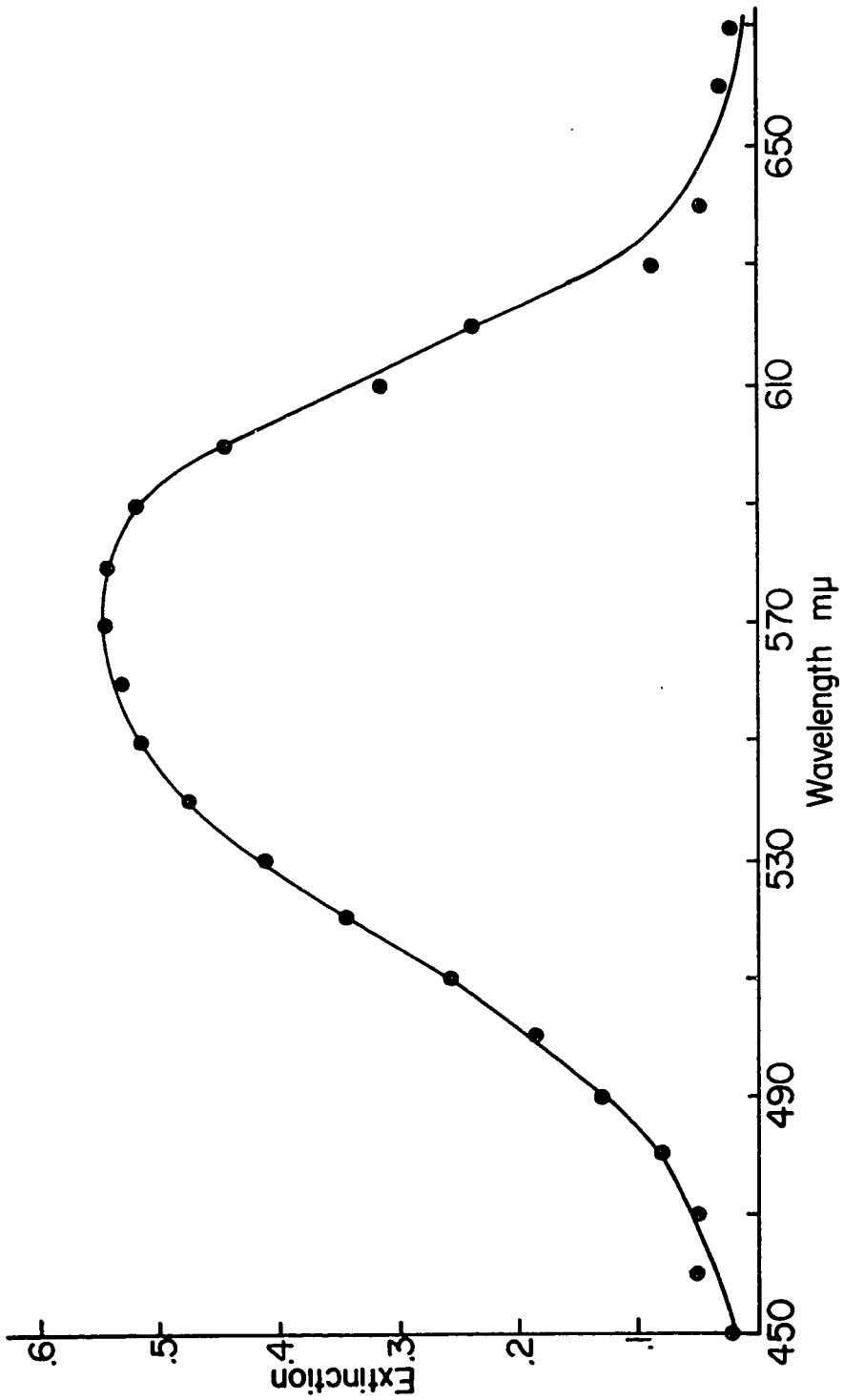


Figure 1

cending slope so that the extinction (E_1) at one wavelength is half the extinction of the other (E_2), thus $2E_1 = E_2$. The wavelengths chosen were λ_1 = 500 mμ and λ_2 = 530 ± 2 mμ, with a few exceptions in the first few experiments.

The above procedures were carried out on every slide measured. Extinction curves were compared to the standard curve of diploid A. strigosa. Most slides gave similar extinction curves. A few slides showing different shapes from the standard were not used. Since the condition of hydrolysis may alter the shape of the Feulgen absorption curve, thus changing the dye intensity, this procedure has assured that all slides measured were in a comparable condition.

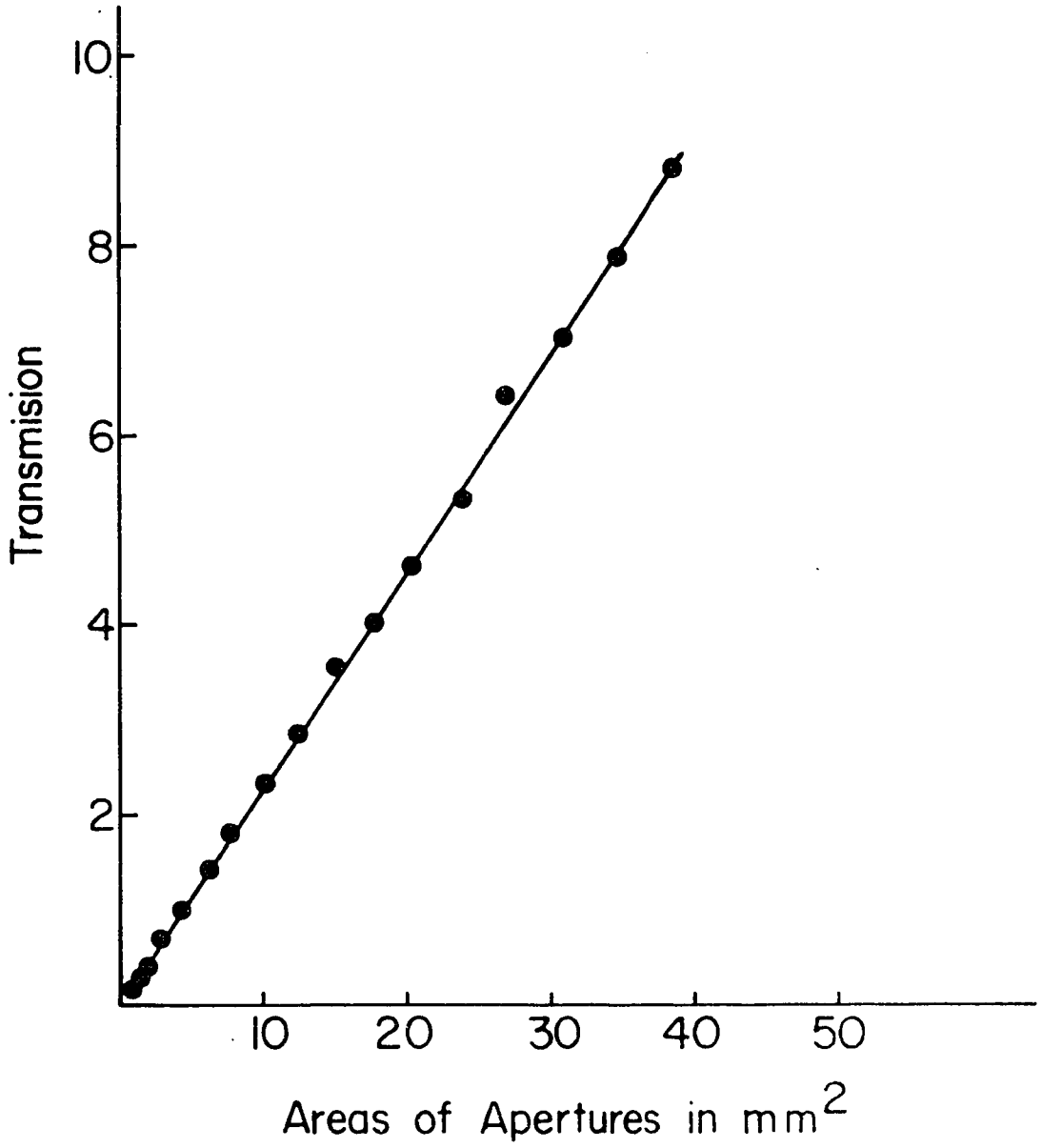
To avoid the errors of instrumentation, the alignment of the various parts of the microspectrophotometer was carefully checked at the beginning of each working day. The linear relationship between the areas of the apertures on the circular disc beneath the phototube and their transmissions was assured. This was done by chosing a clear background and measuring the transmissions of the various apertures at 520 mμ (Figure 2).

The constancy for the performance of the machine was assured by testing a standard slide which had been measured before. Two prophases (P) and two half telophases (T), i.e. one end of a telophase were measured again, the results should be similar to the previous record and should not differ by more than 5% and the values of Ps should be close to twice the values of Ts.

Figure 2

The linear relationship between the measured area and transmission

Figure 2



Extreme care was taken to minimize any error that may be caused by measuring technique. The sequence for measuring a subject was: λ_1 -subject, λ_2 - subject, λ_2 - blank and λ_1 - blank. The blank was always chosen at a spot on the slide as close as possible to the subject. The exit slit of the monochromator was set between 0.03 to 0.06 mm, depending on the size of the nucleus measured.

For testing the reproducability of the data, one nucleus was measured ten times and the mean and standard error obtained were 2.2262 ± 0.0085. The standard error was considerably low and hence was neglected.

5. Experimental design and statistical methods

Completely randomized block design was applied for comparing the relative amounts of nuclear DNA among different species. Each slide was taken as a subunit within a block. The estimates for the relative nuclear DNA content in each species were repeated two to four times. The different experiments carried out at different times were taken as blocks in the analysis of variance.

The DNA content of each nucleus was calculated according to the tables of Mendelsohn (1958) for two wavelength method microspectrophotometry. An arbitrary unit was used for relative comparisons. On most slides at least 10 half telophase (2C) and 10 prophase (4C) nuclei were measured to give the estimates of 2C and 4C values of DNA; 2C was designated for the DNA value of a nucleus before DNA synthesis and 4C for the value after DNA doubling.

The method of unweighted means for disproportionate subclass number was used in the analysis of variance (Snedecor and Cochran 1967), since different numbers of nuclei were measured on each slide. When the F test was significant, the differences among means were located by the method of Duncan's multiple-range test (Duncan 1955).

Experimental results

As already stated in the introduction, one of the purposes of this study is to look for more information about the relationships among the species of Avena. The genus Avena consists of a polyploid series including diploids, tetraploids and hexaploids. Polyploidization, no doubt, has played an important role in the origin of these species. Polyploidization involves hybridization between related species and/or doubling of chromosome of a single species (cf. Dodson 1960). When the origin of a polyploid species is studied on the basis of amounts of nuclear DNA of its tentative diploid donors, it is essential that no quantitative change in nuclear DNA per genome should result from either hybridization or doubling of chromosome per se. Otherwise, it would not be possible to identify the origin of a particular genome in a polyploid species by comparing the amounts of DNA in different species. Hence the following two series of experiments were carried out to test these prerequisites.

1. A. strigosa diploid compared with its autotetraploid

The values of nuclear DNA contents of 2C nuclei of A. strigosa 2x and its autotetraploid (4x) are shown in Table III. The mean values obtained from more than one hundred cells in four repeated experiments show that the value of the autotetraploid is very close to twice that of the diploid, the average DNA value or the autotetraploid

nucleus being 4.239 and of diploid 2.110. This result has also been verified by testing the homogeneity of variances. When the average value of each slide of the diploid strigosa was doubled and their variance was compared with the variance of the samples from the autotetraploid, no significant difference was found. This shows that there is no change in the DNA amount per genome subsequent to the doubling of the chromosomes.

2. Nuclear DNA in A. hirtula, A. sativa and A. hirtula-sativa amphiploid.

A. hirtula-sativa amphiploid is a colchicine doubled F_1 hybrid of A. hirtula and A. sativa. Its genome, according to the conventional designation, should be $A_sA_sAACCDD$. If the amount of nuclear DNA per genome does not change as a consequence of hybridization and chromosome doubling, the nuclear DNA value of the hirtula-sativa amphiploid would be expected to be equal to the sum of A. hirtula and A. sativa. The value of A. hirtula-sativa is 9.2956 while the sum of A. hirtula and A. sativa is 9.2838. Thus, the expected and observed values fit very well (Table IV).

The results of the above two series of experiments have proved that there is no appreciable change in nuclear DNA in each genome in oats species after hybridization nor after chromosome doubling. This also indicates that the technique used here is sufficiently accurate for the purpose of this study.

Table III

DNA values (in arbitrary units) from 2C root tip nuclei in A. strigosa 2x and its autotetraploid.

Experiments	A. strigosa 2x		A. strigosa 4x	
	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.
Series I				
Slide 1	15	2.1077±0.0632	20	4.0896±0.0916
Slide 2	12	1.9971±0.0435	16	3.9716±0.0655
Slide 3	--	---	20	4.2935±0.0925
Series II				
Slide 1	13	2.3022±0.0707	20	4.5094±0.0734
Slide 2	20	2.1846±0.0435	11	4.6254±0.0953
Series III				
Slide 1	16	2.1640±0.0624	16	4.5172±0.0943
Slide 2	17	2.0162±0.0489	17	4.1241±0.1289
Series IV				
Slide 1	17	1.9982±0.0331	11	4.0893±0.0920
Slide 2	--	---	10	3.9310±0.1816
Total No. of nuclei measured	110		141	
Species mean DNA per nucleus		2.1100		4.2390

Table IV

DNA values (in arbitrary units) from 2C nuclei in A. hirtula, A. sativa and A. hirtula-sativa amphiploid.

Experiment	A. hirtula		A. sativa		A. hirtula-sativa	
	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.
Slide 1	10	2.6100±0.1466	19	6.590±0.2679	11	9.6273±0.3803
Slide 2	10	2.6374±0.0877	13	6.730±0.2209	18	9.5042±0.3435
Slide 3	--	---	--	---	12	8.8478 0.1360
Slide 4	--	---	--	---	15	9.2032 0.2066
Total No. of nuclei measured	20		32		56	
Species mean DNA per nucleus		2.6237		6.6601		9.2956

Combined values of A. hirtula and A. sativa = 2.6237 + 6.6601 = 9.2838

3. A. hirtula compared with A. strigosa diploid

The DNA contents of 2C nuclei in A. hirtula and A. strigosa 2x were compared in two experiments. The data are shown in Table V and the analysis of variance in Table VI. There is no significant difference in nuclear DNA content between the two species. This was expected, since according to cytogenetical information A. strigosa and A. hirtula share the same genome, designated A_s.

One point should be clarified here. As can be observed from the analysis of variance, the Block F value is significant, indicating a difference between values obtained in the two experiments. This was because of the different wavelengths used in measuring the nuclei in the two experiments. However, in spite of this difference, the DNA values of the two species are similar and no significant difference was observed in either experiment. Apparently, the different wavelengths used did not affect the relation between the two species. This can also be observed from the nonsignificant F value for the interaction between block (experiment) and species. Since only relative values are concerned here, the results of the two experiments were analyzed together.

4. Comparisons of four diploid species --- A. strigosa, A. longiglumis, A. pilosa and A. ventricosa

The nuclear DNA content of the above four species were compared in three series of experiments. The results of 2C nuclei are shown in Table VII. There is a gradual increase in nuclear DNA content in the sequence of strigosa, longi-

Table V

Comparison of DNA values (in arbitrary units) of 2C nuclei between A. strigosa and A. hirtula.

Experiments	A. strigosa 2x		A. hirtula	
	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.
Series I				
Slide 1	10	2.4410±0.0574	10	2.6100±0.1466
Slide 2	12	2.4621±0.0500	10	2.6374±0.0877
Series II				
Slide 1	15	1.6637±0.0519	13	1.9183±0.0316
Slide 2	12	1.7688±0.0435	14	1.8872±0.0424
total No. of nuclei measured	49		47	
Species mean DNA per nucleus		2.0839		2.2632

Table VI

Analysis of variation of DNA variation between A. strigosa 2x and A. hirtula

Source of variation	Degree of freedom	Sum of squares	Mean squares	F. value
Species	1	0.01666810	0.01666810	4.035*
Blocks	1	0.04595484	0.04595484	11.130**
Bl x Sp	1	0.00184900	0.00184900	< 1
Error	8	0.03532481	0.00441560	

* Not significant

** Significant at 1% level; see text for explanation.

glumis, ventricosa and pilosa with average values of 1.8167, 1.9392, 2.1413 and 2.2413 respectively. The values of the first two as well as the last two species are very close. It seems that these species can be grouped into two classes by their nuclear DNA contents. The average value of pilosa and ventricosa are about 10 to 20 percent higher than those of strigosa and longiglumis.

Statistical analysis (Table VIII) shows that there is no significant difference between strigosa and longiglumis, nor between pilosa and ventricosa. The significant difference lies between the values of strigosa or longiglumis and those of ventricosa or pilosa (Duncan's test in Table VIII).

The data of 4C nuclei appear in Table IX and the analysis of variance and Duncan's test in Table X. The results are consistent with those found in 2C nuclei.

The distributions of the amount of nuclear DNA in 2C and 4C nuclei among the five diploid species are presented in the histograms (Figures 3 and 4). In both cases, the nuclei of hirtula, strigosa and longiglumis occupy the left side of the abscissa with lower DNA values and those of ventricosa and pilosa shift to the right with higher values. The first three species appear as one group, while the last two form another group.

These data have shown that variation of nuclear DNA exists among the diploid species of Avena. At least two distinct groups of species can be differentiated on the basis of the results obtained.

Table VII

DNA values (in arbitrary units) from 2C root tip nuclei in diploid species in Avena

Experiments	A. strigosa No. of nuclei	A. strigosa Mean ± S.E.	A. longiglumis No. of nuclei	A. longiglumis Mean ± S.E.	A. pilosa No. of nuclei	A. pilosa Mean ± S.E.	A. ventricosa No. of nuclei	A. ventricosa Mean ± S.E.
Series I								
Slide 1	15	1.8746±0.0458	12	1.7285±0.0728	16	2.2528±0.0556	14	2.0842±0.0469
Slide 2	12	1.6950±0.0447	12	1.9490±0.0517	13	2.3030±0.0282	14	2.1535±0.0670
Series II								
Slide 1	18	1.8997±0.0648	18	2.0095±0.0346	10	2.2104±0.0316	12	2.1410±0.0360
Slide 2	17	1.9982±0.0331	13	1.8634±0.0574	15	2.2573±0.0519	--	---
Series III								
Slide 1	15	1.6637±0.0519	14	2.0423±0.0412	15	2.2076±0.0360	23	2.1705±0.0400
Slide 2	12	1.7688±0.0435	--	---	--	---	--	---
Total No. of nuclei	89		69		69		63	
Species mean DNA per nucleus		1.8167		1.9392		2.2413		2.1413

TABLE VIII

Analysis of variance and Duncan's test of DNA variation of 2C nuclei among diploid species in Avena

Source of variation	Degree of freedom	Sum of squares	Mean squares	F. values
Species	3	0.33351261	0.11117087	14.6090**
Blocks	2	0.00748465	0.00374232	<1
Bl x Sp	6	0.04565697	0.00760949	1.3784
Error	8	0.04416400	0.0055205	

** Significant at 1% level.

Duncan's Test (5% level)*

Species	strigosa	longiglumis	ventricosa	pilosa
Mean	1.8167	1.9392	2.1435	2.2413

* Significant difference is found between means lying on different lines.

Table IX

DNA values (in arbitrary units) from 4C root tip nuclei in diploid species in Avena

Experiment	A. strigosa		A. longiglumis		A. pilosa		A. ventricosa	
	No. of nuclei	Mean ± S.E.	No. of nuclei	Mean ± S.E.	No. of nuclei	Mean ± S.E.	No. of nuclei	Mean ± S.E.
Series I								
Slide 1	15	3.8950±0.0965	10	3.6716±0.1077	10	4.7145±0.1039	9	4.2555±0.0734
Slide 2	13	3.6192±0.0707	10	3.8953±0.1667	11	4.5342±0.1204	15	4.4587±0.0860
Series II								
Slide 1	10	3.8133±0.0818	17	4.1118±0.0714	11	4.3546±0.0754	10	4.3925±0.1126
Slide 2	11	3.9040±0.0883	13	3.8262±0.0583	14	4.8159±0.1053	--	---
Series III								
Slide 1	8	3.7145±0.0714	10	4.1106±0.0761	13	4.4536±0.0721	18	4.5185±0.0547
Slide 2	11	3.2781±0.0500	--	---	--	---	--	---
Total No. of nuclei measured	68		60		59		52	
Species mean DNA per nucleus		3.7040		3.9544		4.5544		4.4277

Table X

Analysis of variance and Duncan's test of DNA variation of 4C nuclei among diploid species in Avena

Source of variation	Degree of freedom	Sum of squares	Mean squares	F. values
Species	3	1.42432388	0.47477462	17.7789*
Blocks	2	0.01123795	0.00561897	< 1
Bl x Sp	6	0.14287993	0.02381332	< 1
Error	8	0.23098072	0.02887259	

* Significant at 5% level.

Duncan's Test (5% level)*

Species	strigosa	longiglumis	ventricosa	pilosa
Mean	3.7040	3.9544	4.4277	4.5544

* Significant difference is found between means lying on different lines.

Figure 3

Histograms of distributions of DNA amounts estimated in 2C nuclei of diploid oats.

Figure 3

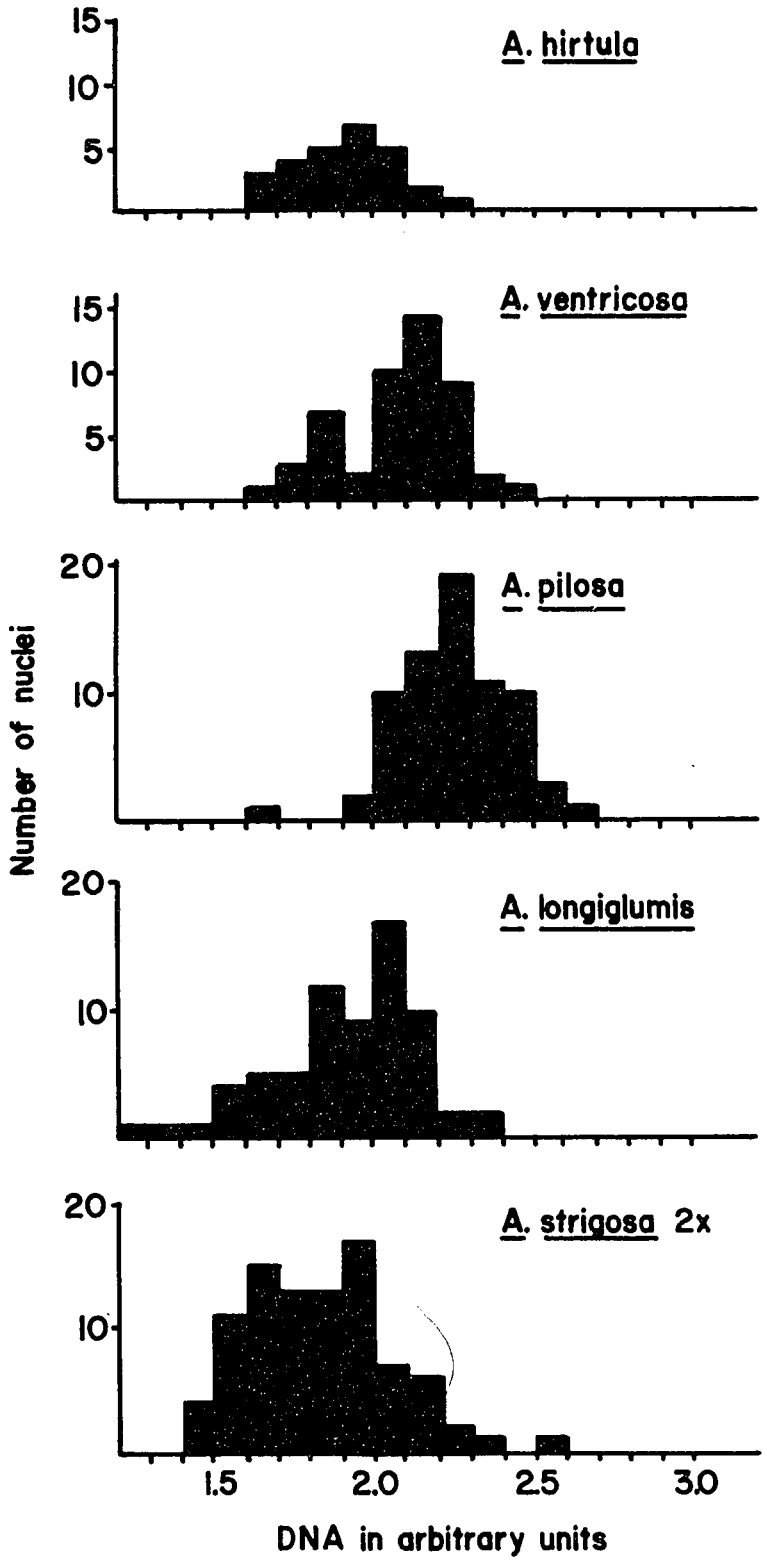
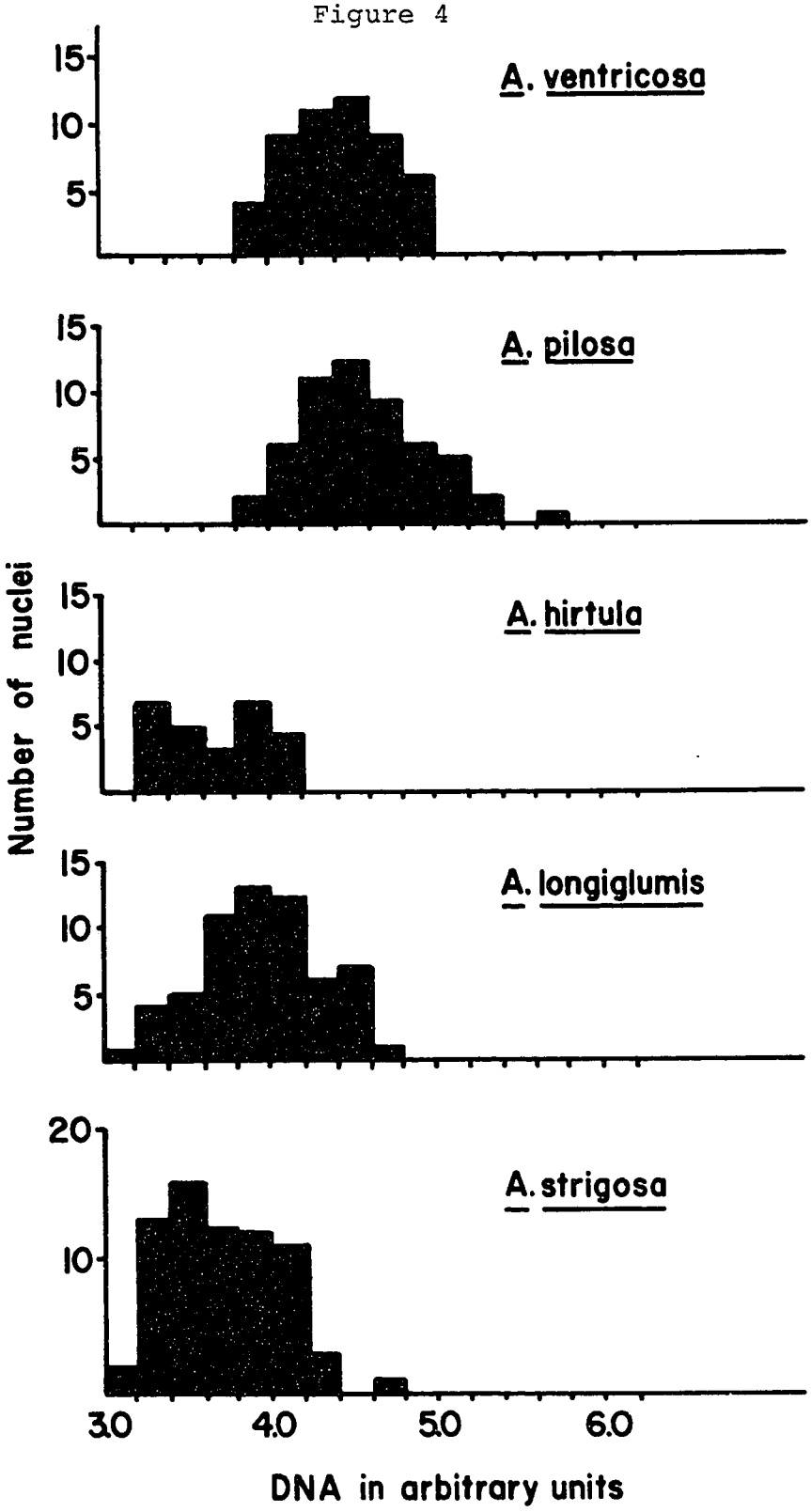

A. hirtula
A. ventricosa
A. pilosa
A. longiglumis
A. strigosa 2x
Number of nuclei
DNA in arbitrary units
1.5
2.0
2.5
3.0

Figure 4

Histograms of distributions of DNA amounts estimated in 4C nuclei in diploid oats.

Figure 4


A. ventricosa
A. pilosa
A. hirtula
A. longiglumis
A. strigosa
Number of nuclei
15
10
5
20
3.0
4.0
5.0
6.0
DNA in arbitrary units

5. The amount of nuclear DNA in tetraploid species

The relative amount of nuclear DNA of autotetraploid A. strigosa and A. barbata were compared in three repeated experiments. The results for 2C nuclei are shown in Table XI and for 4C nuclei in Table XII. The values from the first experiment are different from those of the other two. This is due to the different wavelengths used in the first experiment. Significant difference in the amount of nuclear DNA between the two species has been found in every experiment. As these are relative values, the results of the three experiments were combined.

In both types of nuclei, 2C and 4C, the average amounts of DNA in A. barbata is about 15% lower than that of autotetraploid strigosa. This difference is verified by the analysis of variance in which the species F values are highly significant ($P < 0.01$) (Tables XIII and XIV).

The distributions of DNA amounts in 2C nuclei of the two tetraploids and that of 4C nuclei of diploid strigosa are shown in Figure 5. It may be seen that the amount of DNA in 2C nuclei of A. barbata is lower, while the amounts in the 2C nuclei of autotetraploid strigosa and in the 4C nuclei of diploid strigosa are higher. The cells of the latter two forms, both containing two sets of strigosa genomes, should give the same result as shown in the diagram. The lower nuclear DNA value in A. barbata can also be observed in the histograms of distributions of DNA amounts in the 4C nuclei of the two tetraploid species as shown in Figure 6.

Table XI

Comparison of DNA values (in arbitrary units)of 2C nuclei between A. strigosa autotetraploid and A. barbata

Experiments	A. strigosa 4x		A. barbata	
	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.
Series I				
Slide 1	20	4.5094±0.0734	11	4.0378±0.1400
Slide 2	11	4.6254±0.0953	11	3.7549±0.1749
Series II				
Slide 1	13	3.6249±0.0700	17	3.2374±0.0478
Slide 2	15	3.5130±0.0608	20	2.0448±0.0489
Series III				
Slide 1	9	3.5837±0.0608	21	3.0618±0.0781
Slide 2	17	4.0064±0.0707	--	---
Total No. of nuclei measured	85		80	
Species mean DNA per nucleus		3.9772		3.3664

Table XII

Comparison of DNA values (in arbitrary units) of 4C nuclei between *A. strigosa* autotetraploid and *A. barbata*

Experiments	*A. strigosa* 4x		*A. barbata*	
	No. of nuclei measured	Mean ± S.E.	No. of nuclei measured	Mean ± S.E.
Series I				
Slide 1	8	9.4268±0.1403	9	7.8691±0.2310
Slide 2	11	9.1880±0.1726	11	7.6698±0.2553
Series II				
Slide 1	9	7.5785±0.0547	4	6.8247±0.1600
Slide 2	10	6.0971±0.1000	7	6.0861±0.1754
Series III				
Slide 1	5	7.6250±0.3855	15	6.5823±0.1216
Total No. of nuclei measured	43		46	
Species mean DNA per nucleus		8.0901		6.9357

Table XIII

Analysis of variance of DNA variation in 2C nuclei between *A. strigosa* autotetraploid and *A. barbata*

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F values
Species	1	0.5595	0.5595	32.7193**
Blocks	2	0.9465	0.4733	27.6780*
Bl x Sp	2	0.0260	0.0130	< 1
Error	5	0.0939	0.0188	

* Significant at 5% level.

** Significant at 1% level.

Table XIV

Analysis of variance of DNA variation in 4C nuclei between *A. strigosa* autotetraploid and *A. barbata*

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F. values
Species	1	2.00196420	2.00196420	29.668**
Blocks	2	3.19812811	1.59906405	23.697*
Bl x Sp	2	0.11353053	0.05676526	< 1
Error	4	0.29134062	0.07283515	

* Significant at 5% level.

** Significant at 1% level.

Figure 5

Histograms of distributions of DNA amounts in 2C nuclei of tetraploid and 4C nuclei of diploid oats.

Figure 5

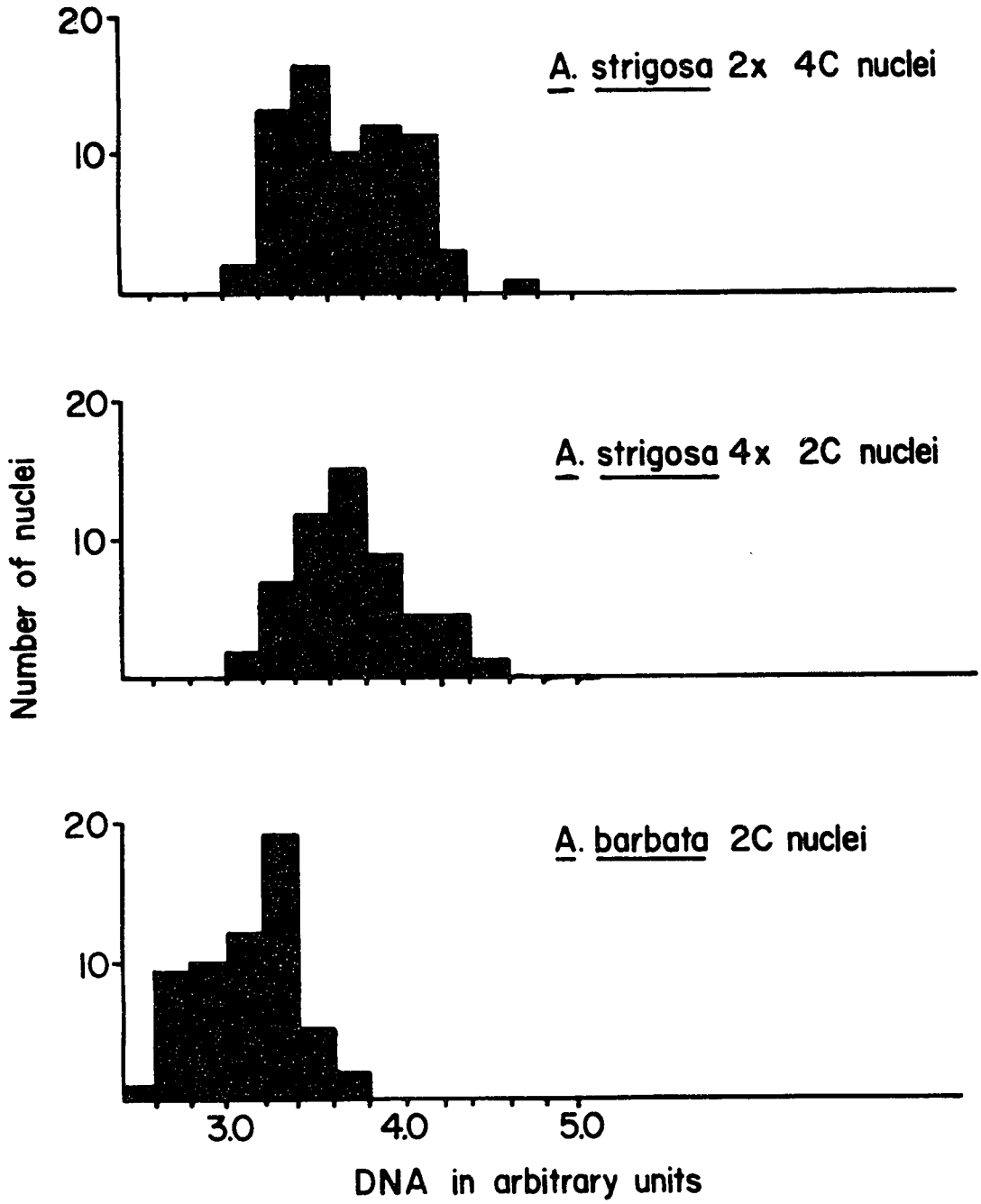

20
10
A. strigosa 2x 4C nuclei
20
10
A. strigosa 4x 2C nuclei
Number of nuclei
20
10
A. barbata 2C nuclei
3.0
4.0
5.0
DNA in arbitrary units

Figure 6

Histograms of distributions of DNA amounts estimated in 4C nuclei of tetraploid oats.

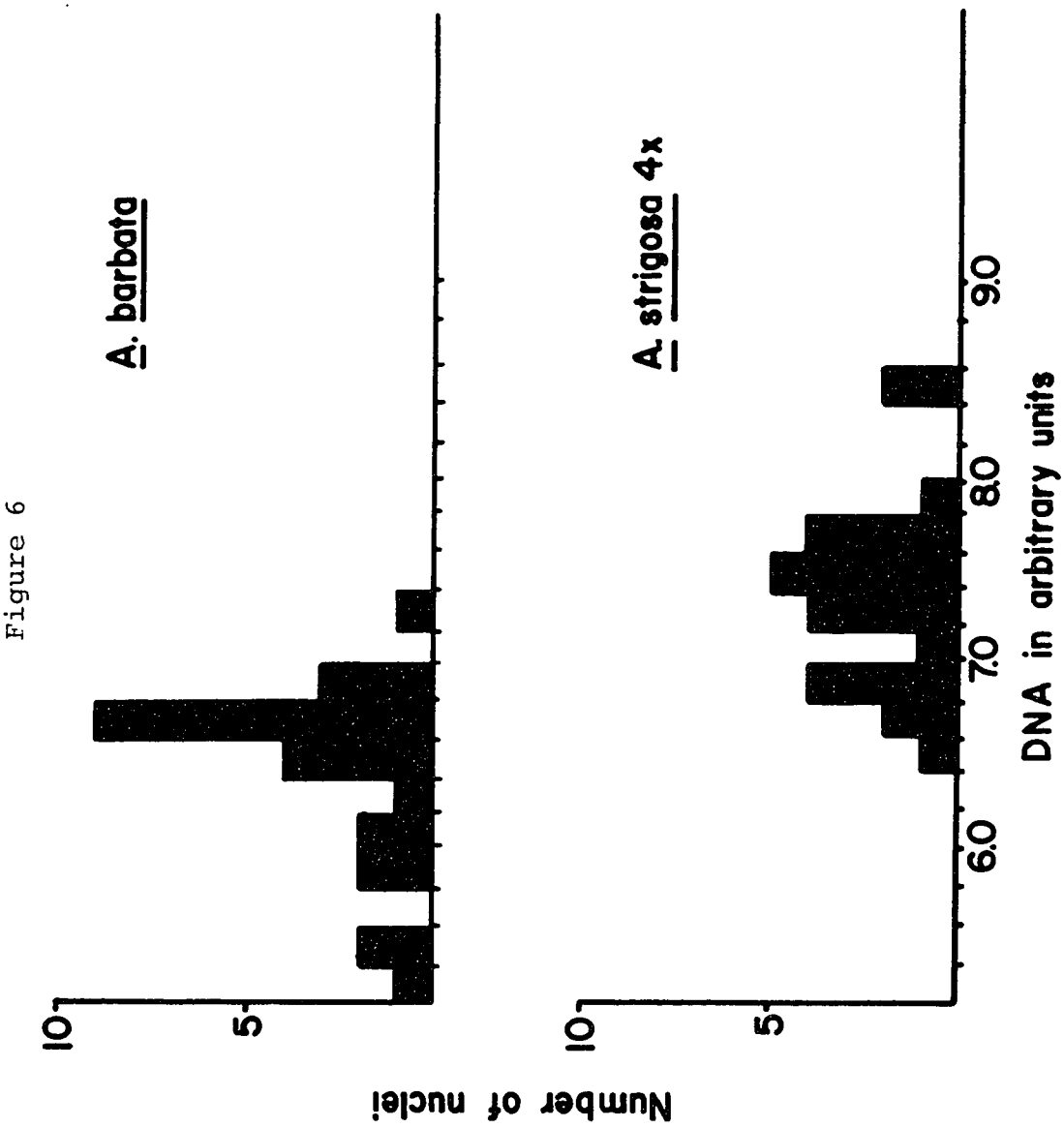

A. barbata
A. strigosa 4x
10
5
10
5
6.0
7.0
8.0
9.0
DNA in arbitrary units
Number of nuclei

Figure 6

6. Ploidy and nuclear DNA

The relative DNA values for all species are presented in Table XV and Figure 7. Diploid A. strigosa was chosen as a standard and the values of the other species were expressed in percent of the standard. As was expected, there appears to be a proportional increase in DNA quantity with the ploidy level. A close inspection of the data, however, reveals important information such as the probable DNA values of some of the component genomes of unknown origin in the polyploid species.

Variation in nuclear DNA content is found among the diploid species with a range from 100 to 121.79. If a common origin for these species is assumed, the difference in their nuclear DNA content could indicate the degree of differentiation from their ancestor. The closer DNA values in some species might suggest a closer phylogenetic relationship than in others.

The relative nuclear DNA value of A. barbata is 178.31. As the A genome in the tetraploid is homologous to A_s, the nuclear DNA value of the unknown B genome would be 78.31, by substracting the value of A_s (100) from that of AABB (178.31). In other words, the nuclear DNA value of the B genome would be about 20% lower than that of A. strigosa 2x. This may be taken as a very helpful indication in the search for the donor of the B genome among the diploid species of oats.

If A. ventricosa is assumed to be the donor of the C genome in the A. sativa complement, the DNA content of the unknown D genome would be expected to be close to the value of the difference between A. sativa and A. strigosa plus A. ventricosa, since the A_s genome is homologous to the A genome in A. sativa. The relative amount of DNA of the D genome then would be about 104.01, very close to the value of the A_s genome.

Table XV

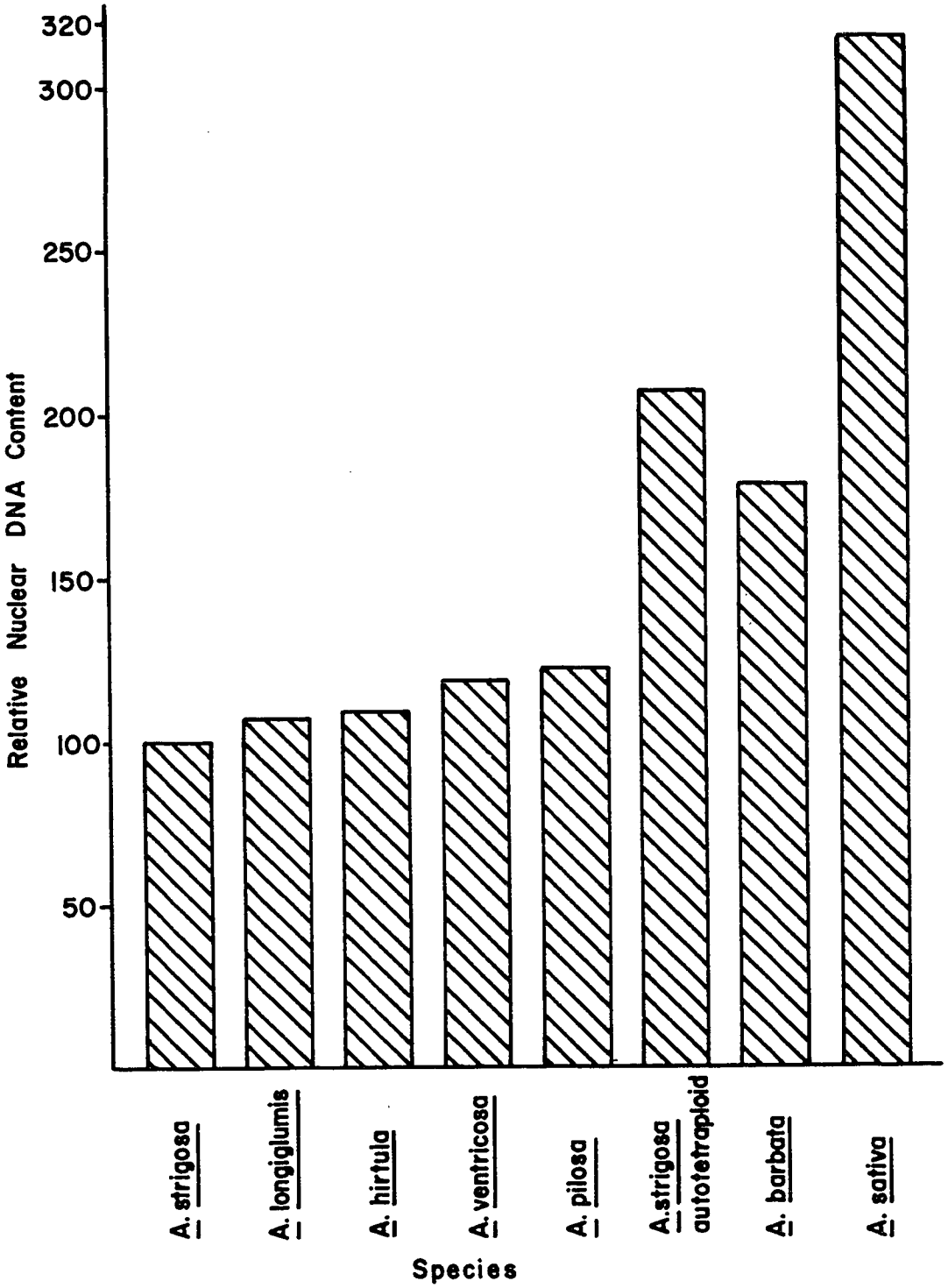
Relative nuclear DNA values in diploids and polyploids

Experiments	strigosa 2x	hirtula	longiglumis	ventricosa	pilosa	strigosa 4x	barbata	sativa
1	100	118.87	103.03	118.72	127.63	210.62	171.55	316.92
2	100	105.33	99.36	109.85	114.85	207.93	183.02	312.36
3	100	107.02	118.99	124.89	118.99	203.59	173.68	--
4	100	103.42	100.70	115.97	123.08	202.95	175.20	--
5	100	--	102.86	113.83	118.83	209.87	184.64	--
6	100	--	117.57	129.24	123.38	217.76	181.77	--
7	100	--	--	117.91	--	201.98	--	--
Mean	100	108.66	107.09	118.63	121.79	207.81	178.31	314.64
± S.E.	--	3.48	3.59	2.49	2.10	0.42	2.24	--

Figure 7

Relative nuclear DNA contents of diploid and of polyploid oats.

Figure 7


320
300
250
200
150
100
50
Relative Nuclear DNA Content
A. strigosa
A. longiglumis
A. hirtula
A. ventricosa
A. pilosa
A. strigosa autotetraploid
A. barbata
A. sativa
Species

Discussion

The results presented reveal that there are substantial quantitative variations in the nuclear DNA of the Avena species. It shows also that hybridication and subsequent doubling of the chromosomes have not caused any appreciable change in the amount of DNA per genome, thus providing another example in the grass family in addition to that of wheat (Rees and Walter 1965).

It is of great interest that the variations in nuclear DNA in the diploid species are associated with chromosomal structural changes and their genetic relationships. The fact that A. hirtula, A. strigosa and A. longiglumis have similar amounts of nuclear DNA supports the cytogenetic conclusion that these species are closely related and may have a common origin. A parallel inference may be drawn from the results of A. pilosa and A. ventricosa, which also contain similar amounts of nuclear DNA.

The significant difference in the amount of nuclear DNA between the strigosa-longiglumis group and the pilosa-ventricosa group substantiates the results of Rajhathy and Thomas (1967), indicating that these two groups are indeed different, and by chromosomal rearrangements they are differentiated into distinct groups. Cross incompatibility isolates these two groups. The amount of nuclear DNA in the pilosa-ventricosa group is about 20% higher than in the strigosa-longiglumis group. This high order of dif-

ference in DNA values could have caused some physiological changes in these species which might be responsible for the difficulties in hybridization.

Genic and chromosomal sterility are well known among hybrids both in plant and animal species. When the genome structures or genetic constitutions of two species have changed so much, eventually it would become impossible to cross with each other (Dobzhansky 1951). It is not known yet whether the difficulties encountered in hybridizing the species between strigosa-longiglumis and pilosa-ventricosa is caused by genic or chromosomal changes. However, in view of such a great difference in nuclear DNA and chromosome structure, one would tend to favor the chromosomal type, i.e. through duplications and translocations or deletions of their chromosomes in the process of speciation. The extensive structural differentiation of the chromosomes between the species of the two groups as revealed from karyotype analysis have been considered responsible, at least partially, for this reproductive isolation (Rajhathy and Dyck 1963).

The association of the difference in nuclear DNA with structural changes in chromosomes and with the cross incompatibility between the species of the strigosa-longiglumis and ventricosa-pilosa groups indicates that these groups are more remotely related. In fact, new designations for the karyotypes of pilosa and ventricosa, C_p and C_v respectively, have been proposed (Rajhathy 1966) to differentiate

them from A_s and A_l of strigosa and longiglumis, respectively. This was confirmed by data on relative quantities of nuclear DNA.

Significantly different amounts of nuclear DNA have been found among diploid species in other plant genera and in animal species, such as Bufo (Ullerich 1966), Lilium (Sunderland and McLeish 1961), Lathyrus (Rees et al. 1966) and Vicia (Martin and Shanks 1966). It seems to be a general phenomenon that the divergence and diversity of these diploid species is associated with their differences in nuclear DNA content. Sometimes, the variations in some diploid species with the same chromosome number may be as great as fortyfold (Rothfels et al. 1966); these may or may not be associated with differences of chromosomal size. Such findings have given rise to speculations about the accompaning structural changes of chromosomes.

Two mechanisms have been considered for the variations of nuclear DNA among the diploid species with the same chromosome number. The first one is segmental changes of chromosomes, i.e. an increase or a decrease in chromosomal length by duplications or deletions (Keyl 1965, Rees and Jones 1967a, 1967b). The second possibility is the degree of polynemy of the chromosomes, i.e. a difference in the number of longitudinal DNA strands along the chromosomes (Christensen 1966, Martin and Shanks 1966, Rothfels et al. 1966, and Schrader and Hughes-Schrader 1956, 1958).

Chromosomal size and volume have not been included in this study. However, it is interesting when compared with the linear difference reported by Rajhathy (1961) between the chromosomes of strigosa and longiglumis; the former is 17 units shorter than the latter, which is equal to the length of the longest chromosome arm in strigosa. The mean differences in their nuclear DNA are 0.1255 (2C nuclei) and 0.2504 (4C nuclei). Although these figures are not statistically significant, they are very close to the significant levels, 0.1743 for 2C nuclei and 0.2859 for 4C nuclei (Tables VI and VII). This could be considered as a probably positive correlation between the total length of the chromosomes and their DNA contents. A similar trend is found between pilosa and ventricosa. The mean DNA value of pilosa is a little higher than that of ventricosa (not significant) while the short arms of the chromosomes of ventricosa are reported to be slightly shorter than those of pilosa (Rajhathy 1967). The data presented here suggest that most probably longitudinal duplications or deletions of chromosome segments are responsible for the different DNA contents among the diploid species of oats. However, the possibility of differential polynemy cannot be ruled out.

The nonsignificant differences in these cases might be due to the limited resolution of the technique used. More sensitive technique and equipment capable of detecting the difference of DNA content of single chromosomes (Carlson et al. 1963) or even of single bands in salivary gland chro-

mosomes have been reported (Keyl 1965). It is possible that, by using these new equipment and techniques, the fine structural differences among the chromosomes of the diploid species of oats may be further resolved and the chromosomal structures in higher plants in general could be elucidated.

The results in tetraploid species have shown that the amount of nuclear DNA in A. barbata is lower than that of autotetraploid strigosa. It is obvious that A. barbata could not be an autotetraploid of diploid strigosa, nor of any other diploid species included in this study, for no other species has a nuclear DNA value lower than strigosa. It seems unlikely that the tetraploid species of oats have evolved from an autotetraploid which resulted from chromosome doubling of a diploid species within the known species of Eubarbatae as was proposed by Ladizinsky and Zohary (1968).

It may be argued, however, that changes in the quantity of nuclear DNA may have occurred after the formation of the tetraploids by gradual structural changes. This is probably not the case because, according to karyotype studies, the complete set of strigosa chromosomes (A) is present in the tetraploids, but prominent differences exist between the chromosomes of the B set and those of the A set (Sadasivaiah and Rajhathy 1968). The changes in the morphology of the chromosomes and consequently of the DNA amount, seem to be restricted to only one genome but not to the other. In other words, the diminution or increase of DNA has not taken

place at random in all the chromosomes of tetraploids but rather specifically in the chromosomes of the B genome. It is very difficult to envisage how this might have happened in an autotetraploid which has the same sets of chromosomes in the same cellular environment.

In fact in tetraploid wheats, Triticum timopheevi, which has a different chromosome constitution (AAGG) from that of T. durum (AABB), was found to have a lower content of nuclear DNA than T. durum. In the tetraploid species with the same chromosome constitution (AABB), the amounts of DNA in their nuclei were also similar (Rees and Walters 1965). Although this difference in the amount of nuclear DNA does not necessarily mean a completely separate ancestry, it does indicate the degree of differentiation of these species from their ancestral species.

It is possible that related species or varieties might have so diverged from the ancestral species by accumulating chromosomal aberrations that resulted in different amounts of nuclear DNA. Then through hybridization and subsequent chromosome doubling the original tetraploid species was established. Such a difference in the amount of nuclear DNA would not always hinder hybridization of these species, for Rees and Jones (1967) have reported that in two species of Lolium, L. perenne and L. temulentum, despite a 30% difference in their nuclear DNA contents, readily hybridized and the hybrids are vigorous and fertile; the extra DNA is considered to be "uninformative". Based on the foregoing

reasoning, the origin of tetraploid oats is more likely through segmental allotetraploidy as proposed by Holden (1966) and Sadasivaiah and Rajhathy (1968).

These authors suggested that the donor of the B genome of tetraploid oats could be probably found in the wild populations of A_s genome (A. hirtula and A. wiestii group, Table I). The results reported in this study provide a useful marker in searching for the donor of the B genome. On the basis of nuclear DNA content the donor of the B genome would be expected to have a value about 20% lower than that of the diploid strigosa. The amount of nuclear DNA in the geographic races in the hirtula-wiestii population have not been studied yet. When such a survey is done, plants having a value close to the expected should be considered as potential donors of the B genome and should be subjected to further tests.

PART TWO

THE AMOUNTS OF NUCLEAR DNA AND THE AVERAGE DURATIONS OF MITOTIC PHASES IN ROOT TIP CELLS OF *AVENA* SPECIES

Introduction

The experiments reported in Part one have shown that there are substantial differences in the amounts of nuclear DNA among the diploid species of Avena. These differences correspond to the gross morphological changes in their chromosomes and might also be associated with their cross-incompatibilities. It seems that the differences in the amounts of nuclear DNA correlate with the genetic affinity between these species. However, there are also examples that closely related species may have much greater differences in their amounts of nuclear DNA (Evans 1964, Rees and Jones 1967) than do more distantly related species. The extra DNA in these cases could be "nonfunctional" or "noninformative". It would be of interest to know whether the quantitative change of the nuclear DNA in Avena species is associated functionally with any structural changes of the chromosomes.

The most direct test for the homology between two chromosomes would be the determinations of the order of bases in their nucleic acids. However, since the fine structures of chromosomes in higher plants and animals are still obscure (Giles 1965), this kind of study is still restricted to only a very few prokaryotes (in terms of Ris and Chandler 1963), for example, the RNA viruses of different genotypes (Reddi 1959). An indirect approach, however, which may be applied in this case, is to compare the patterns of DNA synthesis in the cells of these species.

One of the most striking features of DNA replication in a cell is the specific time interval it occupies during the cell cycle. This was first suggested in the early cytophotometric studies of the amounts of DNA per cell by Alfert (1950) and Swift (1950). Later, Howard and Pelc (1953) confirmed it by using the method of autoradiography and showed that the DNA synthesis in the root tip cells of Vicia occupied only part of the interphase period. The DNA synthetic period was separated from cell division by two gaps, one before and one after DNA synthesis. Thus they divided the interphase period into three phases, the pre DNA synthesis phase (G_1), DNA synthesis phase (S), and post synthetic phase (G_2). Since then, a similar phenomenon has been observed in many other cell types of various origins, both animal and plant (Bender and Prescott 1962; Deeley et al. 1957; Mendelsohn et al. 1960; Siskin 1959; Siskin and Kinosita 1961; Stanner and Till 1960; Taylor 1960a; and Wimber 1960), but the duration of synthetic phases appeared to vary with the cell type.

Van't Hoff (1965, 1967) has studied the relationship between the amount of DNA per cell and the average duration of the S phase, and the average times of mitotic cycle in several diploid plant species. He showed that the amount of DNA per cell was linearly correlated to the average duration of the S phase, the average duration of the mitotic cycle and the rate of DNA synthesis. In other words, the species with larger amounts of DNA per cell had a longer S phase,

longer mitotic cycle time and also a relatively higher rate of DNA synthesis. A similar association of higher DNA content with longer S phase has been also reported in some animal cells (Goldfeder 1965).

Contrary to the above reports, the durations of the S phase in some polyploid and aneuploid cells in animal tumors are the same irrespective of their differences in DNA content (Oehlert et al. 1962). Diploid and haploid cells from normal and induced haploid embryos of Xenopus laevis have the same duration of DNA synthesis (Graham 1966). These apparently contradictory results have raised the question of how the synthesis of DNA in a cell is quantitatively and temporally controlled. It is believed that beyond the level of immediate raw materials which are necessary requirements for DNA synthesis (the four deoxyribonucleoside triphosphates, DNA polymerase, bivalent ions etc.), DNA replication seems to be rigidly controlled at the level of the chromosomes (DNA primer?) (Taylor 1963). This speculation is mainly based on the considerable numbers of reports showing that the replication of chromosomal DNA is not synchronous, but has an ordered sequence; a certain portion of the chromosome replicates at a specific time during the DNA synthetic period.

The asynchrony of DNA replication in plants was first reported by Taylor (1958b) in the root tip cells of Crepis capillaris. He showed that the synthesis was initiated at the distal ends of the chromosomes and progressed toward the centromere. Later, Wimber (1960) observed in Tradescantia

paludosa that the ends of the chromosomes were the last to complete DNA synthesis. The asynchronous pattern of DNA replication was also found among different chromosomes. Lima-de-Faria (1959) first claimed that the X chromosome of a species of grasshopper was the last one to finish its DNA replication within the complement. In tissue culture cells derived from a male embryo of the Chinese hamster, Taylor (1960) found that the long arm of the X chromosome and the Y chromosome replicated later than all the other chromosomes; two pairs of small autosomes and the short arms of two pairs of median sized autosomes also replicated late; other autosomes showed variation of late and early replicating points along the chromosomes. These findings have been confirmed by Hsu (1964) by a different approach in the study of cultured cells of the Chinese hamster. Similar findings were reported in the root cells of Bellevalia and Vicia (Taylor 1963), rye (Darlington and Haque 1964), barley (Kusanagi 1966) as well as in human cells (Moorhead and Defendi 1963; Morishima et al. 1962; Schmid 1963).

It appeared that there were many discrete sites on the chromosome which were able to initiate DNA synthesis. These observations have led Taylor (1963) to postulate the hypothesis that a chromosome consists of many replication units. Cairns (1966) reached a similar conclusion by studying the DNA synthetic rate of chromosomes of cultured human cells (HeLa). The rate of DNA synthesis in these cells was about

0.5μ/minute. At this rate, he calculated that it would require at least 100 sites of duplication in an average human chromosome, which contains about 3 cm of DNA double helix, to complete its replication within the experimentally determined 6 hours S period.

In view of the above findings, it could be explained why a haploid, aneuploid, or polyploid cell may have an S phase time similar to its diploid counterpart. The complete homologous genome should have the same chromosomal structures and the same number of replication units per chromosome. Except in sex chromosomes, the temporal sequence and the rate of DNA synthesis in the homologous chromosomes of higher animals are generally the same (Hsu et al. 1964). In the polytene chromosomes of Drosophila salivary glands, the replication of DNA at homologous loci is closely synchronized (Plaut and Nash 1964). Thus, a haploid set of chromosomes in a genome could be taken as a fundamental unit for the time requirement of the DNA synthesis of the genome. If the basic set of the haploid chromosomes is present and no change of chromosome structure has occured, the haploid, aneuploid or polyploid cells would be expected to have a duration of S phase similar to that of the corresponding diploid.

The following experiments were designed first to test this hypothesis by comparing the durations of various phases in cell cycles of the diploid A. strigosa and its autotetraploid, secondly, to examine whether the differences in the amounts of nuclear DNA of diploid Avena species is associated

with the function of chromosomes in regards to the time requirement for DNA synthesis.

Materials and Methods

1. Plant materials and the germination of seeds

The root meristematic cells of A. strigosa 2X, A. strigosa 4X (autotetraploid) and A. pilosa, which have considerably different amounts of DNA in their nuclei, were used for the study of cell cycles.

The procedures for the germination of seeds were generally those of Ku (1965)[1], with minor modifications. About 80 seeds were soaked in a 9cm Petri dish containing an excess of distilled water for 4 hours in the dark at room temperature, and then the seeds were spread out between a layer of moistened cotton wool and a layer of cheese cloth which was supported by a 3 inch square of screen filter on top of a blackened 250 ml. beaker. The beakers were placed in a black painted 20" x 30" covered container.

Distilled water was added both inside and outside the beakers in the container to a depth of about one inch. The cheese cloth covering the screen filter was extended downward outside to reach the water, thus providing wicks to supply water to the seeds. The container was put into a controlled environment chamber kept at $25^{o} \pm 1^{o}C$ in darkness.

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[1] I would like to thank Dr. P. Weinberger and Miss T. Ku for lending me the equipment for the germination of seeds and for allowing me to use the controlled environment chamber.

After about 48 to 60 hours, when the roots were grown to about 1.5 cm long, seedlings with straight roots of 1.5 ± 0.2 cm in length were carefully selected and transferred to another screen filter supported by a 9 cm Petri dish which contained 40 ml of distilled water. The roots of the seedlings were allowed to pass through the holes of the filter and into the water. The Petri dish with the seedlings was then placed back in the container and returned to the controlled environment chamber for another 2 hours.

2. Labeling of the root meristematic cells

The seedlings with the screen filter were transferred together to another 9 cm Petri dish filled with 40 ml of aqueous solution of tritiated thymidine at a concentration of 1μc/ml (specific activity 2.0 c/mM). The roots were immersed in the H^3-thymidine solution for 30 minutes at room temperature in the dark. At the end of labeling, the roots were washed thoroughly with distilled water and returned to grow in distilled water at 25 ± 1°C in the dark. The darkness, however, was interrupted briefly whenever samples were taken afterwards.

Thirty minutes after the end of H^3-thymidine treatment and subsequently at one or two hours intervals until the 17th hour, samples of the roots were collected and fixed in acetic alcohol (1:3) for 1 hour, then moved through the alcohol series and stored in 70% alcohol.

3. Preparation of slides for autoradiography

The roots were stained by the Feulgen reagent for more than two hours after hydrolyzing in 1N HCl at 60°C for 12 minutes. The terminal 0.5 mm of root cap was cut off. Squashes of these Feulgen stained root tips were made in 45% acetic acid, from the next 2 mm of meristematic tissue, each root being used to make one slide.

The glass slides used for the autoradiographic preparations were first cleaned in acid cleaning fluid, then thoroughly washed in tap and distilled water, finally subbed and dried (subbing mixture: gelatin 0.1 gm, chromium potassium sulfate 0.01 gm, water 100 ml.).

The slides with squash preparations were placed on top of a block of solid carbon dioxide to freeze the cells. After a few minutes, the coverglasses were peeled off with a razor blade. The slides with the cells attached were moved through 95% alcohol and then two changes of absolute alcohol for 5 minutes each and then dipped into 0.3% celloidin (in absolute ethanol/ether, 1/1). After the dipping, they were placed on a wooden rack in a dust free cabinet and allowed to dry in air over night. These slides were then ready for autoradiography.

4. Autoradiography

Kodak NTB-3 liquid emulsion was used for coating all slides. The emulsion was melted at 43°C and poured into a dipping jar in a water bath at 43°C. The dried slides were

warmed to 40°C and then dipped into the emulsion one by one for 4 to 5 seconds. The slides were held vertically for about 4 seconds. The excess emulsion on the side without cells was wiped off. The slides were laid flat and dried at room temperature in the dark for 2 hours.

After drying, the coated slides were stored in black slide boxes (about 20 slides in each box) with Drierite and kept at 4°C for 5.5 days. At the end of the exposure period, the slides were developed in Kodak D_{19} for 10 minutes at 4°C, fixed in Kodak acid hardening fixer for 15 minutes, washed, dehydrated and mounted in oil of low refractive index.

5. The scoring of the slides

The percentage of labeled mitotic cells was scored on each slide sampled at various times of fixation. To avoid subjective bias, all the slides were coded before examination. Autoradiographs were examined by phase-contrast and bright-field microscopy. Microscopic fields under 10X ocular and 40X objectives were chosen at random for scoring the labeled and non-labeled mitotic nuclei until 150 mitotic nuclei were obtained for each slide. The mitotic cells were those which could be definitely identified showing thread-like condensed chromosomes. The late telophases were distinguished from interphases by the help of phase-contrast microscopy, by which an intact cell or two cells could be easily identified.

The labeled cells usually had many more grains on the chromosomes than the background, so there was no difficulty

in identifying them. Whenever a background count was close to those on the cell, an excess of 10 grains per the whole chromosome complement of the mitotic cell over the immediate surroundings was used as a standard for labeling.

Two to four slides were scored at each time of fixation and the standard errors were calculated as errors of a binomial distribution, i.e. S.E. = $\sqrt{pq/n}$, where p is the percentage of labeled mitoses, q = 1-p and n is the number of cell scored (Evans and Scott 1964).

Experimental Results

The data on the proportion of labeled mitotic cells at each fixation time after the H^3-thymidine treatment in the three species are presented in Table XVI. The mitotic labeling curves obtained from plotting the data are shown in Figures 9, 10, and 11.

The three curves all showed two peaks, indicating that at least one mitotic cycle had been covered in all three species. The two peaks found in the two diploid species were similar to those of Vicia faba observed by Howard and Dewey (1960), in which the value of the second peak was roughly half of the first one. In the autotetraploid the second peak was about 90% of the first peak, similar to the mitotic labeling curves reported by Evans and Scott (1964) on the root tip cells of Vicia faba.

The method for estimating the durations of the different phases of the mitotic cycle was mainly based on that of Quaster and Sherman (1959) who used it first on mouse intestinal epithelium. The method was later applied to root meristematic cells by Wimber (1960). The essential of this method is to label the DNA of a group of cells in an asynchronously dividing cell population by supplying tritiated thymidine, so that only those cells in the DNA synthetic period would be labeled. By examining the changes in the percentage of mitotic figures at the various time intervals after the labeling through one complete mitotic cycle, the durations

Table XVI

The proportion of dividing cells labeled after H^3-thymidine treatment in three species of Avena.

fixation time hr. after labeling	A. strigosa 2x		A. strigosa 4x		A. pilosa	
	No. of slides	labeling index ± S.E.	No. of slides	labeling index ± S.E.	No. of slides	labeling index ± S.E.
0.5	4	7.38 ±1.07	4	0	4	0
1	4	6.33 ±0.99	4	7.9 ± 1.56	4	2.35± 0.62
2	2	24.74 ±2.49	-	--	-	--
3	3	69.77 ±2.17	3	8.81±1.34	3	36.69± 2.27
4	2	85.55 ±2.03	-	--	2	74.95± 2.50
5	4	59.83 ±2.00	4	80.69±1.60	4	92.51± 1.07
6	2	48.90 ±2.89	-	--	-	--
7	3	28.86 ±2.14	3	82.11±1.81	3	66.22± 2.23
8	4	29.94 ±1.87	-	--	-	--
9	-	--	4	29.22±2.14	2	56.35± 2.86
10	3	34.92 ±2.25	-	--	3	10.68± 1.46
11	4	24.33 ±1.75	4	29.41±1.86	4	33.10± 1.92
13	4	44.90 ±2.03	2	18.67±2.25	4	50.24± 2.04
15	3	26.44 ±2.08	2	69.29±2.66	2	31.33± 2.68
17	3	31.33 ±2.17	3	39.46±2.30	4	25.83± 1.77

of various mitotic cycle phases may be deduced. If each phase of the cell cycle were of constant duration and no variation between cells, a hypothetic curve as shown in Figure 8 would have been obtained. The duration of each phase could be estimated easily as shown in the figure. However, because of variation in the duration of the cell cycles among the cells, the hypothetic curve is not found. Nevertheless, the average durations of each phase can still be calculated from these curves, and the results are as follows:

1. Mitotic cycle time (T)

This is the time that covers the whole mitotic cycle from the beginning of mitosis of a cell until the beginning of mitosis of the next cell generation. If a cell is labeled during its period of DNA synthesis, it would continue to grow through the rest of the S period, the G_2 period, and finally the mitotic phase. The ascending limb of the first peak of the mitotic labeling index curves represents those cells that were near the end of the S period at the beginning of the H^3-treatment. The slope of the ascending limb depends upon three factors: the duration of labeling, the variation of G_2 and of mitosis, and the length of the mitosis (Wimber 1960).

The ascending limb of the second peak corresponds to those cells which were in the second mitotic division after the labeling. So the two peaks correspond to the first and second waves of the same group of cells as they advance

through the first and second mitosis after the labeling. The interval between these two peaks then could be taken as an estimate of the total mitotic time (T). However, the second peak was not always easy to define and owing to the variation of mitotic cycle time among the cells, the second ascending slope was usually flatter than the first one. The average duration of the total mitotic cycle time would be the time interval between the half maximum labeling index point on the ascending limb of the first peak and the corresponding index point on the ascending limb of the second peak.

In the diploid and autotetraploid strigosa, the time intervals between the 40% intercepts on the two ascending limbs were used to estimate the average durations of mitotic cycle, which gave 9.8 hours for diploid strigosa and 9.9 hours for autotetraploid strigosa. In A. pilosa, the 45% intercepts were used, and the average duration of mitotic cycle of this species was 8.85 hours.

2. Mitotic time (M)

This is the period occupied by cell division, from the beginning of prophase till the end of telophase. Several methods may be used to estimate the duration of mitotic time. One is to read directly from the mitotic labeling index curve by converting it to the hypothetic condition as shown in Figure 8. This was done by drawing a tangent line at the most linear section of the ascending limb of the first peak. The time intervals between the intercepts

Figure 8

Hypothetic labeled mitoses curve. Cells move through the nuclear cycle at the same rate. The total cycle and the various subdivisions may be determined from the curves.

t_{G2} --- duration of G_2. t_m --- duration of mitosis.

t_s --- duration of DNA synthesis. T --- total cycle.

(After D.E. Wimber 1963).

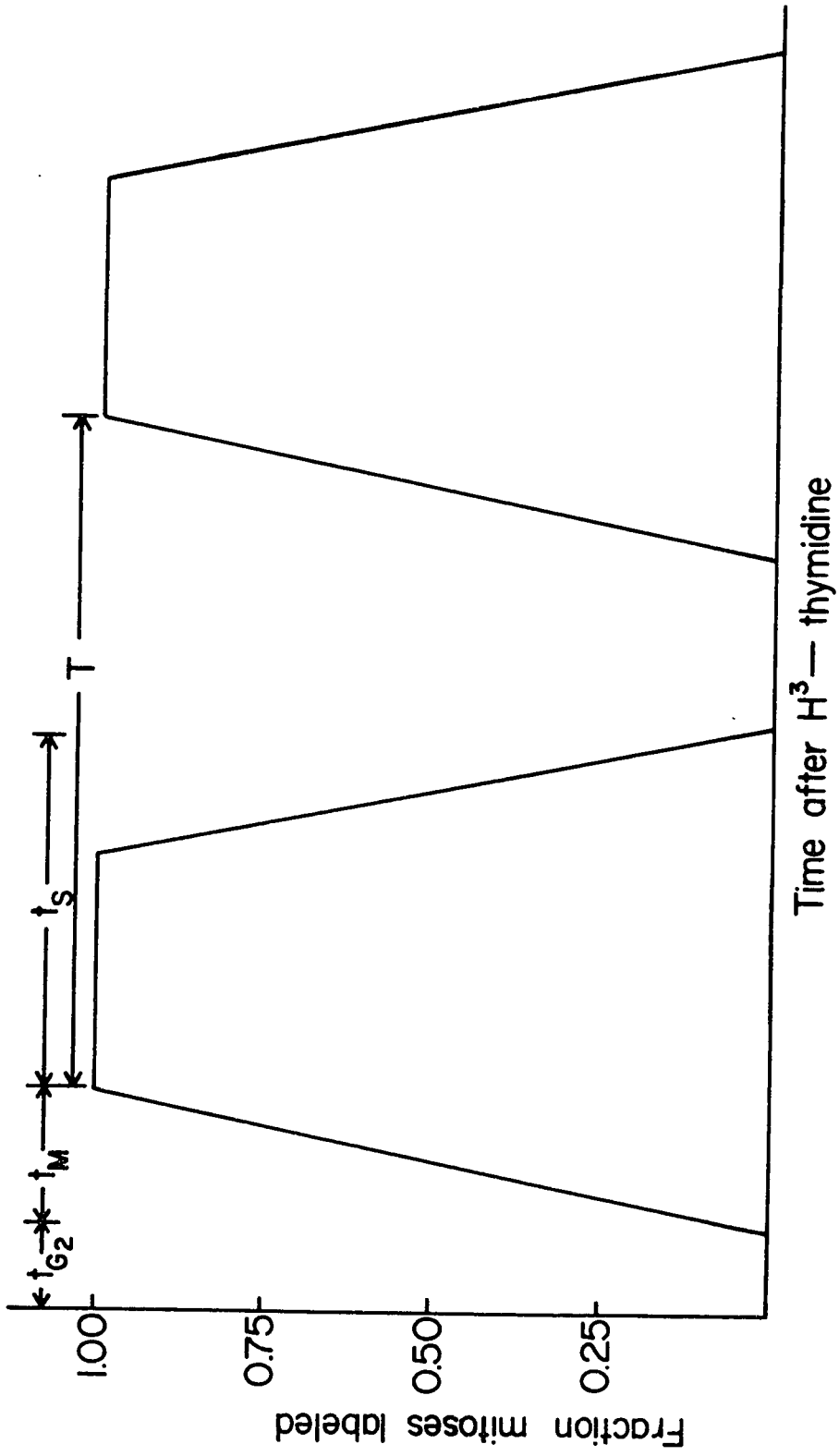

Fraction mitoses labeled
1.00
0.75
0.50
0.25
t_{G_2}
t_M
t_S
T
Time after H^3— thymidine

Figure 8

of this line at the abscissa and at the point where the maximum labeling index was reached would be the approximate duration of mitosis. Accordingly, the mitotic time estimated for diploid A. strigosa was 1.8 hours, for autotetraploid strigosa 1.6 hours, and for A. pilosa 1.7 hours.

3. DNA synthetic phase (S)

It has been stated that the ascending limb of the first peak of the mitotic labeling index curve represent those cells which were near the end of S phase at the beginning of H^3-thymidine treatment. The descending limb, on the other hand, would represent those cells which were at the beginning of S phase at the end of H^3-thymidine treatment. The slopes of the two limbs are due to the variations in the G_2, mitosis and S phases of these cells. The average duration of DNA synthetic phase can be taken as the time interval between the half maximum labelling index in the ascending limb and the corresponding point on the descending limb of the first peak minus the H^3-thymidine labeling time.

The time intervals between the 40% intercepts on the ascending and descending limbs of the first peaks in diploid strigosa was 4.05 hour and in autotetraploid strigosa was 4.25 hour. In A. pilosa the period lasted between 45% intercepts was 4.75 hour. By subtracting the H^3-thymidine labeling time, 0.5 hour, from the above figures, the S phase of diploid A. strigosa was 3.55 hours, of autotetraploid strigosa was 3.75 hours and of A. pilosa was 4.25 hours.

4. Post DNA synthetic phase (G_2)

This phase is the period between the end of DNA synthesis and the beginning of mitosis. Since those cells which were near the end of S phase at the beginning of the H^3-thymidine treatment appeared first as labeled mitotic cells, the minimum duration of G_2 could be estimated by the time interval between the beginning of H^3-thymidine treatment and the appearance of the first labeled dividing cells. However, the durations of G_2 and of mitosis of these cells varied, a better estimation of the G_2 may be had by taking the half maximum mitotic labeling index as a reference point. The period between the commencement of labeling to the half maximum labeling value of the ascending curve of the first peak corresponds to the average $G_2 + 1/2M$. The durations of the M phases were already calculated. The durations of G_2 would be then the time between these two points minus half the time of mitotic phase. The times for G_2 thus calculated were 2.0, 3.55 and 2.9 hours for A. strigosa 2X, A. strigosa 4X and A. pilosa respectively.

5. Pre-DNA synthesis phase (G_1)

This is the period that lasts from the end of mitosis to the beginning of DNA synthesis and it can be estimated by substracting the sum of G_2, S and M durations from the total mitotic time (T). The durations of G_1 of the three species were calculated as follows:

A. strigosa 2X

$$G_1 = T-(S + G_2 + M)$$
$$= 9.8 - (3.55 + 2.00 + 1.80)$$
$$= 9.8 - 7.35$$
$$= 2.4 \text{ (hr)}$$

A. strigosa 4X

$$G_1 = T - (S + G_2 + M)$$
$$= 9.9 - (3.75 + 3.55 + 1.6)$$
$$= 9.9 - 8.9$$
$$= 1.00 \text{ (hr)}$$

A. pilosa

$$G_1 = T - (S + G_2 + M)$$
$$= 8.85 - (4.25 + 2.90 + 1.70)$$
$$= 8.85 - 8.85$$
$$= 0 \text{ (hr)}$$

To summarize the above information, the average durations of various phases in the mitotic cycles of the root tip cells of the three Avena species are given in Table XVII.

Table XVII

Duration of various mitotic phases of the root tip cells of Avena species. (in hours)

Species	G_1	S	G_2	M	T
A. strigosa 2X	2.45	3.55	2.00	1.80	9.80
A. strigosa 4X	1.00	3.75	3.55	1.60	9.90
A. pilosa	0.0	4.25	2.90	1.70	8.85

Figure 9

Mitotic labeling index curve showing the relationship between the proportion of mitotic cells labeled and time after removal from H^3-thymidine in the roots of Avena strigosa 2X.

Figure 9

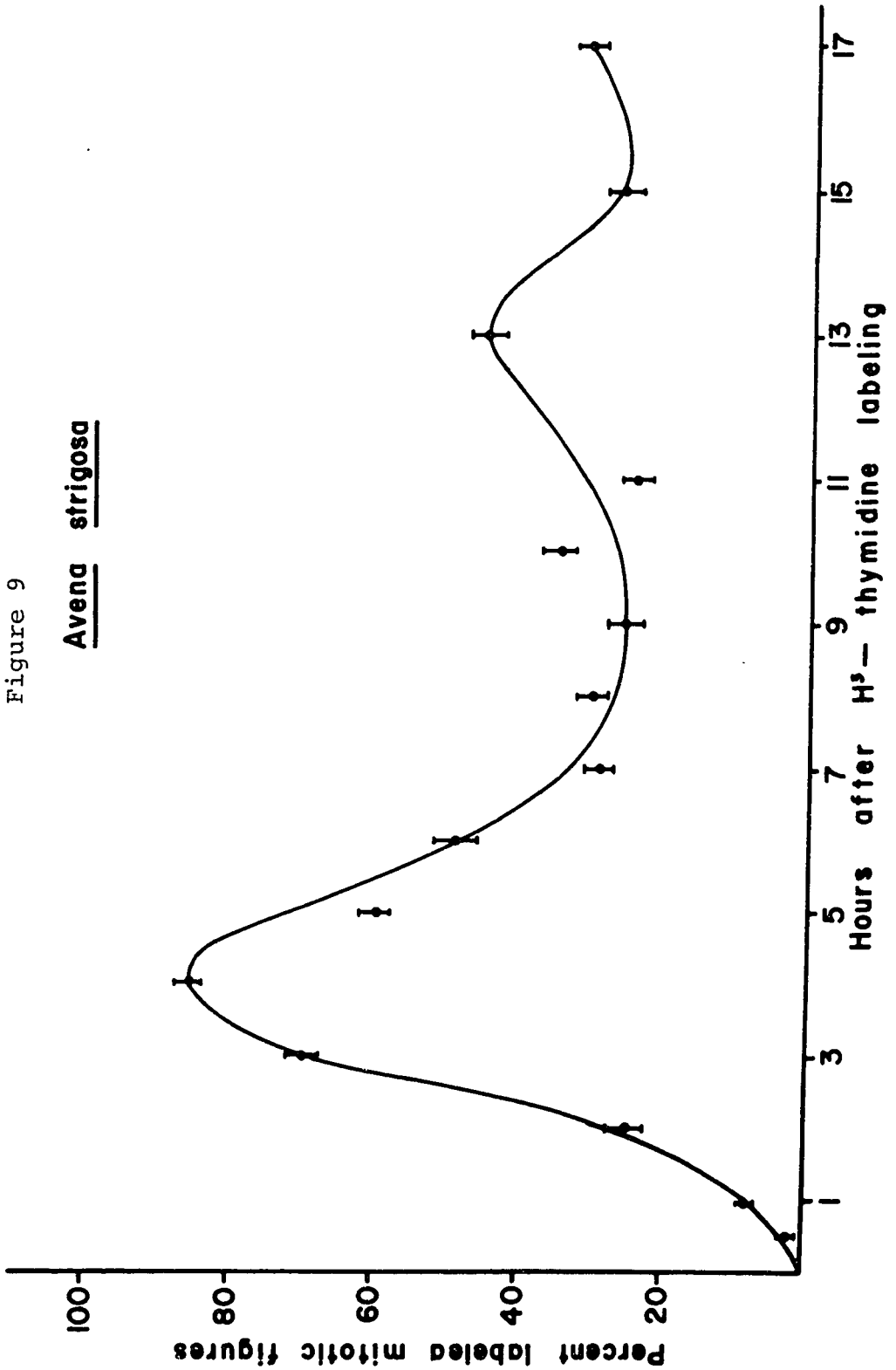

Avena strigosa
Percent labeled mitotic figures
100
80
60
40
20
1
3
5
7
9
11
13
15
17
Hours after H³— thymidine labeling

Figure 10

Mitotic labeling index curve showing the relationship between the proportion of mitotic cells labeled and time after removal from H^3-thymidine in the roots of Avena strigosa 4X.

Figure 10

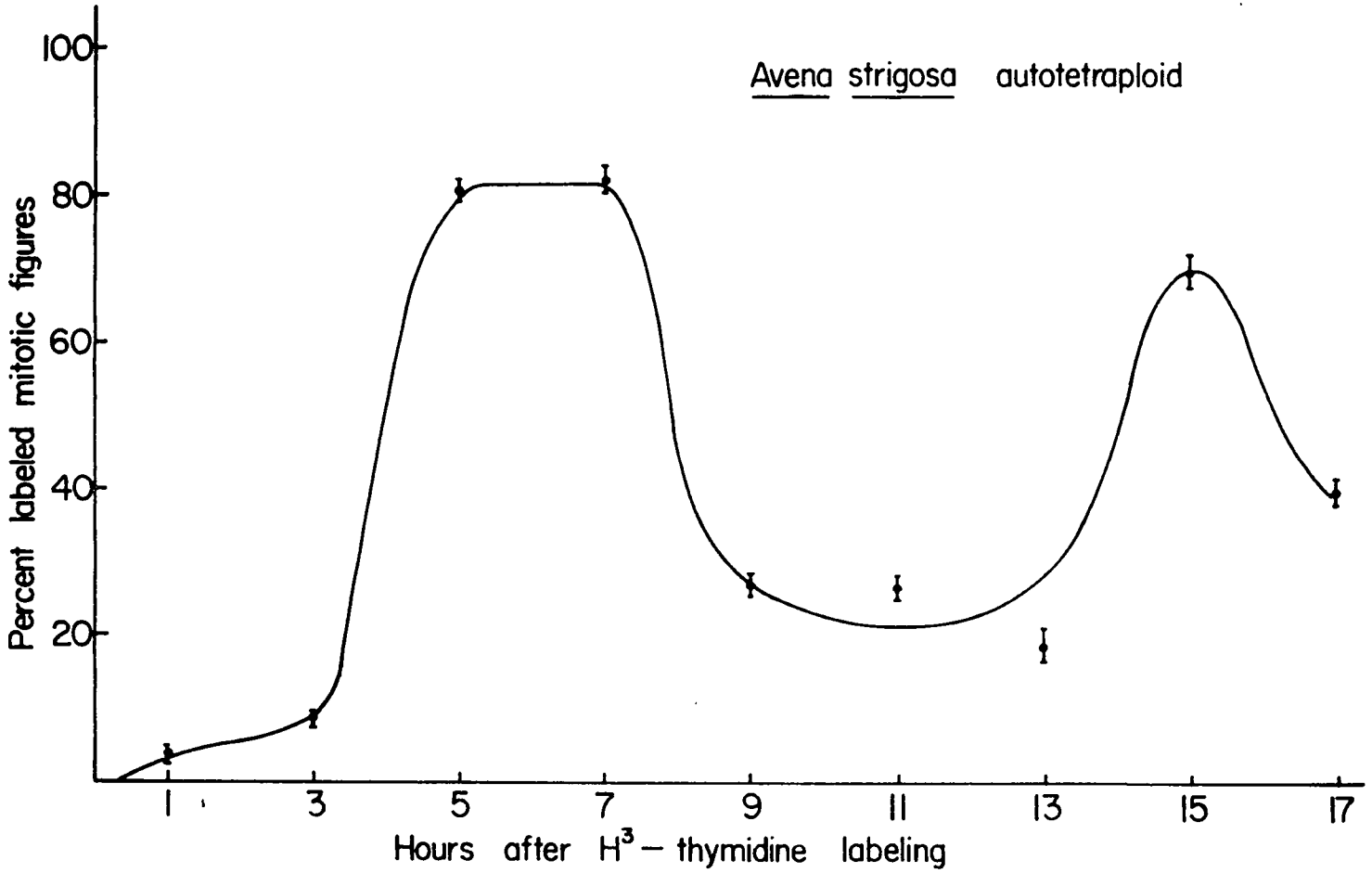
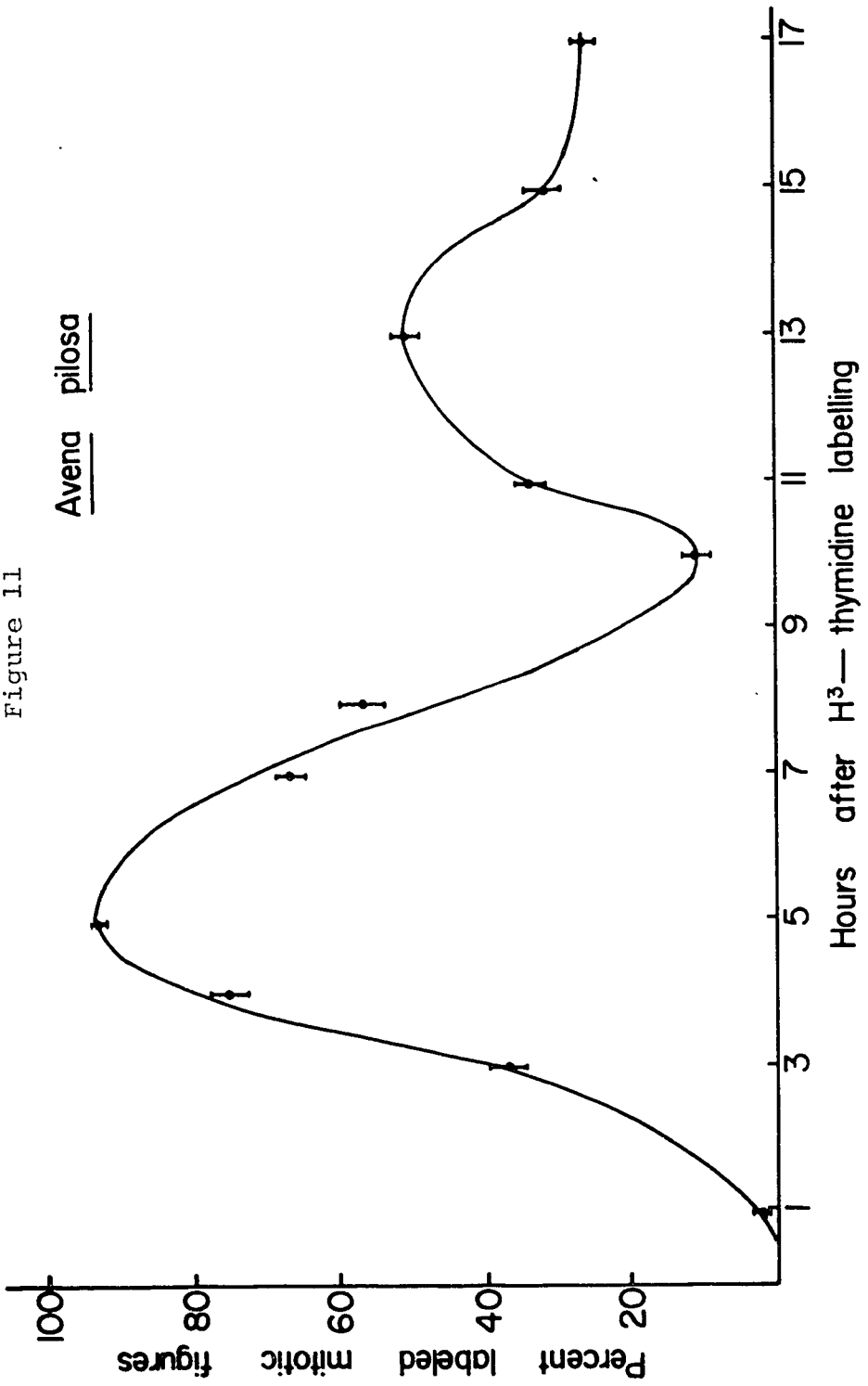

Avena strigosa autotetraploid
Percent labeled mitotic figures
100
80
60
40
20
1
3
5
7
9
11
13
15
17
Hours after H³ – thymidine labeling

Figure 11

Mitotic labeling index curve showing the relationship between the proportion of mitotic cycle labeled and time after removal from H^3-thymidine in the roots of Avena pilosa.

Figure 11


Avena pilosa
Percent labeled mitotic figures
100
80
60
40
20
1
3
5
7
9
11
13
15
17
Hours after H³— thymidine labelling

Discussion

The results indicate that the duration of the DNA synthetic phases (S) of diploid and autotetraploid strigosa are similar (3.55 and 3.75 hr respectively). The 0.2 hr (12 minutes) difference between the two is probably due to experimental variation rather than to any real difference. This supports the hypothesis that homologous genomes replicate in synchrony. The cause of the similar S phase in diploid and autotetraploid strigosa is probably the increased number of DNA replication units in the autotetraploid resulting from the doubling of the genome, as suggested in the introduction.

A similar condition was reported by Cameron and Stone (1964), who found that in three strains of Tetrahymena pyriformis, the macronuclei of two strains had higher DNA amounts than the third one, but the S phases of the three were the same; their results were consistent with the hypothesis that the macronucleus of Tetrahymena contains multiples of genomic sets and each set replicates synchronously.

Van't Hoff (1966) has also reported that in the colchicine induced tetraploid cells in the root of Pisum sativum the S phase is similar in duration to the diploid cells in the same roots. Thus, in the tetraploid nuclei, twice as much DNA is synthesized per unit time as in the diploid nuclei. He accounted for the increased rate of DNA synthesis in the tetraploid cells by a supposed proportional increase of the

enzyme systems associated with DNA synthesis. Of course, by doubling the genome, the part of genome that codes for the production of these enzymes is also doubled, hence more enzymes could be produced.

That the structures of chromosomes were involved in controlling the timing of DNA synthesis was also suggested by the data on A. pilosa, whose S phase is longer (4.25 hr) than that of autotetraploid strigosa (3.75 hr) despite the much higher nuclear DNA content of the latter. It is apparent that the duration of the S phase may be influenced by other factors even more than by the amount of DNA in the nuclei when polyploidy is involved in related species.

The S phase of A. pilosa is also longer than that of diploid A. strigosa. These results agree with the conclusion made by Van't Hoff (1965) that cells of different diploid species with higher DNA content have a longer S period. It is of interest that the ratio of the amounts of nuclear DNA of A. pilosa and A. strigosa (121.79) is very close to the ratio of the times required for the synthesis of their DNA (119.72).

The total mitotic cycle times of diploid and autotetraploid cells of strigosa are 9.8 and 9.9 hr respectively, not significantly different. These data do not agree with the results on mammalian cells in vitro which showed that the tetraploid cells of old animals (Post and Hoffman 1964) and of old ascites tumors (Hauschka et al 1957) both had a longer mitotic cycle than their diploid counterparts. The discre-

pancy could be due to the different types of cells studied or due to the differences of age. In fact, the young ascites tumor cells all have similar duration of the mitotic cycle regardless of their ploidy (Table 4, Hauschka et al. 1957). These different results have pointed out that the duration of mitotic cycle may be controlled by a very complex system, perhaps involving the interaction of many factors, both intracellular and intercellular.

The mitotic cycle time of A. pilosa is shorter than that of A. strigosa 2X. This result does not support the conclusion of Van't Hoff (1965) that in different diploid plant species the cells with higher DNA content would have a longer duration of mitotic cycle. Actually, an exception was found in his own studies (Table 1. Van't Hoff 1965); in Impatiens balsamina the cellular DNA was higher than that of Crepis capillaris, but the duration of mitotic cycle of the former was shorter than that of the latter. These examples have again indicated that besides the quantity of DNA per cell some other factors are involved in the timing of the mitotic cycle. Giméney-Martín et al. (1966) found that the durations of mitotic cycles of the diploid, and caffein induced binucleate and tetraploid cells in onion roots were 13.5, 14.0 and 14.5 hr respectively. They ascribed the different ratios of nuclear-surface area and nuclear volume to the different durations of mitotic cycles of the three types of cells.

The most variable phase in the cell cycles of the three

species studied is G_1. These data are consistent with the reports on most mammalian cell types (Defendi and Manson 1963). The most striking point, however, is that there is no detectable G_1 phase in the cells of A. pilosa, and it is for this reason that the mitotic cycle of this species is shorter than that of A. strigosa 2X.

Although most cell types reported in the literature do have a G_1 phase, there are also some other reports showing the absence of a G_1 period in various types of cells. For example, the G_1 does not exist in slime mold (Nyggard et al. 1960). Xenopus eggs start DNA synthesis during telophase (Graham 1966) and in neuroblasts of grasshoppers, the S phase fills the entire interphase (Gaulden 1956). The absence of G_1 has been shown in mammalian cells too, such as Ehrlich ascites tumor cells (Basergo 1963; Lala and Patt 1966), pre-enamel cell of the young rabbit (Starkey 1963), and the lung cells of Chinese hamster in cultures (Robbins and Scharff 1967). In higher plants, the generative nucleus of pollen grains starts its DNA synthesis in the early interphase (Taylor 1958a; Woodward 1958); even in the much studied root tip cells of Vicia faba, G_1 is variable and may be missing (0 to 2 hr), as reported by the pioneer workers in this field, Howard and Dewey (1960). The foregoing examples show that the lack of G_1 in some types of cells may not be abnormal but rather it may be an inherited variation of that type of cells. One must be prepared to accept wide differences among cell types.

One more note that could be added here is that the absence of the G_1 phase in the root cells of A. pilosa has solved a puzzle which arose when the amount of nuclear DNA of the cells was measured, because some of the late telophase-like nuclei gave intermediate values between 2C and 4C; it might be that those cells were already in early interphase, and because no G_1 period exists, these cells had already started DNA synthesis.

The data of these experiments have shown that the doubling of nuclear DNA resulting from polyploidization in Avena species has not altered the function of the chromosome in relation to temporal control of DNA replication. Furthermore, it also indicates that the extra DNA in A. pilosa may be associated with late replicating regions along the chromosomes. Van't Hoff (1967) has reported that three related varieties of Pisum have similar durations of mitotic phases in their cell cycles. The difference in the durations of mitotic phases of A. pilosa and A. strigosa may be taken as another indication of the degree of divergence between these two species.

As may be seen from the similar duration of S phases of diploid and autotetraploid strigosa, the homologous chromosomes within the Avena genome may have a similar pattern of DNA synthesis in a synchronous fashion irrespective of ploidy. This speculation could be further tested by direct comparison of the pattern of DNA synthesis in the chromosomes of these species, similar to the studies of Darlington and Haque (1964) in rye root, of Hsu et al. (1964) in Chinese hamster cells

and of German (1964) in human cells. If this is true, then the origin of a particular genome in hexaploid oats may be further resolved by comparing the replication patterns of chromosomes of different diploid species and those of the hexaploids.

For instance, the presumptive donor of the C genome, A. ventricosa, could be tested by this method. It has been shown that the regions of secondary constrictions are usually late replicating (Schmid 1963; Bianchi 1966; Bianchi and Molina 1967). Chromosome SM_{15} in the A. sativa complement was suspected of having evolved from the presumed second satellited chromosome of A. ventricosa by losing its satellite (Rajhathy 1966). It is quite possible that, by studying the replication patterns of the two chromosomes, their homology may be proved or disproved, because, if the presumed satellited chromosome of A. ventricosa is the donor of SM_{15} in A. sativa, the distal regions of the short arm of the two chromosomes might be both late replicating and the other parts of the chromosomes might also show the same pattern.

REFERENCES

Alfert, M., 1950. A cytochemical study of oogenesis and cleavage in the mouse. J. Cellular Comp. Physiol., 36, 381-409.

Baserga, M.A., 1963. Mitotic cycle of ascites tumor cells. Arch. Pathol., 75, 156.

Bender, M.A. and Prescott, D.M., 1962. DNA synthesis and mitosis in cultures of human peripheral leukocytes. Expt. Cell Res., 27, 221.

Bianchi, N.O., 1966. Chromosome of the rat. II. DNA replication sequence of bone marrow chromosomes in vivo. Cytologia, 31, 276-293.

_____, and Molina, O.J., 1967. Chronology and pattern of replication in the bone marrow chromosomes of Gallus domesticus. Chromosoma (Berl.), 21, 387-397.

Brachet, J., 1947. Nucleic acids in the cell and the embryo. Symp. Soc. Exp. Biol., 1, 207.

Cairns, J., 1966. Autoradiography of HeLa cell DNA. J. Molecular Biol., 15, 372-373.

Cameron, I.L. and Stone, G.E., 1964. Relation between the amount of DNA per cell and the duration of DNA synthesis in three strains of Tetrahymena pyriformis. Expt. Cell Res., 36, 510-514.

Carlson, L., Caspersson, T., Foley, G.E., Kodynowsky, J., Lonakka, G., Simonsson, E., and Sörén, L., 1963. The application of quantitative cytochemical techniques to the study of individual mammalian chromosome. Expt. Cell Res., 31, 589-594.

Carr, J.G., 1945. Mechanism of the Feulgen reaction. Nature, 156, 143.

Catcheside, D. and Holmes, B., 1947. The action of enzymes on chromosomes. Sym. Soc. Exp. Biol., 1, 225.

Christensen, B., 1966. Cytophotometric studies on the DNA content in diploid and polyploid Enchytraeidae (Oligochaeta). Chromosoma (Berl.) 18, 305-315.

Conger, A.D. and Fairchild, L.M., 1953. A quick-freeze method for making smear slides permanent. Stain Tech., 28, 281-283.

Cosson, M.E., 1854. Classification des espèces du genre Avena du groupe de l'Avena sativa (Avena, dect. Avenatypus), et considérations sur la composition et la structure de l'épillet dans la famille des Graminées. Bull. Soc. Bot. France, 1, 11-17.

Darlington, C.D. and Haque, A., 1964. Organization of DNA synthesis in rye chromosomes. in "Chromosomes Today", C.D.Darlington and D.R. Lewis, ed., pp. 102-107. Plenum Press, New York.

Deeley, E.M., Davies, H.G. and Chayen, J., 1957. The DNA content of cells in the root of Vicia faba. Expt. Cell Res., 12, 582.

Defendi, V. and Manson, L.H., 1963. Analysis of the life-cycle in mammalian cells. Nature, 198, 359.

Dobzhansky, T.G., 1951. Chapter 8, in "Genetics and the Origin of Species" third edition, Columbia University Press.

Dodson, E.O., 1946. Some evidence for the specificity of the Feulgen reaction. Stain Tech., 21, 103-105.

Dodson, E.O., 1960. Chapter 19, in "Evolution: Process and Product", Reinhold, New York.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11, 1-42.

Ellison, W., 1940. The cytology of certain diploid, triploid and tetraploid Avena hybrids. Genetica, 22, 409-418.

Emme, H., 1932. Cytology of pentaploid oat hybrids. Bull. Appl. Bot., Genet. and Plant-Breed., Ser. II - 1, 169-176.

Evans, H., 1965. Discussion on W.J. Peacock's paper, "Chromosome replication", Natl. Cancer Inst., Mono., No. 18, 131.

Evans, H. and Scott, D., 1964. Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in Vicia faba. Genetics, 49, 17-38.

Gaulden, M.E., 1956. DNA synthesis and X-ray effects at different mitotic stages in grasshopper neuroblasts. Genetics, 41, 645.

German, J., 1964. The pattern of DNA synthesis in the chromosomes of human blood cells. J. Cell Biol., 20, 37-56.

Giles, N.H., 1965. Symposium on structure and function of genes and chromosomes: a summation. Natl. Cancer Inst. Mono., No. 18, 341-354.

Giménez-Martín, G., Gonzalez-Fernandez, A. and Lopez-Saez, J.F., 1966. Duration of the division cycle in diploid, binucleate and tetraploid cells. Expt. Cell Res., 43, 293-300.

Goldfeder, A., 1965. Biological properties and radiosensitivity of tumors: determination of the cell-cycle and time of synthesis of deoxyribonucleic acid using tritiated thymidine and autoradiography. Nature, 207, 612-614.

Graham, C.F., 1966. The effect of cell size and DNA content on the cellular regulation of DNA synthesis in haploid and diploid embryos. Expt. Cell Res., 43, 13-19.

Griffiths, D.F., Rowlands, D.W., and Peregrome, W.T.H., 1959. Cytogenetic relationships of certain artificial and natural species of Avena. J. Agri. Sci., 52, 189-199.

Hauschka, T.S., Grinnell, S.T., Révész, L. and Klein, G., 1957. Quantitative studies on the multiplication of neoplastic cells in vitro. IV. Influence of doubled chromosome number on growth rate and final population size. J. Natl. Cancer Inst., U.S. 19, 13.

Holden, J.H.W., 1966. Species relationships in the Avenae. Chromosoma (Berl.), 20, 75-124.

Howard, A. and Dewey, D.L., 1960. Variation in the period proceeding deoxyribonucleic acid synthesis in bean root cells. In "The Cell Nucleus", J.S. Michell ed. Butterworth, London, pp. 155-162.

_____, and Pelc, S.R., 1953. Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. Heredity, 6 (Suppl.), 261-273.

Hsu, T.C., 1964. Mammalian chromosomes in vitro. XVII. DNA replication sequence in the Chinese hamster. J. Cell Biol., 23, 53-62.

_____, Schmid, W. and Stubblefield, E., 1964. DNA replication sequences in higher animals. in "The Role of Chromosomes in Development", N. Locke ed. Acad. Press, New York, pp. 83-112.

Hughes-Schrader, S., 1951. The desoxyribonucleic acid content of the nucleus as a taxonomic character in mantids (Orthoptera, Mantoidea). Biol. Bull., 100, 178-187.

_____, 1953. The nuclear content of desoxyribonucleic acid and interspecific relationships in the mantid genus Liturguosa (Orthoptera, Mantoidea). Chromosoma (Berl.), 5, 544-554.

_____, 1957. The DNA content of the nucleus as a tool in the taxonomic study of insects. Xth Inter. Congr. Entomology, pp. 935-944.

Huskins, C.L., 1931. A cytological study of Vilmonius unifixable dwarf wheat. J. Genet., 25, 113-124.

Johansen, D.A., 1940. Plant Microtechnique, New York, McGraw Hill.

John, B. and Hewitt, G.M., 1966. Karyotype stability and DNA variability in the Acrididae. Chromosoma (Berl.), 20, 155-172.

Kasten, F.H., 1956. Chromatin and the Feulgen reaction. J. Histochem. Cytochem., 4, 310-317.

Keyl, H.-G., 1965. A demonstrable local and geometric increase in the chromosomal DNA of Chironomus. Experienta (Basel), 21, 191-193.

Kihara, H. and Nishiyama, I., 1932. The genetics and cytology of certain cereals. III. Different compatibility in reciprocal crosses of Avena, with reference to tetraploid hybrids between hexaploid and diploid species. Japan. J. Bot., 6, 245-305.

Ku, T.W., 1965. The effect of imbibition and vernalization on some parameters of root growth. M.Sc. thesis, Department of Biology, University of Ottawa, Ottawa, Canada.

Kusanagi, A., 1966. Rate of DNA replication in the DNA synthetic period of the barley chromosomes. Chromosoma (Berl.), 20, 125-132.

Ladizinsky, G. and Zohary, D., 1968. Genetic relationships between diploid and tetraploid in series Eubarbatae of Avena. Can. J. Genet. Cytol., 10, 68-81.

LaLa, P.K. and Patt, H.M., 1966. Cytokinetic analysis of tumor growth. Proc. Natl. Acad. Sci. (Wash), 56, 1735 .

Lima-de-Faria, A., 1959. Differential uptake of tritiated thymidine into hetero- and euchromatin in Melanoplus and Secale. J. Biophys. Biochem. Cytol., 6, 457-466.

Matlzew, A.I., 1930. Wild and cultivated oats. Section Euavena Griseb. Bull. Appl. Bot. Genet. Plant Breeding (Leningrad), Suppl. 38, 1-522.

Marshall, J.G. and Myers, W.M., 1961. A cytogenetic study of certain interspecific Avena hybrids and the inheritance of resistance in diploid and tetraploid varieties to races of crown rust. Crop. Sci., 1, 29-34.

Martin, P.G. and Shanks, R., 1966. Does Vicia faba have multistanded chromosomes? Nature, 211, 650-651.

McGinnis, R.C., 1966. Establishing a monosomic series in Avena sativa L. In "Chromosome manipulations and plant genetics", Oliver and Boyd, Edinburgh and London, pp. 86-97.

McLeish, J. and Sunderland, N., 1961. Measurement of DNA in

higher plants by Feulgen photometry and chemical methods. Expt. Cell Res., 24, 527-540.

Mendelsohn, M.L., 1958. The two wavelengths method of microspectrophotometry. II. A set of tables to facilitate the calculations. Biophys. and Biochem. Cytol., 4, 415-424.

_____, Dohan, F.C. Jr., and Moore, H.A. Jr., 1960. Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C^3H mouse. I. Typical cell cycle and timing of DNA synthesis. J. Natl. Cancer Inst., 25, 477.

Moorhead, P.S. and Defendi, V., Asynchrony of DNA synthesis in chromosomes of human diploid cells. J. Cell Biol., 16,202.

Morishima, A., Grumbach, M.M. and Taylor, J.H., 1962. Asynchronous duplication of human chromosomes and the origin of sex chromatin. Proc. Natl. Acad. Sci., U.S. 48, 756.

Nishiyama, I., 1929. The genetics and cytology of certain cereals. I. Morphological and cytological studies on triploid, pentaploid and hexaploid Avena hybrids, Japan. J. Gen., 5, 1-48.

_____, 1934. Ibid. VI. Chromosome behaviour and its bearing on inheritance in triploid Avena hybrids. Mem. Coll. Agr. Kyoto Univ., 32, 1-257.

_____, 1936. Cytological studies in Avena. I. Chromosome association in hybrids between A. barbata Pott and autotetraploids of A. strigosa Schreb. Cytologia, 7, 276-281.

Nygaard, O.F., Guttes, S., and Rush, H.P., 1960. Nucleic acid metabolism in a slime mold with synchronous mitosis. Biochem. et Biophys. Acta., 38, 298.

Oehlert, W., Seemayer, N. and Lauf, P., 1962. Autoradiographic research on the cell generation cycle of the Ehrlich ascites carcinoma in the white mouse. Beitr. Pathol. Anat., 127, 63-68.

Ohno, S., and Atkin, N.B., 1966. Comparative DNA values and chromosome complements of eight species of fishes. Chromosoma (Berl.), 18, 455-466.

_____, Maramoto, J. and Christian, L., 1967. Diploid-tetraploid relationship among old- world members of the fish family Cyprinidae. Chromosoma (Berl.), 23, 1-9.

_____, Klein, J. and Atkin, N.B., 1968. Evolution from fish to mammals by gene duplication. Hereditas (Lund), 59, 169-187.

Patau, K., 1953. Absorption microphotometry of irregular-shaped objects. Chromosoma (Berl.), 5, 341-362.

Patau, K. and Swift, H., 1953. The DNA content (Feulgen) of nuclei during mitosis in a root tip of onion. Chromosoma (Berl.), 6, 149.

Pearse, E.G., 1960. Histochemistry, theoretical and applied. London, Churchill.

Plaut, K. and Nash, D. Localized DNA synthesis in polytene chromosomes and its implications. in "The Role of Chromosomes in Development" M. Locke ed. Acad. Press pp. 113-135.

Post, J. and Hoffman, J., 1964. Changes in the replication times and patterns of the liver cell during the life of the rat. Expt. Cell Res., 36, 111-123.

Quaster, H. and Sherman, F.G., 1959. Cell population kinetics in the intestinal epithelium of the mouse. Expt. Cell Res., 17, 420-438.

Rajhathy, T., 1961. Chromosomal differentiation and speciation in diploid Avena. Can. J. Genet. Cytol., 3, 372-377.

_____, 1963. A standard karyotype for Avena sativa. Can. J. Genet. Cytol., 5, 127-132.

_____, 1966, Evidence and an hypothesis for the origin of the C genome of hexaploid Avena. Can. J. Genet. Cytol., 8, 774-779.

_____, and Morrison, J.W., 1959. Chromosome morphology in the genus Avena, Can. J. Bot., 37, 331-337.

_____, and _____, 1960. Genome homology in the genus Avena. Can. J. Genet. Cytol., 2, 278-285.

Rajhathy, T. and Dyck, P.L., 1963. Chromosomal differentiation and speciation in diploid Avena. 2. Karyotype of A. pilosa. Can. J. Genet. Cytol. 3, 372-377.

_____, and Thomas, H., 1967. Ibid. 3. Mediterranean wild populations. Can. J. Genet. Cytol., 9, 52-68.

Reddi, K.K., 1959. The arrangement of purine and pyrimidine nucleotides in tobacco mosaic virus nucleic acid. Proc. Natl. Acad. Sci., 45, 293-300.

Rees, H. and Jones, G.H., 1967a. Chromosome evolution in Lolium. Heredity, 22, 1-18.

_____, and Jones, G.H., 1967b. Structural basis of quantitative variation in nuclear DNA. Nature, 216, 825-826.

Rees, H. and Walters, M.R., 1965. Nuclear DNA and the evolution of wheat. Heredity, 20, 73-82.

_____, Cameron, R.M., Jones, F.H. and Hazarika, M.H., 1966. Nuclear variation between diploid Angiosperms. Nature, 211, 828-830.

Riley, R. and Chapman, V., 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature, 182, 713-715.

Ris, H. and Mirsky, A.E., 1949. Quantitative cytochemical determination of deoxyribonucleic acid with Feulgen nucleal reaction. J. Gen. Physiol., 33, 125-146.

_____, and Chandler, B.L., 1963. The ultrastructure of genetic system in prokaryotes and eukaryotes. Cold Spring Harbor Symp., 28, 1-8.

Robbins, E. and Scharff, M.D., 1967. The absence of detectable G_1 phase in a cultured strain of Chinese hamster lung cell. J. Cell Biol., 34, 684.

Rothfels, K., Sexsmith, E., Heimburger, M. and Krause, M.O., Chromosome size and DNA content of species of Anemone L. and related genera (Ranunculaceae). Chromosoma (Berl.), 20, 54-74.

Sadasivaiah, R.S. and Rajhathy, T., 1968. Genome relationships in tetraploid Avena. Can. J. Genet. Cytol., (in press).

Schimid, W., 1963. DNA replication pattern of human chromosomes. Cytogenetics, 2, 175.

Schrader, F. and Hughes-Schrader, S., 1956. Polyploidy and fragmentation in the chromosomal evolution of various species of Thyanta (Hemiptera). Chromosoma, 7, 469-496.

Schrader, F. and Hughes-Schrader, S., 1958. Chromatid autonomy in Banasa (Hemiptera: Pentatomidae). Chromosoma (Berl.), 9, 193-215.

Sharma, A.K. and Sharma, A., 1965. Chromosome Techniques, Theory and Practice, Butterworth, London.

Sisken, J.E., 1959. The synthesis of nucleic acid and proteins in the nuclei of Tradescantia root tips. Expt. Cell Res., 16, 602.

Sisken, J.E. and Kinosita, R., 1961. Timing of DNA synthesis in the mitotic cycle in vitro. J. Biophys. Biochem. Cytol., 9, 509.

Snedecor, C.W. and Cochran, W.G., 1967 "Statistical Methods" 6th ed. The Iowa State College Press, Ames, Iowa.

Stanner, C.P. and Till, J.E., 1960. DNA synthesis in individual L-strain mouse cells. Biochem. et Biophys. Acta, 37, 406.

Stanton, T.R., 1953. Production, harvesting, processing, utilization and economic importance of oats. Econ. Bot., 7, 43-64.

Starkey, W.E., 1963. The migration and renewal of tritium labeled cells in the developing enamel organ of rabbits. J. Brit. Dental Ass., 115, 143.

Stebbins, G.L., 1947. Types of polyploids: their classification and significance. Advance Genet., 1, 403-429.

Stedman, E. and Stedman, E., 1950. The cytological interpretation of the Feulgen reaction. Biochem. J., 47, 508-512.

Sunderland, N. and McLeish, J., 1961. Nucleic acid content

and concentration in root cells of higher plants. Expt. Cell Res., 24, 541-554.

Swami, U.B.S. and Thomas, H., 1966. Studies on the relationship of Avena sativa (6x) and Avena hirtula (2x). Can. J. Genet. Cytol., 8, 51-56.

Swift, H., 1950. The constancy of deoxyribose nucleic acid in plant nuclei. Proc. Natl. Acad. Sci., U.S.,36, 643-654.

_____ and Rash, E., 1956. Microphotometry with visible light. In Physical techniques in biological research. Edited by G. Osler and A. Pollister, Acad. Press, New York.

Taylor, H.J., 1958a. Incorporation of phosphorus-32 into nucleic acids and proteins during microgametogenesis of Tulbaghis. Am. J. Bot., 45, 123.

_____, 1958b. The mode of chromosome duplication in Crepis capillaris. Expt. Cell Res., 15, 350-357.

_____, 1960a. Nucleic acid synthesis in relation to the cell division cycle. Ann. N.Y.Acad. Sci., 90, 409.

_____, 1960b. Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. J. Biophys. Biochem. Cytol., 7, 455.

_____, 1963. Control mechanism for chromosome replication in the cell cycle. Symp. Int. Soc. Cell Biol., 2, 161-177.

Thellung, A., 1911. Uber die Abstammung, den sustematischen Wert und die Kulturgeschichte der Saathafer-Arten (Avenae sativae Cosson) Viertelj. der Natur. Ges., 56, 293-350.

Thomas, H. and Jones, M.L., 1964. Cytological studies of penta-

ploid hybrids and a synthetic decaploid in Avena. Chromosoma (Berl.), 15, 132-139.

Thomas, H. and Jones, M.L. 1965. Chromosomal differentiation in diploid species of Avena. Can. J. Genet. Cytol., 7, 108-111.

_____, and Rajhathy, T., 1967. Chromosome relationships between Avena sativa (6x) and Avena pilosa (2x). Can. J. Genet. Cytol., 9, 154-162.

Ullerich, F.-H., 1966. Karyotp und DNS-Gehalt von Bufo bufo. B. viridis, B. bufo X B. viridis und B. calamita (Amphibia, Anura). Chromosoma (Berl.), 18, 316-342.

Van't Hoff, 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. Expt. Cell Res., 39, 48-58.

Van't Hoff, J., 1966. Comparative cell population kinetics of H^3-T labeled diploid and colchicine-induced tetraploid cells in the same tissue of Pisum. Expt. Cell Res., 41, 274-288.

_____, 1967. Studies on the relationships between cell population and growth kinetics of root meristems. Expt. Cell Res., 46, 335-347.

Wimber, D.E., 1960. Duration of the nuclear cycle in Tradescantia paludosa root tips as measured with H^3-thymidine. Am. J. Bot., 47, 828-834.

_____, 1961. Asynchronous replication of deoxyribonucleic acid in root tip chromosomes of Tradescantia paludosa.

Expt. Cell Res., 23, 402-407.

Wimber, D.E., 1963. Methods for studying cell proliferation with emphasis on DNA labels. In "Cell Proliferation". L.F. Lamerton and R.J.M. Fry ed. Blackwell, Oxford, pp. 1-17.

Woodard, J.W., 1958. Intracellular amounts of nucleic acids and protein during pollen grain growth in Tradescantia. J. Biophys. Biochem. Cytol., 4, 383.