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PACHYTENE CHROMOSOME STUDIES IN THE GENUS *MEDICAGO*

by

© CHRISTOPHER BOB GILLIES

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Pachytene Chromosome Studies in the Genus *Medicago*" submitted by Christopher Bob Gillies in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Supervisor

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ABSTRACT

Twelve perennial species of *Medicago* were examined with the light microscope at the pachytene stage of meiosis, and idiograms were constructed describing the karyotypes of 11 of them and of some of their interspecific hybrids. Five diploid species - *M. sativa, M. falcata, M. glomerata, M. coerulea* and *M. glandulosa* were found to have karyotypes which could be described by a common idiogram designated as the coenospecies *M. sativa sensu lato (s.l.)*. The karyotype of diploid *M. prostrata* was very similar to this group. The pachytene chromosomes of tetraploid *M. glutinosa* were similar to those of *M. sativa* and quadrivalents indicated probable autotetraploid origin.

The karyotypes of *M. daghestanica, M. pironae* and their hybrid were almost identical, as were the karyotypes of *M. rhodopaea* and the hybrid *M. rhodopaea* X *M. rupestris*. Both groups had karyotypes fairly similar to *M. sativa s.l.* and hence probably share a common ancestry with the *M. sativa* coenspecies. The idiograms of *M. hybrida, M. suffruticosa* and their hybrid were almost identical but differed markedly from those of all other perennials studied and appear unrelated to all other perennial species studied.

Pachytene studies of diploid, tetraploid and hexaploid species of *M. sativa s.l.* showed that there was a decided contraction of chromosomes with the increase in ploidy level. Investigation of dihaploid plants derived from tetraploid alfalfa showed that these were similar in karyotype to diploid *M. sativa s.l.* and hence the parents can be considered autotetraploids. Using the *M. sativa s.l.* idiogram the chromosomes present in triplicate in eight trisomics and one double trisomic

of diploid alfalfa were tentatively identified.

Five annual species of *Medicago* having 2n = 14 chromosome numbers were analysed at pachytene and idiograms prepared. *M. murex* was represented by both 2n = 14 and 2n = 16 accessions, and it was found that the 14 chromosome karyotype was probably derived from the 16 chromosome karyotype by unequal reciprocal translocations. Differences between the two suggest that they should be classified as two species. Three of the other 2n = 14 idiograms - *M. polymorpha*, *M. praecox* and *M. rigidula* had one extremely long chromosome which was hypothesized to have been formed by translocation from two chromosomes of 2n = 16 ancestors. *M. constricta* did not have such an unusually long chromosome.

Early prophase sporocytes of the double trisomic TR39^{L}2 were examined with the electron microscope and the synaptonemal complexes formed by synapsed chromosomes were described. The fine structure of the centromere was studied and found to be similar to previous descriptions of mammalian mitotic chromosome centromeres. The association of the nucleolar chromosomes with the nucleolus was similar in structure to the centromere but no synaptonemal complexes were found at the chromosome-nucleolus junction.

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INTRODUCTION

The genus *Medicago* includes 34 annual species and 27 perennial species (Bolton, 1962). The annuals, usually prostrate or semi-prostrate herbs with spiny, coiled pods, are found in the areas surrounding the Mediterranean Sea. The perennials are mostly upright to decumbent herbs having both smooth and spiny pods varying from coiled to crescent shaped. Most of the perennials appear to have originated around the Black Sea, the Caspian Sea and parts of Southern Russia, the Ukraine and Persia.

Although Linnaeus described nine species of the genus *Medicago*, Urban (1873) was the first and only person to publish a comprehensive monograph of all the then known species in the genus. Urban described 46 species, but his grouping of species within the genus was artificial since annuals and perennials (which are not closely related) were placed in the same sections. Much confusion has continued to occur due to several factors: 1. in annuals due to incorrect identification and a multiplicity of species name synonyms. 2. in perennials due to the affording of species rank to geographical forms, and to ease of inter-specific crosses. 3. confusion over where the genera *Medicago*, *Trigonella* and *Melilotus* overlap.

The difficulty of cytological study in *Medicago* has deterred workers from undertaking detailed analysis of chromosomes, so that until recently most reports were confined to chromosome counts. These were mostly n = 8, with a few species n = 7 and some limited polyploidy. These studies did little to explain relationships within the genus. Heyn (1963) revised the taxonomy of the annual species of *Medicago* so that accurate identification of them is now possible. Following this,

Simon (1965) described the karyotypes of 22 annual species using somatic chromosomes, but the smallness and uniformity of the chromosomes limited the usefulness of the idiograms for evaluating species relationships. In perennial species the discovery of different ploidy levels in single species, and the crossability of many "species" have, if anything, led to an even more confused picture of species relationships.

In the late 1950's there appeared the first studies which showed that analysis of pachytene chromosomes in *Medicago* was possible (Sprague, 1959), and might be a more productive means of comparing chromosomes from different species. The last few years have seen this field reach fruition with the publication of pachytene idiograms of several perennial species. With these technical problems overcome the way was now open for the use of pachytene chromosome karyotypes in the study of species relationships in the genus *Medicago*.

A number of perennial species readily cross with either diploid or tetraploid *M. sativa*, and hence these species were studied to determine what degree of similarity there was between karyotypes of such interfertile species. Other groups of perennials which are unrelated to the *M. sativa* group were studied to determine how they differed in karyotype from *M. sativa*. As the technique used was also satisfactory for annual species, those annuals with 14 chromosomes were studied in an effort to determine the origin of the 14 chromosome forms. *M. murex* accessions with both 14 and 16 chromosomes had been found and these were studied in an attempt to determine the relationship of the two karyotypes.

Since the karyotypes of members of the *M. sativa* complex had been determined, it was possible to study the effect of different ploidy levels on the morphology of certain chromosomes. The pachytene chromo-

somes of dihaploids derived from tetraploid alfalfa were studied and measured. Using these measurements the likelihood of allo- or autotetraploid origin of the parents was investigated. Pachytene chromosomes were also used in an attempt to identify the chromosomes present in triplicate in a number of trisomic lines of diploid alfalfa.

The pachytene chromosomes of perennial *Medicago* are divided into distinct chromatic and achromatic regions. They appear to be ideal for study of any effects that differences in chromosome structure might have on the synaptonemal complexes formed during meiotic pairing of chromosomes. For this reason electron microscope studies of pachytene stages were undertaken. A plant was chosen which was trisomic for both the highly chromatic nucleolar organizing chromosome and a second non-nucleolar chromosome. The trisomic chromosomes form trivalents at pachytene and these were thought to offer a means of recognizing chromosomes in electron microscope preparations. The nucleolus would identify the N.O. chromosome, and the morphology of this region could be studied.

A. LIGHT MICROSCOPE STUDIES

I. GENERAL REVIEW OF *MEDICAGO* CYTOGENETICS AND PACHYTENE STUDIES

1. Chromosome numbers and ploidy levels in the genus

Fryer (1930) appears to have been the first to count the chromosomes of a wide range of *Medicago* species. He quotes two previous reports of chromosome counts, Karpechenko (1925) - cited by Tischler (1927), and Ghimpu (1928), but these reports were in Russian and French respectively and not widely known prior to his work. Fryer found 2n = 16 and 2n = 32 for most species, with *M. rigidula* and *M. hispida* (syn. *M. polymorpha*) having 2n = 14. He measured chromosomes and reported length differences between species. Cooper (1936) and Senn (1938) reported chromosome numbers for further species.

In 1956 Heyn studied the chromosome numbers of 19 species and reported n = 7, 8 and 9, although the latter number is disputed by Clement (1962) as probably being an error. In his extensive study of both perennial and annual species in 1962, Clement found species with n = 7, 8 and 16. He found a great deal of confusion and erroneous nomenclature amongst the annual species and concluded that only n = 7 was to be found in the species *M. polymorpha, M. rigidula* and *M. coronata.* Lesins and Lesins (1962) reported similar confusion and listed the first two species plus *M. praecox* as being those with n = 7. Heyn (1963) has given a fairly complete summary of all the available cytological data on annual *Medicagos* and lists *M. polymorpha, M. rigidula* and *M. praecox* as the species with n = 7. Lesins and Lesins (1963a)

added *M. globosa* (syn. *M. constricta*) to the list of n = 7 annual species.

The occurrence of a 2n = 16 *M. sativa* was reported by Bolton and Greenshields (1950). It had two satellited chromosomes and resembled the tetraploid *M. sativa* in flower and pod characters. It crossed normally with diploid *M. falcata* but set little seed in crosses with tetraploid *M. sativa*. They reported also four satellites in tetraploid *M. sativa* and suggested this as evidence for an autotetraploid origin. Sprague (1959) found two satellites in diploid *M. falcata* and *M. gaetula** and found these two species cytologically indistinguishable from diploid *M. sativa*. He suggested that all three diploid species might have been involved in the origin of tetraploid *M. sativa*.

Lesins (1959, 1963) has described two naturally occurring hexaploids, *M.cancellata* and *M. saxatilis*. Recently, Bingham and Binek (1969a) found two hexaploid *M. sativa* plants among a number of commercial tetraploid *M. sativa* plants. Another source of interest in ploidy change has been the occurrence of dihaploid (2n = 2x = 16), also called haploid, plants derived from tetraploid plants. There have been four reports of obtaining this type of plant from tetraploid *M. sativa* in the last thirteen years. The dihaploids reported by Lesins (1957), Stanford and Clement (1958), and Clement and Lehman (1962) all arose naturally, while Bingham (1969) obtained a number of dihaploids by a

* Lesins (personal communication) suggests this may have been *M. coerulea* since *M. gaetula* is considered to be a tetraploid form of *M. sativa*.

deliberate programme of 4n x 2n crosses, using male sterile tetraploids carrying recessive markers to facilitate rapid screening of progeny for dihaploid plants.

The three earlier reported dihaploids were subjected to meiotic studies and crossed with other diploid species. Lesins (1957) reported some meiotic irregularities, while Stanford and Clement (1958) found largely regular chromosome pairing, although they did detect a quadrivalent and univalents. The dihaploid found in a polyembryonic plant of the variety 'African' by Clement and Lehman (1962) was self sterile but highly fertile in crosses with other diploids. It had normal metaphase I pairing but the nucleolus organizing chromosomes appeared to differ by a duplication which caused unequal satellites and resulted in a loop forming at pachytene.

Since 1960, Lesins and Lesins (1960, 1961, 1963a, 1965, 1966) have described a large number of species and given their somatic chromosome numbers and sizes. Included have been *M. prostrata* (2n = 16 and 2n = 32), *M. daghestanica*, *M. pironae*, *M. hybrida*, *M. rupestris*, and *M. glomerata* (all 2n = 16).

2. *Medicago* species relationships

The common perennial species *M. sativa* and *M. falcata* have often been crossed. Ledingham (1940) crossed tetraploid *M. sativa* with diploid *M. falcata* and obtained triploid hybrids which had eight bivalents and eight univalents at metaphase I of meiosis. He also obtained some tetraploid hybrids when the diploid was used as female parent. These tetraploid hybrids formed 16 bivalents and were fertile.

Ledingham considered the chromosome pairing in the tetraploid hybrids to be at random. Nilan (1951) found that the parents of the variety 'Rhizoma' were tetraploid hybrids of a similar origin to Ledingham's.

Lesins (1952) found it possible to cross *M. sativa* with *M. falcata, M. glutinosa* and *M. coerulea* at the same ploidy level. Oldemeyer and Brink (1953) crossed autotetraploid *M. falcata* with *M. media* and obtained hybrids which were more fertile than the autotetraploids. Sprague (1959), as mentioned above, obtained interspecific and trispecific hybrids between diploid *M. sativa, M. falcata* and *M. gaetula*. All hybrids had regular meiosis and were highly fertile. Cleveland and Stanford (1959) obtained essentially the same results as Ledingham (1940) in crosses between diploid *M. falcata* and tetraploid *M. sativa*. They also obtained tetraploid hybrids using *M. falcata* as male parent in the cross, and suggested these were due to unreduced *M. falcata* gametes.

The dihaploid *M. sativa* plant found by Lesins (1957) was crossed with diploid *M. falcata* and the hybrid obtained was more fertile than the dihaploid parent, having 85% normal chromosome pairing at diakinesis. Clement and Stanford (1961a) obtained a better seed set when they crossed their dihaploid *M. sativa* with tetraploids than when they crossed it with diploids. In crosses with tetraploids they obtained some pentaploids. These they explained as the result of fertilization of 24 chromosome gametes caused by abnormal partition of meiotic products in the dihaploid. McLennan, Armstrong and Kasha (1966) obtained triploid and tetraploid hybrids in crosses between male sterile tetraploid *M. sativa* and diploid *M. sativa, M. falcata, M. coerulea* and

M. hemicycla. The triploid plants were used to produce trisomics (Kasha and McLennan, 1967). The tetraploid hybrids had high pollen fertility and up to 14 bivalents at metaphase I of meiosis. The transfer of diploid *M. sativa* and *M. falcata* germ plasm to tetraploid *M. sativa* was found by Bingham (1968c) to entail few difficulties.

The relationship of tetraploid *M. sativa* to diploid species, and its auto- or allo-tetraploid origin are problems which have engaged many workers. Ledingham (1940), Julen (1944), and Grun (1951) all found quadrivalents in meiotic studies of tetraploid *M. sativa* and put forward the idea of an autotetraploid origin.

Armstrong (1954), after studying pairing in 4n, 6n and 8n progeny of common alfalfa, decided that the genomes were not completely homologous. He concluded that common tetraploid alfalfa was probably an allotetraploid, derived from related diploid species which differed by well marked morphological characters. Cleveland and Stanford (1959) compared a tetraploid *M. sativa* with a colchicine - induced autotetraploid *M. falcata.* The frequency of quadrivalents in the *M. falcata* was 2.74 per cell and in the *M. sativa* 1.74 per cell, a result which they interpreted as being evidence of diploidization of a former autotetraploid. They quoted Gilles and Randolf (1951) who had found a reduction in quadrivalent frequency over a period of ten years in an autotetraploid line of maize. Cleveland and Stanford (1959) suggested that genetic and structural chromosomal changes have reduced the quadrivalent frequency of *M. sativa* from the originally higher figure it would have had as a new autotetraploid. Nicoloff(1962a) found that in the tetraploid alfalfa he studied, about one third of pollen mother

cells had four quadrivalents, but 48% of cells had 16 bivalents. Gillies (1970b) noted only three different quadrivalents at pachytene in the tetraploid *M. sativa* he studied. Clement and Stanford (1961b) suggested that the lower chiasma frequency they had noticed in the more chromatic knobbed arm of alfalfa chromosomes would result in fewer quadrivalents being formed.

The fairly good pairing of the eight bivalents found in diploid *M. sativa* by Stanford and Clement (1958) led them to conclude that *M. sativa* was essentially an autotetraploid. They interpreted the reciprocal translocation present as evidence of diploidization having occurred. Gillies (1970b) encountered difficulty in pairing some of the bivalents of the tetraploid *M. sativa* but found at least four pairs which were virtually identical. Urata and Britten (1965) pointed to non-homologous pachytene pairing and the presence of translocations in their tetraploid *M. sativa*, as evidence for some alloploid origin.

The reports of tetrasomic inheritance of flower colour (Stanford, 1951) and of tetrasomic dosage effects (Stanford, 1959a), have led to reappraisal of many inheritance studies in tetraploid *M. sativa*. Barnes and Hanson (1967) list 32 tetrasomic and seven disomic characters, and this has added to the evidence in favour of an autotetraploid origin of tetraploid *M. sativa*.

A number of crosses have been carried out between *M. sativa* - *M. falcata* and more distantly related species, and between other species in the genus, to determine their relationships. Oldemeyer (1956) found that crosses between species in the section Falcago were generally

successful. He obtained seed in crosses of tetraploid *M. sativa* and *M. hemicycla*. Lesins (1956, 1961a) crossed *M. dzhawakhetica* (2n = 32) with tetraploid *M. sativa* and obtained hexaploid hybrids. In crosses of the tetraploid *M. dzhawakhetica* with diploid *M. sativa* he obtained triploids. It appeared that a genomic ratio of two *dzhawakhetica* to one *sativa* was necessary for successful crossing. Hexaploid hybrids gave pentaploids when back crossed to tetraploid *M. sativa*.

Clement (1963) studied a hybrid between diploid *M. dzhawakhetica* and diploid *M. sativa*. This plant was 2n = 16 and had fairly regular meiotic pairing. Chiasma were noted in both arms of some bivalents and chromosome homology at pachytene was good. A few seeds were obtained in crosses of the hybrid with diploid *M. sativa*. Clement concluded that the 2:1 genomic ratio proposed by Lesins (1961a) was not essential and that desirable characters such as disease resistance could be transferred from *M. dzhawakhetica* to *M. sativa*.

In crosses of *M. cancellata* (2n = 48) with tetraploid and artificial hexaploid *M. sativa*, Lesins (1961b) obtained pentaploid and near hexaploid progeny respectively. Similar results were obtained with hexaploid *M. saxatilis* by Lesins (1970). Segregation of anthocyanin in the *M. cancellata* x *M. sativa* hybrids (Lesins, 1961b) suggested some pairing and crossing over between the species chromosomes in the hybrid. Lesins (1962) found that crosses of diploid and tetraploid *M. prostrata* with diploid and tetraploid *M. sativa* respectively gave good seed set when the *M. sativa* plants were the female parents, but the reciprocals produced shrunken seed. The chromosome pairing and disjunction in the diploid hybrid appeared normal, but in the tetraploid

hybrid deviation from expected ratios of anthocyanin segregation led Lesins to suggest that preferential pairing was occurring within the *M. sativa* and *M. prostrata* genomes of the hybrid. Lesins (1968) also studied crosses of *M. glomerata* with *M. sativa, M. falcata* and *M. prostrata*, and found that *M. glomerata* was more closely related to *M. sativa* and *M. falcata* than was *M. prostrata*.

Other perennial species crossed by Lesins included *M. daghestanica* x *M. pironae* (Lesins and Gillies, 1968) in which sterile hybrids were produced, and *M. hybrida* x *M. suffruticosa* (Lesins, 1969) which produced fully fertile, normally segregating hybrids. Lesins also studied hybrids between the annuals *M. striata* and *M. littoralis* (Lesins and Erac, 1968a) and found reciprocal differences and hybrid mortality, but the hybrids between *M. aschersoniana* and *M. laciniata* (Lesins and Erac, 1968b) were of high fertility and segregated normally. Lilienfeld (1965) noted reciprocal plastid differences and malfunctioning plastids in crosses between two races of *M. truncatula*.

Simon has studied the relationships of annual species of *Medicago* by several methods, including comparison of somatic chromosome idiograms (Simon and Simon, 1965), grafting affinities (Simon, 1967a), one and two dimensional chromatography of phenolics (Simon, 1967b; Simon and Goodall, 1968), and by serological studies (Simon, 1969). Lesins and Lesins (1963c) compared pollen morphology of 53 types including 49 species, and found this character to be as useful as other characters in determining phylogenetic relationships.

3. Pachytene studies

The technique of pachytene analysis of the chromosomes of a plant was pioneered by McClintock in 1930 and 1931, when she cytologically demonstrated translocations, inversions and duplications in maize. The chromosome complement of maize was extensively studied by this means and Longley (1941) gave a complete description of the pachytene karyotype. Brown (1949) and Barton (1950) used the technique to analyse the chromosomes of tomato, and they advanced the idea of using chromomeres and knobs for distinguishing chromosomes. They found that the centromeres were surrounded by chromatic zones (Brown, 1949) which stained more heavily than the smaller chromomeres in the achromatic regions (Barton, 1950). Brown also noted telomeric chromomeres, and a slower pairing in the chromatic regions compared with the centromere and achromatic regions. Gottchalk (1954) produced an extensive study of 26 species of the related genus *Solanum* and compared these with several other genera.

Following the work of Brown and Barton, a considerable increase in the range of plants which have been subject to pachytene analysis has occurred. A detailed pachytene map of rye in which particular attention was paid to chromomere structure was published by Lima-de-Faria in 1952. He reported no essential difference in the organization of chromatic and achromatic regions at the light microscope level of magnification, and decided that differences reported by other workers could be explained by variations in chromomere size and fibrillae staining. Shastry, Rao and Misra (1960) analysed *Oryza sativa* at pachytene and identified the 12 bivalents on the basis of length and arm ratio.

Das and Shastry (1963) analysed three forms of *Oryza perennis* and found variations in total chromosome length within and between forms. They also noted differences between forms in number and morphology of nucleolus organizing chromosomes.

Pachytene chromosomes have been divided into two broad morphological classes by Rick and Khush (1966). The differentiated tomato type appears to be the norm for dicotyledons, as it is found in ten other Angiosperm families. The *Zea* type, which has dispersed chromatin, appears to be more common in monocotyledons. It is the type found in rye by Lima-de-Faria (1952) and in rice by Shastry et al (1960). Lima-de-Faria and Sarvella (1962) compared the pachytene karyotypes of *Zea*, *Solanum* and *Salvia*. They found a gradient of decreasing chromomere size on each side of the centromere in all *Solanum* chromosomes except the nucleolus organizing chromosome. *Salvia* had knobs surrounding the centromere and a less distinct gradient, while *Zea* had little gradient in chromomere size along the chromosomes.

Rhoades (1955) noted that maize karyotypes from somatic and pachytene chromosomes had the same chromosome order except for two reversals. Hyde (1953) reported that in *Plantago ovata* the chromosome order was similar in mitotic prophase and pachytene idiograms. Lima-de-Faria (1952) found that the order of pachytene chromosomes in rye was the same as that reported earlier for somatic chromosomes by Lewitsky (1931), but that the nucleolus organizing chromosome was relatively shorter at pachytene.

Brown (1949) and Barton (1950) had suggested that chromatic areas of chromosomes would form a greater proportion of somatic chromo-

some lengths than would achromatic areas, due to differential contraction. This was confirmed by Ramanna and Prakken (1967) who found contraction rates of achromatic parts of tomato chromosomes averaged about six times those in chromatic parts. This could cause a considerable change in arm ratios and relative chromosome length from a pachytene to a somatic idiogram.

The paucity of pachytene studies until very recently in such an important crop as alfalfa, may be due to the inherent difficulties in obtaining good cytological preparations of meiotic material in *Medicago*. Sprague (1959) examined a trispecies hybrid between diploid *M. sativa, M. falcata* and *M. gaetula*. He noted apparently normal synapsis and gave a range of chromosome lengths of 33 microns to 54.7 microns. One bivalent was attached to the nucleolus at diakinesis. Clement and Stanford (1961b) noted that the shorter arms of *M. sativa* pachytene chromosomes appeared more chromatic and that chromosomes had knobs at both ends.

In 1963 Clement and Stanford published the first attempt at a pachytene idiogram for diploid *M. sativa*. The plants studied were a dihaploid *M. sativa* and crosses of it with diploid *M. sativa* and *M. falcata*. Two of the chromosomes were found to be associated in a translocation quadrivalent. Two more chromosomes were not identifiable and the remaining four ranged in length from 50 microns to 37.4 microns. The shortest was the satellited nucleolus organizing chromosome. Each chromosome had a knob at only one end (at the end of the short arm, if the centromere was submedian). The knobbed arm was more chromatic than the unknobbed, and several interstitial knobs

were found on most chromosomes. Arm ratios ranged from 0.93 to 2.42, the knobbed arm being used as divisor. The satellited chromosome was much more chromatic than the other chromosomes.

The results of Urata and Britten (1965) who worked on a tetraploid *M. sativa*, largely agreed with those of Clement and Stanford (1963), but chromosomes were more chromatic in their shorter arms. Urata and Britten concluded from differences in chromosome morphology that their tetraploid alfalfa was of different origin from Clement and Stanford's diploid material. Urata and Britten also noted non-homologous pairing and precocious separation of achromatic areas of bivalents at pachytene.

Buss and Cleveland (1968b) and Gillies (1968) published complete idiograms of the pachytene chromosomes of diploid *M. sativa*, Buss and Cleveland after first attempting a somatic chromosome analysis (1968a). These two pachytene idiograms agreed well in terms of chromosome lengths, total haploid complement lengths, arm ratios and general morphology of chromosome knobs. However, the order of the chromosomes, based on relative lengths, was not the same. Buss (1967) had found considerable differences between somatic and pachytene karyotypes of the diploid *M. sativa* reported by Buss and Cleveland (1968a, b), particularly in the relative lengths of the nucleolus chromosomes, which were shortest at pachytene but were fifth in length in somatic metaphases. They also differed considerably in arm ratios between the two stages. Other chromosomes in the complement could be matched between the two stages, mainly on the basis of arm ratios, so that the order by relative lengths did not agree in the two stages analysed.

Gillies (1970 a, b) has recently published idiograms of diploid *M. falcata* and tetraploid *M. sativa*. The former species was very similar to the diploid *M. sativa* described above, both in lengths and arm ratios of chromosomes, and in general chromosome morphology, although the chromosomes were not as chromatic as those of *M. sativa* and appeared to lack some of the terminal knobs of that species. The chromosomes of the tetraploid *M. sativa* (Gillies, 1970b) were much shorter than those of either diploid species and much more chromatic, although the pattern of chromomeres and the arm ratios were quite similar to those of the diploids, suggesting a greater degree of contraction. Gillies was able to identify three different quadrivalents at pachytene in the tetraploid.

Meanwhile Ho (1969) had studied other lines of diploid *M. sativa* and *M. falcata* and their hybrids. The pachytene chromosomes for *M. sativa* reported by Ho were much shorter than those reported by Buss and Cleveland (1968b) and Gillies (1968), but the *M. falcata* was very similar in karyotype to that reported by Gillies (1970a). The hybrids' chromosomes were intermediate in length between those of the parents, being much closer to the shorter (*M. sativa*) parent. Ho's photographs were similar to Gillies' (1970a) in showing that *M. falcata* chromosomes appeared less chromatic than *M. sativa* chromosomes.

II. MATERIALS AND METHODS

1. Materials

The various accessions of perennial and annual species studied were from the University of Alberta Department of Genetics collection. They are listed in Table 1. Where possible, the place of collection or the earliest known source of the accession is given. With two exceptions, ('Saskatchewan White' *M. sativa* and *M. polymorpha* N. 3176) all are referred to by their University of Alberta accession numbers.

Table 2 lists other plants derived from perennial species, which were used in pachytene analyses. The hybrids were produced by Dr. K. Lesins by crosses in the glasshouse at the University of Alberta. The autotetraploids and autohexaploids were also produced by Dr. Lesins. The trisomics were grown from cuttings supplied by Dr. K. Kasha of the University of Guelph. Buds from the dihaploids were supplied by Dr. E.T. Bingham from plants grown by him in the glasshouse at the Department of Agronomy of the University of Wisconsin.

All plants (except the dihaploids) were grown in pots in the glasshouse at the University of Alberta and maintained under extended daylength in the winter by the use of artificial lighting.

2. Cytological Techniques

All studies were carried out on pollen mother cell squashes from young flower buds. Buds were collected before 9 a.m. from plants in the glasshouse and fixed in a mixture of 3 parts methyl alcohol: 1 part glacial acetic acid. After 24 hours the fixative was drained off and the buds were rinsed three times at hourly intervals with 80% methyl alcohol.

TABLE 1. ACCESSIONS OF *MEDICAGO* SPECIES USED IN PACHYTENE STUDIES

(a) Perennials

Species	Acc. No.	Source or other details of origin
M. coerulea Less.	221	Stalingrad, U.S.S.R.
	1975	Caucasus, U.S.S.R.
	2010	Near Mt. Ararat, Turkey.
	2011	" " " "
M. daghestanica Ruprecht	67	Botanical Institute, Tphilisi, U.S.S.R.
M. falcata L.	135	Omsk, Siberia, U.S.S.R.
	136	Alaska (possibly P.I. 179615).
	1830	Sozopol, Bulgaria.
M. glandulosa David	80	Dunav (Danube?) R. Valley, Bulgaria
M. glomerata Balb.	1529	Mt. Madonna Delle Nave, Badalucco, Italy.
	1530	" " " " " "
M. glutinosa M.B.	1852	Ukraine S.S.R., U.S.S.R.
M. hybrida Trautv.	2028	St. Antoine de Galamus, Pyrenees, France.
M. lavrenkoi Vass.	256	Leningrad (Kara-Alma), U.S.S.R.
M. prostrata Jacq.	1682	Pacentro, Italy.
	1690	Campo di Joeve, Italy.
M. pironae De Visiani	1908	Ames, Iowa (originally) from Jugoslavia).
M. rhodopaea Velen.	493	Sofia, Bulgaria.
M. rupestris M.B.	1847	Crimea, U.S.S.R.
M. sativa L.	505	Ames, Iowa (originally from Turkey).
	506	Kashan, Iran.
	-	'Saskatchewan White' variety.
M. suffruticosa Ramond	1544	Near Tosa, Pyrenees, Spain.
	1549	" " " "

(b) Annuals

Species	Acc. No.	Source or other details of origin
M. constricta Dur.	1447	Thessaloniki, Greece.
M. murex Willd.	353	Institut Scientifique, Cherifien, Algeria.
	1075	Capoterra, Italy.
	1521	Cannes, France.
	1980	Perth, W. Australia (originally from Bot. Garden, Coimbra, Portugal).
	1981	Perth, W. Australia (originally from Portugal).
	1982	Unknown origin (possibly either Corsica or Turkey).
	1983	Perth, W. Australia (originally from Sheron Beitlid, Israel).
	2065	Isle Pantelleria, Italy.
	2143	" " "
M. polymorpha L.	401	Al 'Azair, Iraq.
	409	Botanical Garden, Czechoslovakia.
	427	" " "
	-	N. 3176 (J.P. Simon's No.), Perth, W. Australia (originally from Kefar Nashum, Israel).
M. praecox D.C.	461	Canberra, Australia.
M. rigidula (L.) All.	479	Botanical Garden, Poland.
	1324	Sofar, Lebanon.

TABLE 2. HYBRIDS AND OTHER PLANTS DERIVED FROM PERENNIAL SPECIES OF *MEDICAGO*

(a) Hybrids - all hybrids were produced by K. Lesins. Hybridity was verified by use of standard markers such as flower colour, pod coiling, etc.

M. glomerata 1529 X *M. falcata* 1830.

M. glomerata 1529 X *M. sativa* 506 (206-10...a self-sterile line)

M. sativa ('Saskatchewan White') X *M. glomerata* 1529.

M. lavrenkois 256 X *M. glomerata* 1529.

M. daghestanica 67 X *M. pironae* 1908.

M. hybrida 2028 X *M. suffruticosa* 1544.

M. rhodopaea 493 X *M. rupestris* 1847.

M. sativa 506 (206-10) X 4n *M. rhodopaea* (colchicine induced from #493).

(b) Polyploids

Autotetraploids

1-5-2:- colchicine induced from *M. falcata* 136 by K. Lesins.

10-3-1:- " " " *M. falcata* 135 " " "

Hexaploid

Hexa 8-13:- derived from a cross of colchicine induced octoploid of commercial tetraploid *M. sativa* X commercial tetraploid *M. sativa*. Produced by K. Lesins.

(c) Dihaploids - supplied by E.T. Bingham, Dept. of Agronomy, University of Wisconsin, Madison, Wisconsin. For additional information see Bingham (1969) and Bingham and Binek (1969b).

H-1 dihaploid from tetraploid *M. sativa* T-1c (a male sterile, green hypocotyl tetraploid, derived from the variety 'Flemish')

H-2)
H-3) dihaploids from tetraploid *M. sativa* T-27 (a male sterile, yellow flowered tetraploid, derived from 'Flandria' and 'Vernal' varieties by W.H. Davis, Teweles Seed Co., Milwaukee, Wisconsin).

H-6)
H-7) dihaploids from tetraploid *M. sativa* T-9 (a male sterile, purple flowered tetraploid, derived from 'Duristan' X 'Ranger' by W.H. Davis).
H-8)

H-7 X *M. falcata* D48 (a diploid *M. falcata* - U.S.D.A. P.I.22506.)

(d) Trisomics - supplied by K.J. Kasha, Crop Science Dept., University of Guelph, Guelph, Ontario. Two cuttings of each plant were supplied. For further information concerning the parentage of these trisomics see Kasha and McLennan (1967).

TR 6-3 - trisomic.

TR 19-3 X Du Puit - trisomic for satellited chromosome.

TR $33^{L}1$ - trisomic.

TR $34^{S}3$ - "

TR $37^{L}12$ - "

TR $37^{L}15$ - "

TR $37^{L}17$ - "

TR $39^{L}2$ - double trisomic.

TR $39^{S}3$ - trisomic.

The buds were stained in vials in a modification of Snow's (1963) alcoholic hydrochloric acid carmine, in which methyl alcohol had been substituted for ethyl alcohol. This modification was developed by Urata and Britten (personal communication). Excess 80% methanol was blotted from the buds before they were placed in the stain. The staining period varied with the species being used and was determined by sampling at intervals until staining was adequate. In general, perennials were stained in 2-3 weeks while annuals required about 6 weeks. After staining, the buds were transferred to 80% methyl alcohol for at least a few days before squashes were made. This period in 80% methyl alcohol was found to enhance spreading and resulted in some clearing of the cytoplasmic background.

In preparing a squash, the anthers from a bud were teased out in a drop of 45% acetic acid on a slide, and a circular No.1 coverglass applied. The slide was then warmed on a hot plate at about 50^{o}C for a minute before a blunt needle was used to apply pressure to the coverglass over each anther in turn, causing them to burst and the sporocytes to spread. At this point, the slide was rapidly scanned under low power on the microscope. If no pachytene stages were present, younger or older buds on the raceme were examined until pachytene stages were found. If pachytene stages were present, the cells were squashed by applying pressure with the thumb over the coverglass. Well prepared slides had no air bubbles under the coverglass and could be examined for up to twenty minutes before bubbles developed.

Pachytene stage cells were examined under oil immersion optics. Cells in which all or a reasonable number of chromosomes could be followed, were photographed, and where necessary notes and drawings were

made to assist in interpretation. It was found that cells with all chromosomes in one focal plane, and hence suitable for photography, were regularly obtained only if no air bubbles were present under the coverglass and if no re-irrigation of the slide was carried out after squashing. After photography was completed, slides were made semi-permanent by irrigation with Rattenbury's (1956) permanent squash medium.

Photography was carried out on Kodak High Contrast Copy film with the use of a Leica 35 mm camera. A Microsix exposure metre was employed so that constant exposure time could be used by adjustment of lighting conditions. All photography was done with oil immersion optics and hence at a constant magnification, which was determined by photographing a stage micrometer. Film was developed and fixed by a routine method using Kodak D-19 Developer and Kodak Acid Fixer.

3. Karyotype Analysis

A standardized procedure was adopted for studying the chromosome morphology of all species. The photographs were printed from 35 mm negatives at a constant magnification of 2000. Rough drawings were then made of the chromosomes* in each cell of a species and arbitrary numbers assigned to the chromosomes for purposes of identification. Using a pair of dividers set at a constant aperture of 1 mm (equivalent to 0.5 microns at 2000X) each chromosome was measured, and the length, position of centromere and of each chromomere and

* Throughout this thesis where 'chromosome' is used at the pachytene stage, it will refer to the paired pachytene chromosomes (pachytene bivalents) unless otherwise stated.

chromatic area were marked on millimetre graph paper, to give a linear representation of each chromosome in a cell.

On completion of this for all distinguishable chromosomes in each cell, the linear drawings were compared. Where possible, initial comparisons were made between cells in which all of the chromosomes were measurable. Using such cells, a tentative standard set of haploid chromosomes classes was established with which chromosomes from incomplete cells were compared. In making comparisons, chromosomes were identified by using the criteria of relative length (as a proportion of the complement length or relative to other chromosomes in the cell), ratio of long arm/short arm, and position of chromatic areas and chromomeres. The presence of terminal knobs and the attachment to the nucleolus were also useful in identification.

After the chromosomes had been assigned to classes, the mean length, mean arm ratio and the standard errors of means were calculated for each chromosome class. Means and standard errors (S_E) were calculated for the satellite length (distance from the nucleolus organizer to the end of the chromosome arm on which it is situated) and for the length of the haploid chromosome complement of complete cells. In some cases, the proportional length of each chromosome in the idiogram was included. This was calculated as:-

$$\frac{\text{the mean length of the chromosome}}{\text{the sum of the mean lengths of all the chromosomes in the idiogram}}.$$

The chromosomes were then arranged and numbered in descending order of length. Construction of an idiogram for the species followed. This

showed the lengths, arm ratios, and positions of prominent chromomeres and chromatic areas of chromosomes, which were arranged with short arm uppermost.

In describing the chromosomes in idiograms, the nomenclature of Levan, Fredga and Sandberg (1964) was used when referring to centromere position. Thus the terms "median centromere" and "metacentric chromosome" were used when the arm ratio was 1.0 to 1.7, and "submedian centromere" and "submetacentric chromosome" when the arm ratio was 1.7 to 3.0. Since very few arm ratios over 3.0 were encountered, these were included in the latter class. The term "symmetrical" was used to describe chromosomes in which chromatic areas were approximately equally distributed on the two chromosome arms on each side of the centromere, and "asymmetrical" was used to describe chromosomes in which one arm (usually the shorter) was considerably more chromatic than the other.

Comparisons of both chromomere morphology and length and arm ratio means, were carried out between chromosomes of different accessions of a species, and between chromosomes of different species (particularly those considered to be closely related). Appropriate t tests and F tests were carried out and critical differences estimated by Duncan's new multiple range tests (Steel and Torrie, 1960) where necessary.

III. KARYOTYPE STUDIES OF THE COENOSPECIES *M. SATIVA S.L.*

1. Review of literature

As mentioned above, this group has been subject to a great deal of cytogenetic analysis over the years, but this has not led to clarification of the many problems of relationships within the group. Bolton (1962) states that since crosses at similar ploidy levels between *M. sativa, M. falcata, M. glutinosa, M. hemicycla, M. coerulea* and *M. trautvetteri* are successful and give fertile hybrids, Sinskaya's (1940) idea of a coenspecies is a logical one. To these species should be added *M. glandulosa* (often considered part of *M. falcata* or *M. glutinosa*) and *M. glomerata*. The relationship of the latter to the *M. sativa - falcata* complex has been demonstrated by Lesins (1968). The only barrier between members of this coenospecies appears to be an incomplete one formed by ploidy differences. Lesins (1961a) proposed the term *M. sativa sensu lato* (*s.l.* - in the broad sense) for all freely interbreeding species of this group, with *M. sativa sensu stricto* (*s.s.* - in the strict sense) being confined in usage to the purple flowered, coiled pod taxa of cultivated and wild alfalfas. This group probably includes *M. coerulea*.

Numerous cytological studies and crosses in the species *M. sativa* and *M. falcata* have been carried out (Bolton and Greenshields, 1950; Nilan, 1951; Lesins, 1952, 1957; Sprague, 1959; Stanford and Clement, 1958; Cleveland and Stanford, 1959; McLennan, Armstrong and Kasha, 1966; Bingham, 1968c; Bingham and Binek, 1969a; Ho, 1969), so that their close relationship is beyond question. Their main distinguishing characters, flower colour and pod coiling, appear to be

controlled by fairly simple genetic systems, and intermediate forms are common *(M. media)*. With this probable close relationship the similarities between pachytene chromosomes of the two species noted by Ho (1969) and Gillies (1970a) are no surprise.

Some variations in the pachytene karyotypes of diploid *M. sativa* have been reported (Clement and Stanford, 1963; Buss and Cleveland, 1968b; Gillies, 1968; Ho, 1969). In general, these reports agree on the arm ratios, chromomere patterns and relative lengths of chromosomes but differ widely on absolute lengths of chromosomes. This is quite probably partly a reflection of a diversified genetic background of the material studied, as would be expected in an outbreeding species like *M. sativa*. The reports of Buss and Cleveland (1968b) and Gillies (1968) did not disagree markedly on chromosome lengths and both used plants originating from Turkey. The chromosome lengths reported by Ho are by far the shortest for diploid *M. sativa* but they are fairly close to those given by Gillies (1970b) for tetraploid *M. sativa*.

The evidence for an autotetraploid origin of tetraploid *M. sativa* has been mentioned earlier, and with this type of origin it is not surprising that viable dihaploids (haploids) have been obtained from tetraploid alfalfa (Lesins, 1957; Stanford and Clement, 1958; Clement and Lehman, 1962; Bingham, 1969). In his study of pachytene in a tetraploid *M. sativa*, Gillies (1970b) was able to identify several pairs of homoeologous bivalents which formed quadrivalents. At least six of the eight pairs of homoeologous bivalents that he postulated were morphologically very similar. The fertility of the dihaploids with other diploids and tetraploids, *M. sativa* and *M. falcata* (Lesins, 1957;

Stanford and Clement, 1958; Clement and Lehman, 1962), while not especially high, has been further evidence for the concept of a single coenospecies with several ploidy levels.

Hexaploid alfalfa (*M. sativa*) has been reported by Bingham and Binek (1969a). They found mostly bivalent chromosome pairing at metaphase I, with some univalents and quadrivalents. In crosses with diploid *M. falcata* and triploid *M. falcata* X *M. sativa* hybrids, tetraploid and near hexaploid progeny respectively were obtained. Nicoloff (1962b) has found about twenty bivalents, one quadrivalent and two to three univalents at diakinesis in a hexaploid *M. sativa*. More multivalents were present at metaphase I.

A number of crosses between other members of the coenospecies have been reported by Lesins. In 1952, he reported crosses of *M. coerulea* and *M. glutinosa* with *M. sativa*. Both crosses gave fully fertile hybrids. *M. coerulea* also crossed normally with *M. falcata*. Lesins and Lesins (1966) described an accession of *M. glomerata* from N.W. Italy, noting its similarity to other members and intermediate types in the *M. sativa - falcata* complex. They stated that *M. glomerata* may be important as a common ancestor of *M. sativa* and *M. falcata*. Lesins (1968) studied a number of crosses between *M. glomerata* and *M. sativa*, *M. falcata* and *M. prostrata*. He found a high affinity between *M. glomerata* and *M. sativa* and *M. falcata*, and although there were some meiotic irregularities and reduction in fertility in the hybrids, the meiotic disturbances were not significantly more than had been previously noted in *M. sativa* X *M. falcata* crosses. Lesins (1968) concluded that on morphological grounds *M. glomerata* could be considered a separate

species and that it was closely related to the *M. sativa - falcata* complex, but not as closely as *M. falcata* was to *M. sativa*.

Lesins and Lesins (1960) reported the finding of two ploidy levels in *M. prostrata*, 2n = 16 accessions from Italy, and 2n = 32 from Czechoslovakia. Later Lesins (1962) reported the results of breeding and cytological studies of crosses of *M. prostrata* with *M. sativa* and *M. falcata* at both ploidy levels. If *M. prostrata* was used as male parent in crosses, the seed that set were smaller than those of either parent. The reciprocal cross produced fewer seeds and they were poorly developed or shrivelled. Hybrids all had very high metaphase I pairing of chromosomes. There was some evidence of preferential pairing within genomes of tetraploid hybrids. Lesins was of the opinion that there was justification for placing *M. prostrata* in a separate species, on the basis of the impaired fertility in crosses with the *M. sativa - falcata* complex, but he considered that under artificial conditions transfer of genetic material between the two species was relatively easy.

In crosses with *M. glomerata*, Lesins (1968) found that *M. prostrata* behaved similarly in giving reciprocal differences in seed set, although in this case no shrivelled seed was produced. This led Lesins (1968) to suggest that *M. glomerata* was closer in affinity to *M. prostrata* than were *M. sativa* and *M. falcata*.

2. Analysis of chromosome karyotypes and their relationships

a. Diploid *M. sativa* L.

The results presented for this species are essentially the same as those reported by Gillies (1968) but some changes have been

TABLE 3. MEAN LENGTHS AND ARM RATIOS OF PACHYTENE CHROMOSOMES OF DIPLOID *M. SATIVA* L.

Chromosome	Mean Length ± S_E (in μ)	Mean Arm Ratio ± S_E	Number Measured
1	39.6 ± 1.59	1.40 ± 0.108	15
2	38.2 ± 1.80	1.80 ± 0.123	14
3	37.4 ± 1.86	2.48 ± 0.138	14
4	35.7 ± 1.21	2.40 ± 0.123	17
5	33.8 ± 1.24	1.33 ± 0.084	15
6	31.7 ± 1.29	1.76 ± 0.109	14
7	31.3 ± 1.11	1.34 ± 0.093	16
8	29.8 ± 1.00	1.13 ± 0.037	15
Sum of Mean Lengths	277.5	-	-
Satellite (chromosome 8)	8.4 ± 0.29	-	15
Haploid Complement	284.4 ±11.20	-	11

made in the presentation of data - notably of chromosome 8 (the nucleolus organizing chromosome).

A total of twenty cells at the pachytene stage were analysed from a plant of Acc. No.505, and of these, eleven had all eight chromosomes distinguishable. One of these cells is shown in Figure 1, with an interpretative drawing of the chromosomes. The mean lengths and arm ratios and their respective standard errors (S_E) are given in Table 3. The mean of the total haploid complement length from eleven complete cells was 284.4 $\pm$ 11.20 microns, which agreed well with the sum of the eight mean lengths (277.5 microns).

The idiogram constructed from these mean values, including the positions of chromomeres and chromatic areas, is given in Figure 2. It differs from that given by Gillies (1968) in having chromosome 8 inverted so that the short arm is uppermost as in the other seven chromosomes. A brief description of the chromosomes is as follows.

Chromosome 1: the longest chromosome, it has a near median centromere and symmetrical distribution of chromatic areas adjacent to the centromere. The shorter arm has a telomeric knob and there is a knob about one third of the way along the long arm.

Chromosome 2: almost as long as chromosome 1 but a submetacentric type. A greater proportion of the short arm is more chromatic than in chromosome 1, but the terminal third to a half of the short arm is achromatic and the telomeric knob is not pronounced.

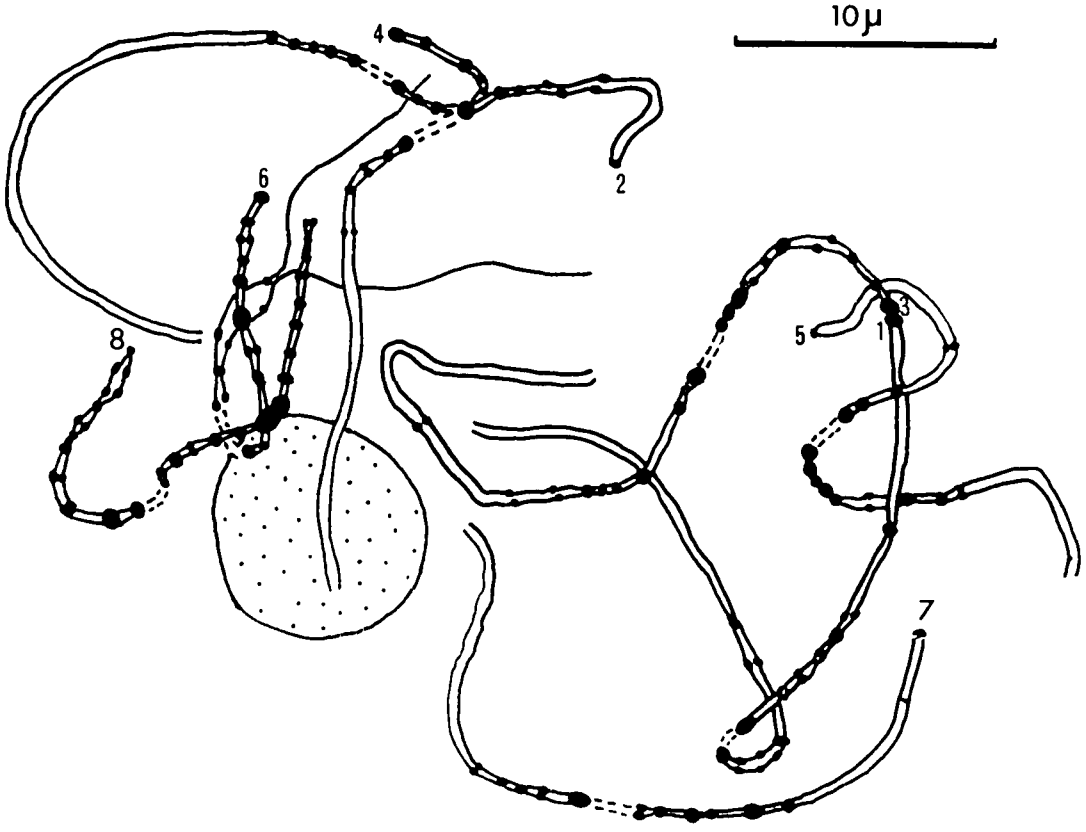
Chromosome 3: a submetacentric chromosome which approaches the previous two in length. The short arm is entirely composed of chromatic knobs

Figure 1

Pachytene cell of diploid *M. sativa* L. a. Arrows indicate positions of centromeres. b. Interpretative drawing of a. with chromosomes numbered at short arm telomeres, and centromeres indicated by broken lines. X3250.

a

b



and the proximal third to a half of the long arm is also chromatic, but not as heavily as the short arm.

Chromosome 4: similar to chromosome 3 but shorter and having the chromatic part of the long arm confined to a smaller proportion adjacent to the centromere.

Chromosome 5: a median centromere and fairly symmetrical distribution of chromatic areas on each side of the centromere are characteristic of this chromosome. Distal portions of both arms are achromatic but the short arm has a telomeric knob.

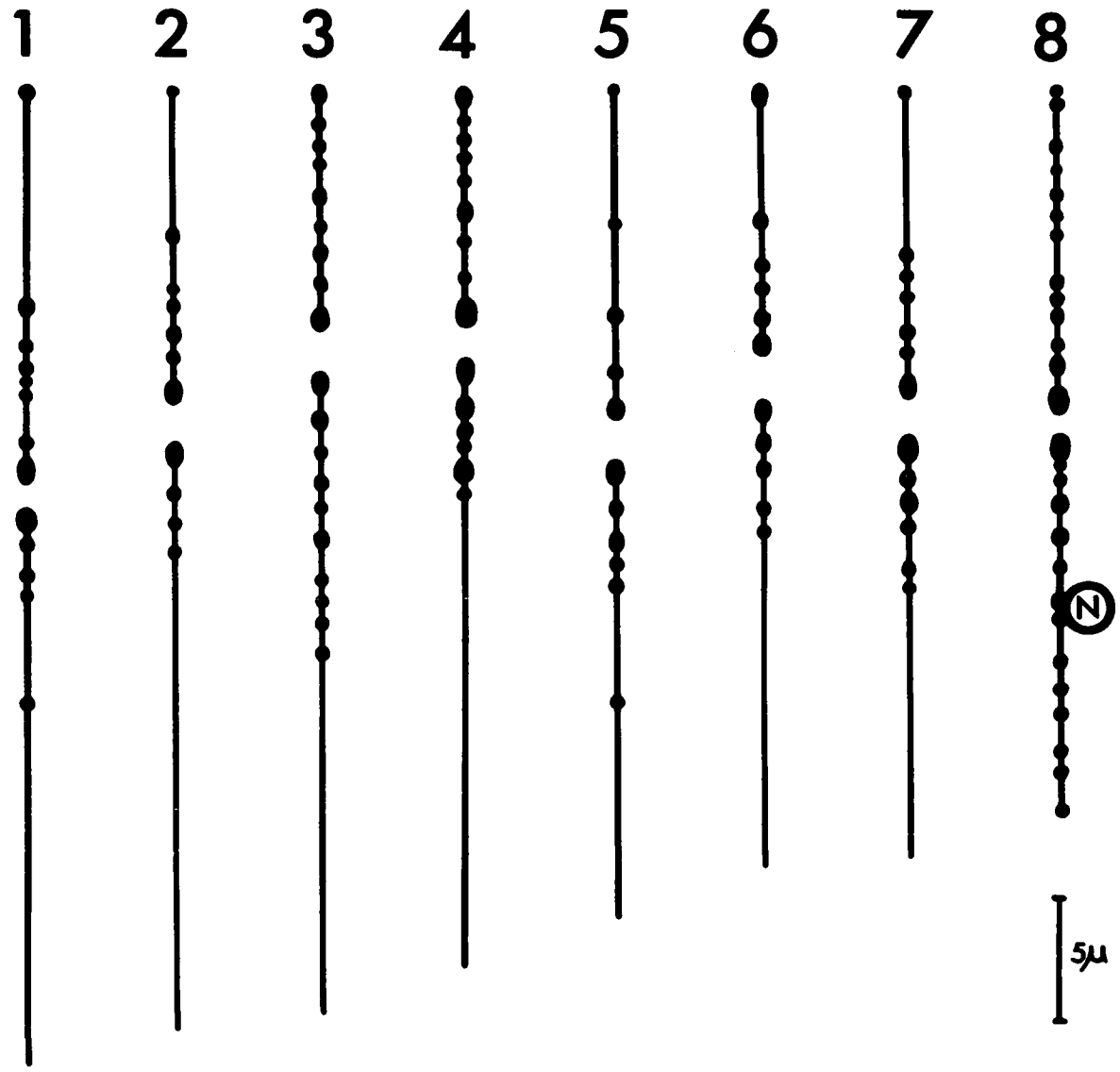
Chromosome 6: a submetacentric chromosome, it differs from chromosome 2 in being much shorter and having a higher proportion of its short arm chromatic. It is not as chromatic as chromosomes 3 and 4 but it does possess a prominent telomeric knob.

Chromosome 7: about the same length as chromosome 6, but having a more median centromere and less chromatic short arm. The long arm and short arm are almost equally chromatic adjacent to the centromere. The short arm has a terminal knob.

Chromosome 8: readily distinguishable by its attachment to the nucleolus and by its largely chromatic nature throughout most of its length. It has a near median centromere and the nucleolus organizer is located almost midway along the longer arm, the satellite being 8.4 microns long. The satellite is not as chromatic as the rest of the long arm. The nucleolus organizer (N.O.) is marked by a large chromatic knob which sometimes can be seen to consist of two knobs with a slight gap between, probably representing the N.O. proper.

Figure 2

Idiogram of pachytene chromosomes of diploid *M. sativa* L. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3250.


1
2
3
4
5
6
7
8
N
5μ

In the report of Gillies (1968) this karyotype was compared with the incomplete dihaploid karyotype produced by Clement and Stanford (1963). The chromosomes of diploid *M. sativa* were found to be similar in gross chromomeric morphology to the dihaploids, but the dihaploid chromosomes were much longer. It was suggested that this may have been partly due to differences in technique. Since that time a number of other workers have produced idiograms of several different diploid *M. sativa*s, and some measure of the variability to be expected between different lines can be obtained by comparing the results. Buss and Cleveland (1968b) published a karyotype of an *M. sativa* which also was originally from Turkey. Ho (1969) studied a plant obtained from the C.D.A. Research Station, Ottawa, as Accession No.1943-770, while Stringam (unpublished, personal communication) used a plant S2128 (C.D.A. Research Station, Saskatoon Accession Number), originally from Erovan, Russia.

All reports are essentially the same in their description of the general characteristics of *M. sativa* pachytene chromosomes, but there are some differences in the relative lengths of chromosomes, so that the order of chromosomes varies in the different reports. Gillies (1970a) has made a comparison of the *M. sativa* idiogram reported here with that of Buss and Cleveland (1968b) and suggested a common order of chromosomes. A further comparison of the present idiogram with those mentioned above, follows. The chromosomes which are suggested to be similar to chromosomes in Buss and Cleveland's (1968b) idiogram have been altered somewhat from Gillies' (1970a) comparison.

Chromosome Numbers of Equivalent Chromosomes

Acc. No. 505 (this study)	PI 172981 (Buss & Cleveland 1968b)	1943-770 (Ho, 1969)	S2128 (Stringam)	Dihaploid (Clement & Stanford 1963)
1	4	2	4	1 & 2
2	2	4	3	
3	1	3	2	3
4	3	1	1	5
5	6	7 (6?)	5	4 ?
6	5	5	6	6
7	7	6 (7?)	7 (inverted)	7
8	8	8	8	8

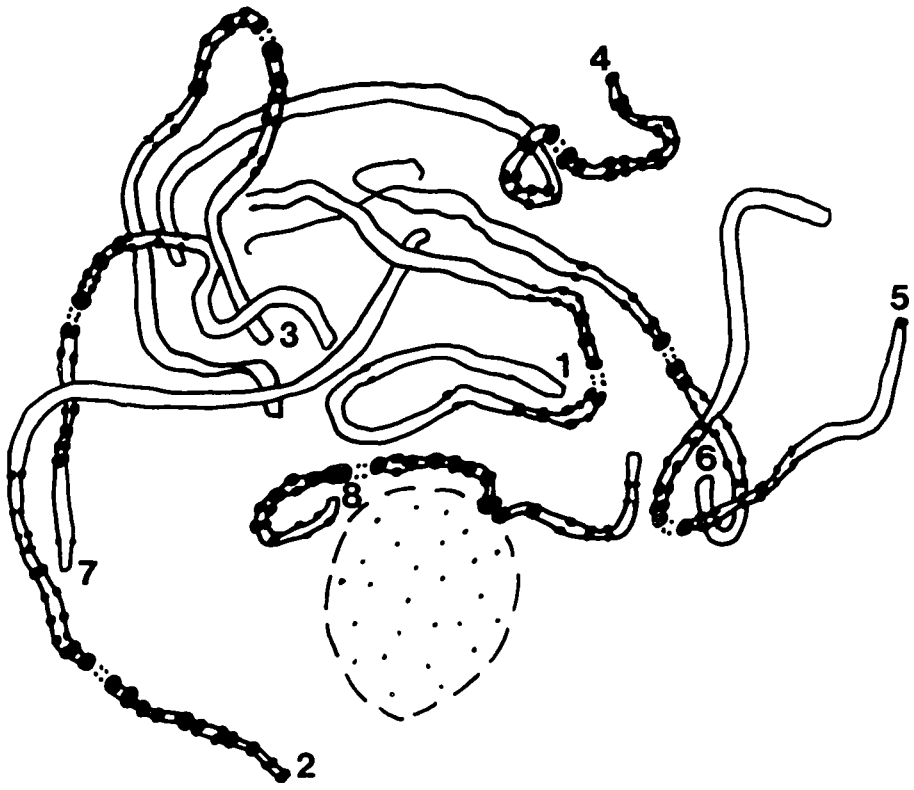
These comparisons were based on arm ratios and chromomere patterns with reference to relative length of chromosomes. Chromosomes 3, 4 and 6 were selected on the basis of their high arm ratios in combination with their largely chromatic short arms. Buss and Cleveland's chromosomes 1, 3 and 5 fit this category better than 1, 5 and 6, as proposed by Gillies (1970a). Buss and Cleveland's chromosomes 4 and 6 are symetrical chromosomes like chromosomes 1 and 5 of the present study. Ho (1969) presented the same matching of Buss and Cleveland's (1968b) and Gillies' (1968) idiograms as did Gillies (1970a). Ho's comparisons of his own idiogram with Gillies' (1968) agreed with the present one in the matching of his chromosomes 2, 3, 6 and 8, but he placed his chromosomes 1, 4, 5 and 7 in different positions. Gillies (1970b) compared the pachytene chromosomes of tetraploid *M. sativa* in pairs with the standard order he proposed for *M. sativa* and *M. falcata*

Figure 3

a. Pachytene cell of diploid *M. falcata* L. a'. Interpretative drawing of a. with chromosomes numbered at short arm telomeres, and centromeres represented by dotted gaps. X3250.

a

a'



(Gillies 1970a) and found most of them matched well for arm ratio and chromatic pattern, although the tetraploid's chromosomes were shorter and more chromatic than the diploids.

The haploid complement lengths of the four *M. sativa* karyotypes compared above were respectively 277.5 microns, 256 microns, 149 microns and 290 microns (the latter is my calculation from Stringam's idiogram). It can be seen that all but Ho's are of similar magnitude to those of the present study. Ho (1969) explained this difference in length between his idiogram and those of Buss and Cleveland (1968b) and Gillies (1968) as possibly being due to genetically controlled differences in chromosome contraction, and pointed to the much higher chromaticity of his material compared with that of the longer chromosomes of the other reports. This explanation is quite plausible in a species such as *M. sativa* which is morphologically very variable.

b. Diploid *M. falcata* L.

Two plants of Accession No.136 were used to supply buds. A total of 31 pachytene cells were analysed, 6 of them with the complete haploid complement measurable (e.g. Fig. 3). The mean lengths and arm ratios of the eight chromosomes are given in order of mean length in Table 4. The sum of the eight mean lengths is 250.8 microns while the mean of the complement lengths of the six complete cells was 238.8 microns. The idiogram constructed from this data is shown in Figure 4.

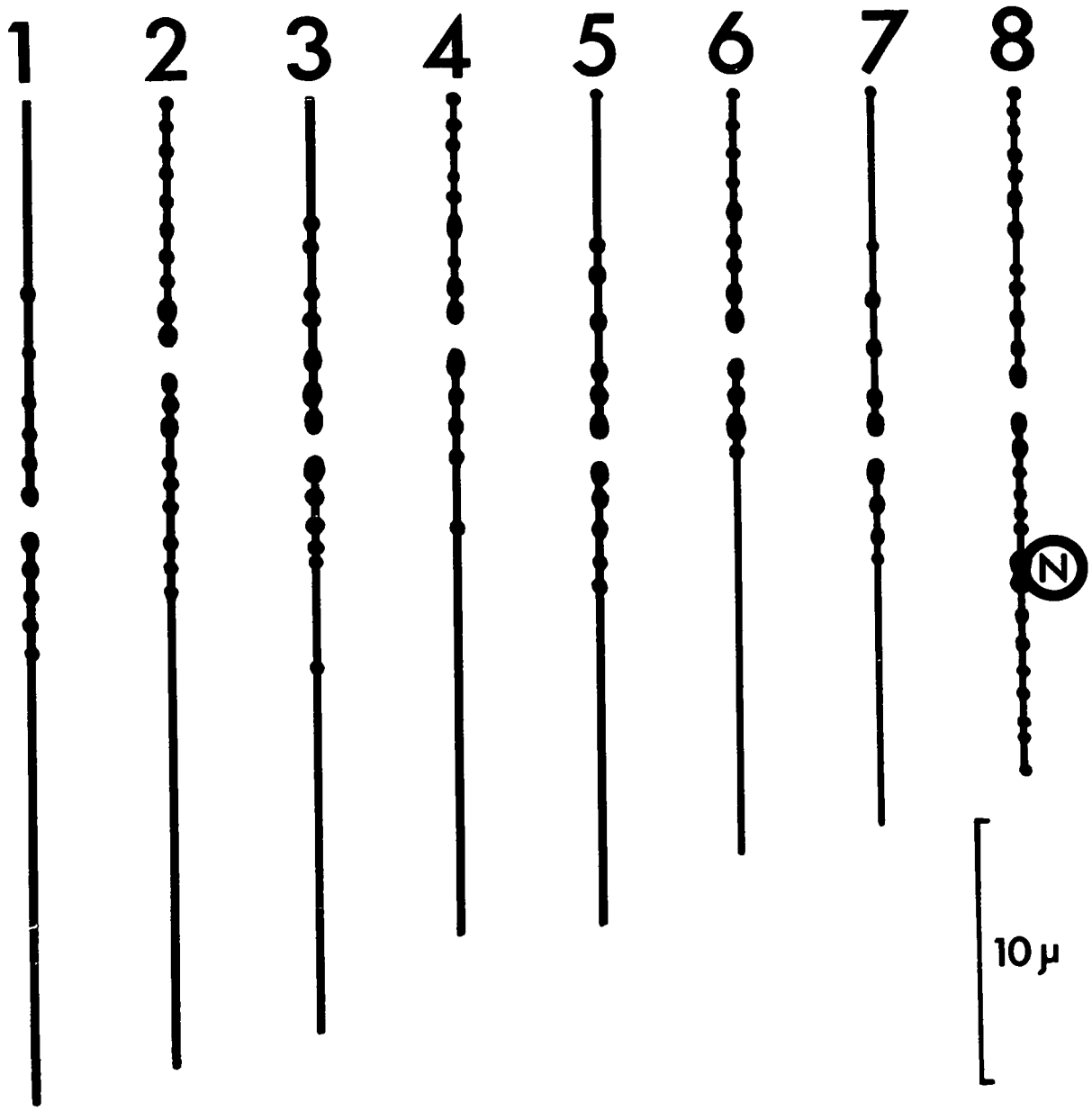
Not all chromosomes have telomeric knobs consistently present in their short arms, chromosomes 2, 4, 5, 6 and 8 being the ones in which terminal knobs regularly occur. The size of chromosomes and their

TABLE 4. MEAN LENGTHS OF ARM RATIOS OF PACHYTENE CHROMOSOMES OF DIPLOID *M. FALCATA* L.

Chromosome	Mean Length ± SE (in μ)	Mean Arm Ratio ± S_E	Number Measured
1	37.4 ± 1.15	1.41 ± 0.079	14
2	36.1 ± 1.03	2.82 ± 0.138	21
3	34.8 ± 0.88	1.71 ± 0.064	20
4	31.1 ± 0.82	2.55 ± 0.090	17
5	30.8 ± 0.71	1.36 ± 0.076	19
6	28.1 ± 1.10	2.03 ± 0.120	15
7	27.3 ± 0.87	1.07 ± 0.048	15
8	25.2 ± 0.95	1.20 ± 0.036	15
Sum of Mean Lengths	250.8	-	-
Satellite (chromosome 8)	7.7 ± 0.23	-	15
Haploid Complement	238.8 ± 10.77	-	6

Figure 4

Idiogram of pachytene chromosomes of diploid *M. falcata* L. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3750.


1
2
3
4
5
6
7
8
N
10 μ

intensity of staining (i.e. the chromaticity of the chromosomes) does not appear as great as in *M. sativa*. The description given above for the diploid *M. sativa* chromosomes can be applied fairly accurately to the *M. falcata* chromosomes if the order of chromosomes 2 and 3 is reversed. Chromosome 3 resembles chromosome 1 of *M. sativa* in having a knob midway along its long arm. In the short arm of chromosome 6 the amount of chromatin decreases towards its end where there is only a relatively small telomeric knob. The satellite of chromosome 8 is not as chromatic as the rest of the long arm of this chromosome.

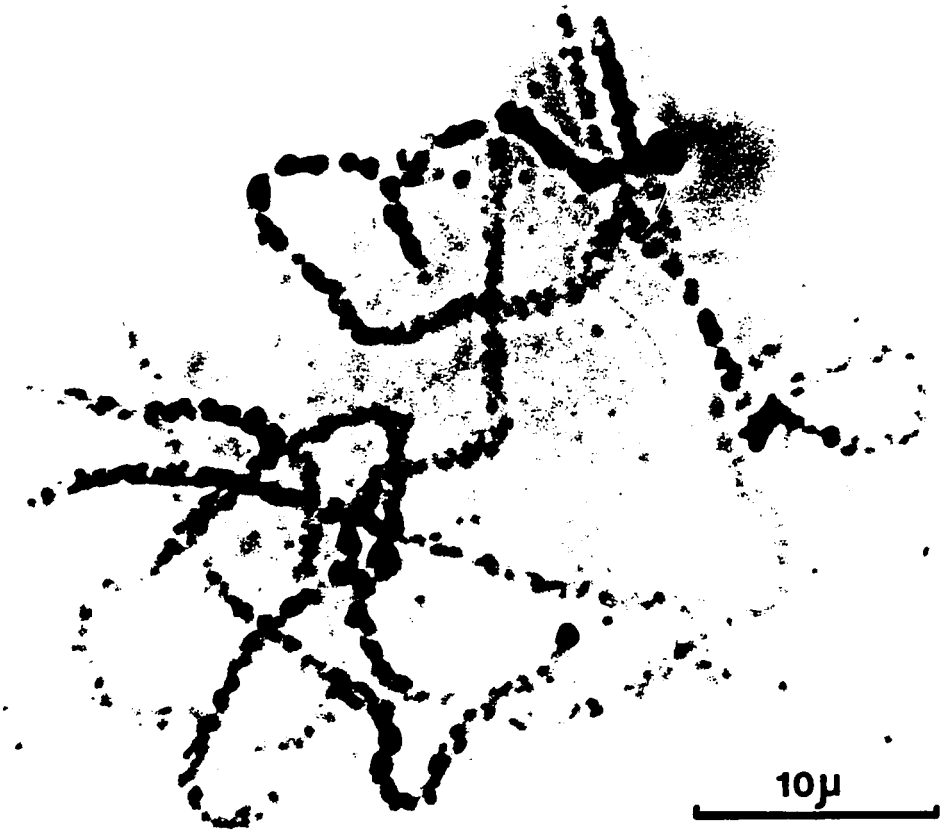
The two reports of diploid *M. falcata* pachytene idiograms by Ho (1969) and Gillies (1970a) are quite similar to the present one. Both agree that *M. falcata* chromosomes are less chromatic than those of *M. sativa*. The haploid complement lengths reported, 258.9 microns (Ho, 1969) and 275.5 microns (Gillies, 1970a), agree well with the value of 250.9 microns obtained here. Comparisons of the chromosome morphology and arm ratios show that apart from differences in chromosome order the idiograms have few gross differences. The suggested equivalent chromosomes are as follows.

Present Study	Ho (1969)	Gillies (1970a)
1	2	1
2	3	3
3	4	2
4	1	7
5	6	4
6	5	5
7	7	6
8	8	8

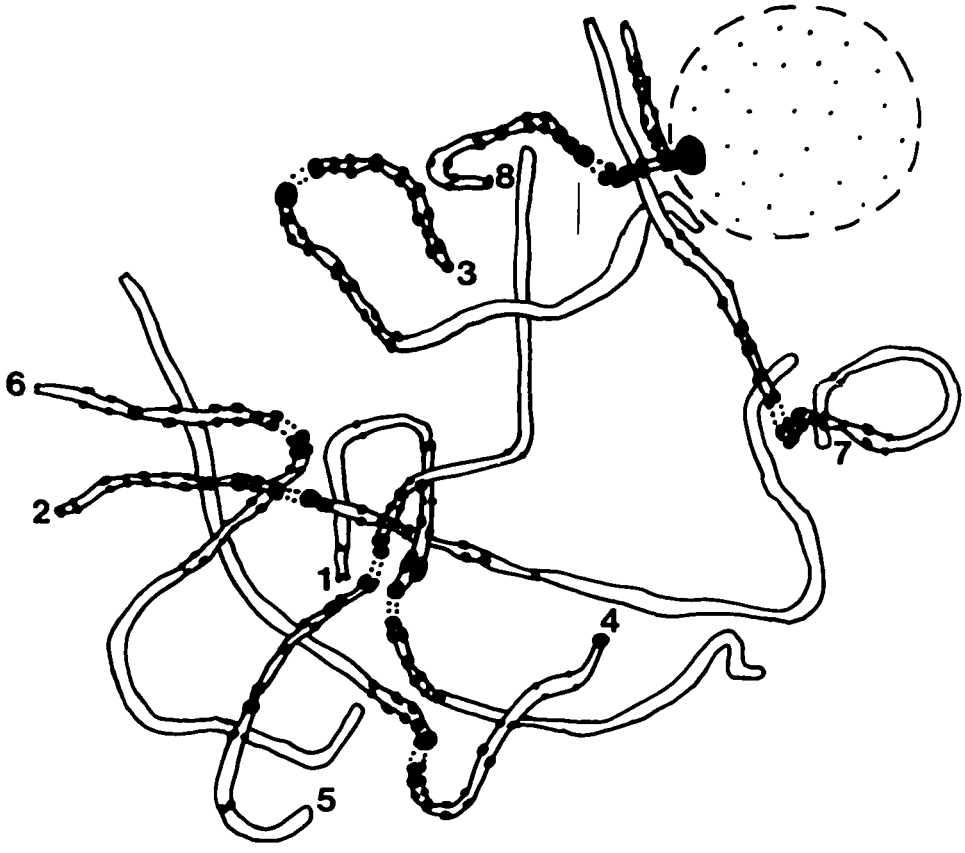
Figure 5

a. Pachytene cell of *M. glomerata*. a! Interpretative drawing of a. with chromosomes numbered at short arm telomeres, and centromeres represented by dotted gaps. X3100.

a



a'



The chromosome 8 shown by Gillies (1970a) differed from those of Ho and the present study in having the N.O. on the shorter arm. In view of the closeness of arm ratios to unity in all these studies it is possible that this difference may be due to differential contraction of the arms of the chromosomes in the different plants. However, the similarity in lengths of the chromosome 8's in the different studies tends to oppose this. Gillies (1970a) suggested that internal rearrangement of the chromosome could explain the difference in the position of the N.O. in the *M. falcata* he studied.

Gillies (1970a, b) compared his *M. falcata* idiogram with those for *M. sativa* and decided that the two species were sufficiently similar to be described by a common set of chromosome lengths and arm ratios. The present *M. falcata* idiogram and that constructed by Ho (1969) also fit this suggested common idiogram (this is discussed further in section h. under species relationships). Ho (1969) also decided that diploid *M. sativa* and *M. falcata* idiograms were identical, and this was substantiated by the fact that the hybrid between the two species also had an idiogram similar to those of the two parents and intermediate in length. Although Ho found the *M. sativa* and *M. falcata* idiograms differed in length, the relative lengths of equivalent chromosomes in each were approximately the same.

c. *M. glomerata* Balb.

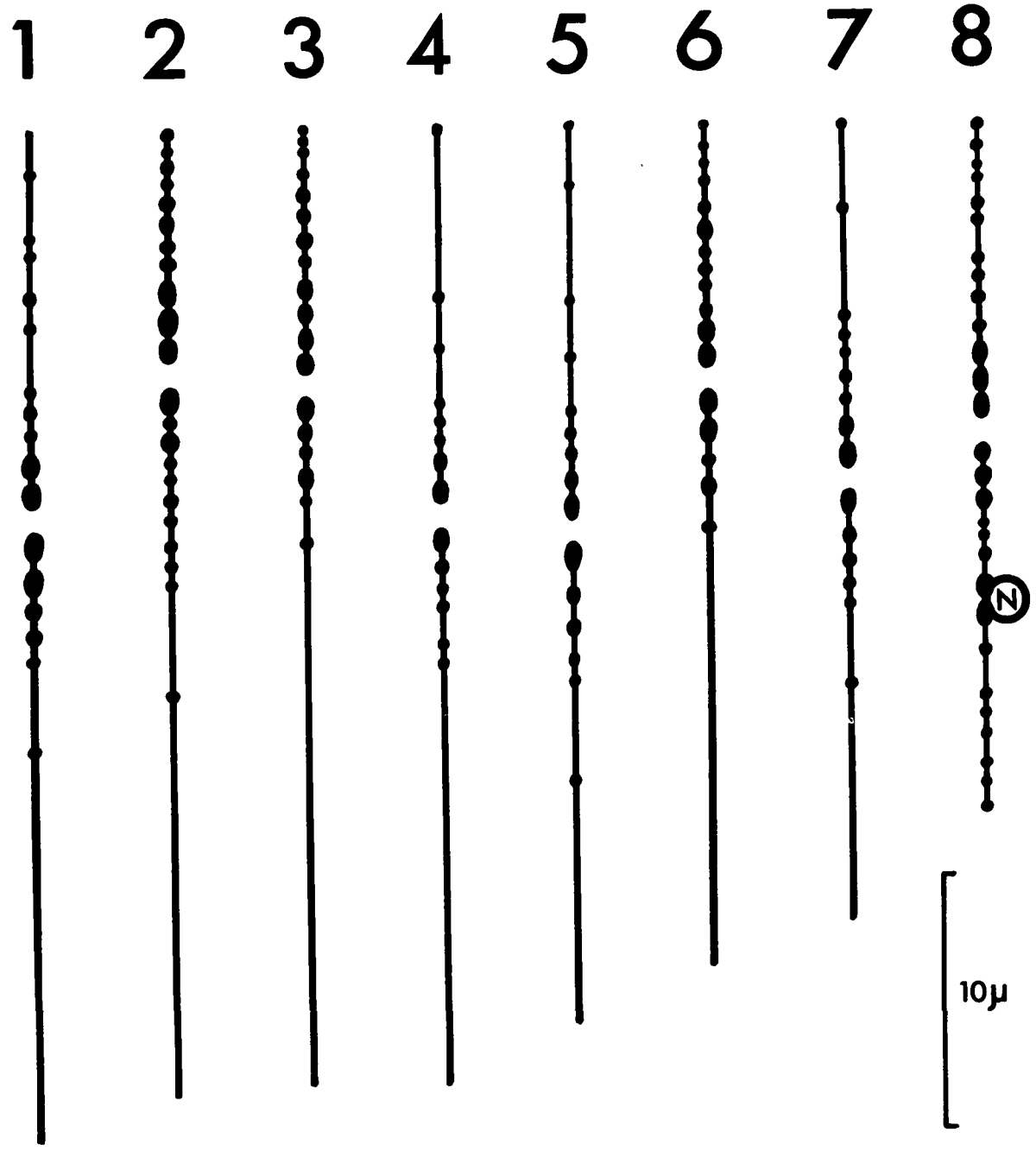
Two accessions of *M. glomerata* were grown but one (No. 1529) provided most of the material for pachytene analysis. In view of the fact that both accessions were collected in the same location, the few results from No. 1530 were analysed together with those from No. 1529. Forty-seven cells were analysed and 15 complete cells were

TABLE 5. MEAN LENGTHS OF ARM RATIOS OF PACHYTENE CHROMOSOMES OF *M. GLOMERATA*

Chromosome	Mean Length ± S_E (in μ)	Mean Arm Ratio ± S_E	Number Measured
1	39.4 ± 0.88	1.62 ± 0.089	27
2	37.6 ± 1.05	3.01 ± 0.107	28
3	37.2 ± 0.70	2.78 ± 0.067	29
4	37.2 ± 0.77	1.47 ± 0.072	32
5	34.9 ± 0.50	1.22 ± 0.036	34
6	32.7 ± 1.04	2.35 ± 0.093	25
7	30.7 ± 0.57	1.22 ± 0.034	23
8	26.6 ± 0.71	1.24 ± 0.048	30
Sum of Mean Lengths	276.3	-	-
Satellite (Chromosome 8)	8.4 ± 0.27	-	30
Haploid Complement	273.6 ± 7.81	-	15

Figure 6

Idiogram of pachytene chromosomes of *M. glomerata*. Short arms are uppermost and gaps indicate centromere position. N is the position of the nucleolus organizer. X 3750.


1
2
3
4
5
6
7
8
N
10μ

found, one of which is shown in Fig. 5. The mean chromosome lengths and arm ratios are given in Table 5. Using these values the idiogram in Figure 6 was constructed. The mean complement length was 273.6 microns while the sum of the eight mean chromosome lengths was 276.3 microns.

The chromosomes were more chromatic than those of *M. falcata*, being very similar in appearance to those of *M. sativa*, but without such prominent telomeric knobs as the latter. The descriptions of the *M. sativa* chromosomes fit the *M. glomerata* chromosomes except that chromosomes 1, 2, 3 and 4 of *M. sativa* are equivalent to chromosomes 4, 1, 2 and 3 respectively of *M. glomerata*. The arm ratios of the submetacentric chromosomes of *M. glomerata* are a little higher than those of the equivalent *M. sativa* chromosomes and the telomeric knob on chromosome 6 is not as pronounced in *M. glomerata*. The satellite of chromosome 8 in *M. glomerata* is the same length as that of *M. sativa* but is slightly more chromatic.

The *M. glomerata* haploid complement is almost identical in length with that of *M. sativa* and also with that of *M. falcata* published by Gillies (1970a). In view of the closeness of relationship of *M. glomerata* and *M. sativa - falcata* (Lesins, 1968) these findings are not surprising. This will be discussed further in sections g. and h.

d. *M. coerulea* Less.

Four diploid accessions of *M. coerulea* were examined (Table 1). Sufficient good preparations were obtained for analysis of three of these (Nos. 221, 2010 and 2011), but insufficient cells of No.1975 were

Figure 7

a. Pachytene cell of *M. coerulea* Acc. No. 221. b. Pachytene cell of *M. coerulea* Acc. No. 2011. a! and b! Interpretative drawings of a. and b. with chromosomes numbered at short arm telomeres, and centromeres represented by dotted gaps. X2100.

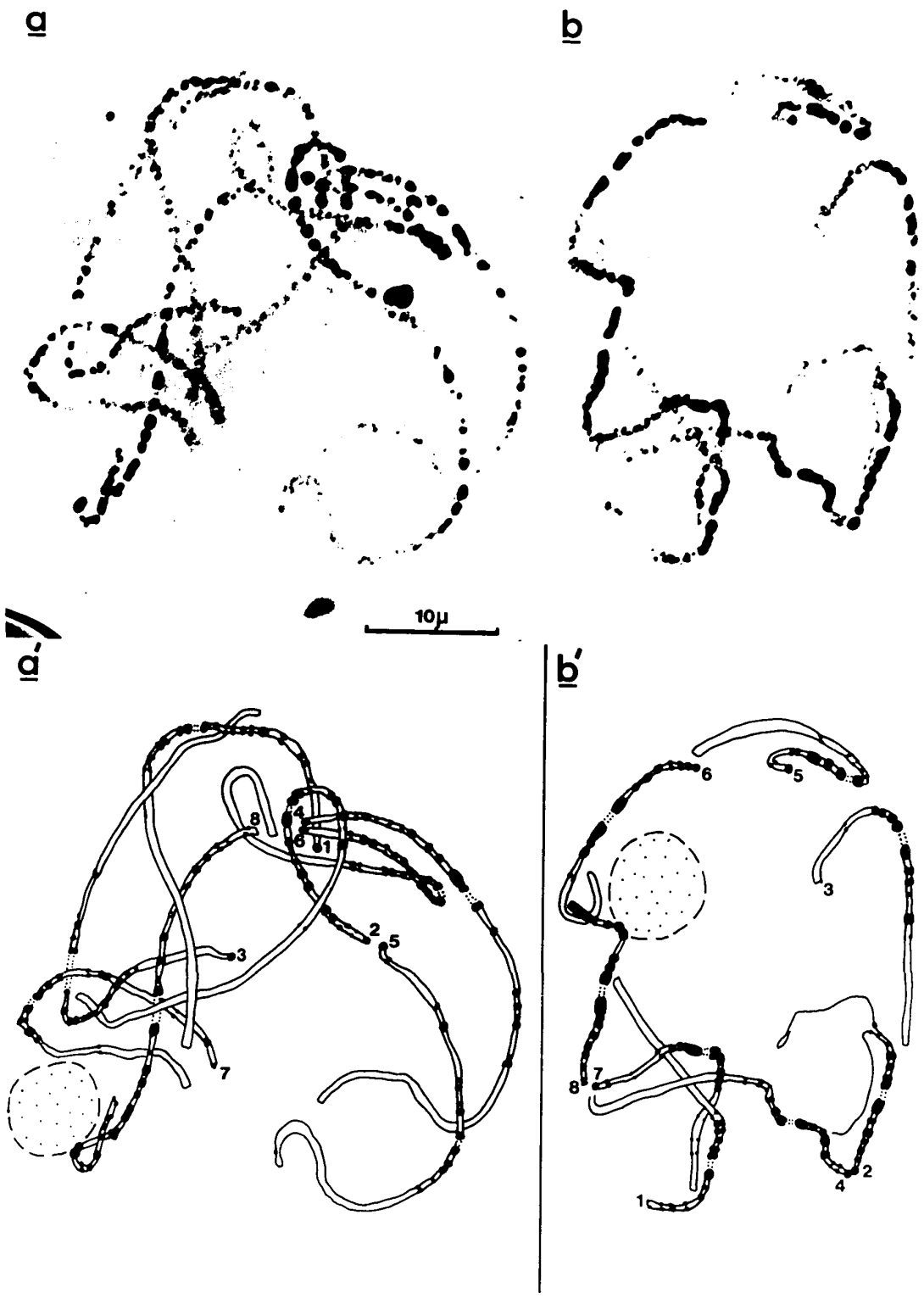

a
b
10µ
a'
b'
8
4
6
1
2
5
3
7
6
5
3
8
7
1
4
2

Figure 8

a. Pachytene cell of *M. coerulea* Acc. No. 1975. b. Pachytene cell of *M. coerulea* Acc. No. 2010. c. Nucleolar chromosome and chromosome 3 of *M. coerulea* Acc. No. 2010. a', b' and c'. Interpretative drawings of a, b and c with chromosomes numbered at short arm telomeres, and centromeres represented by dotted gaps. X2100.

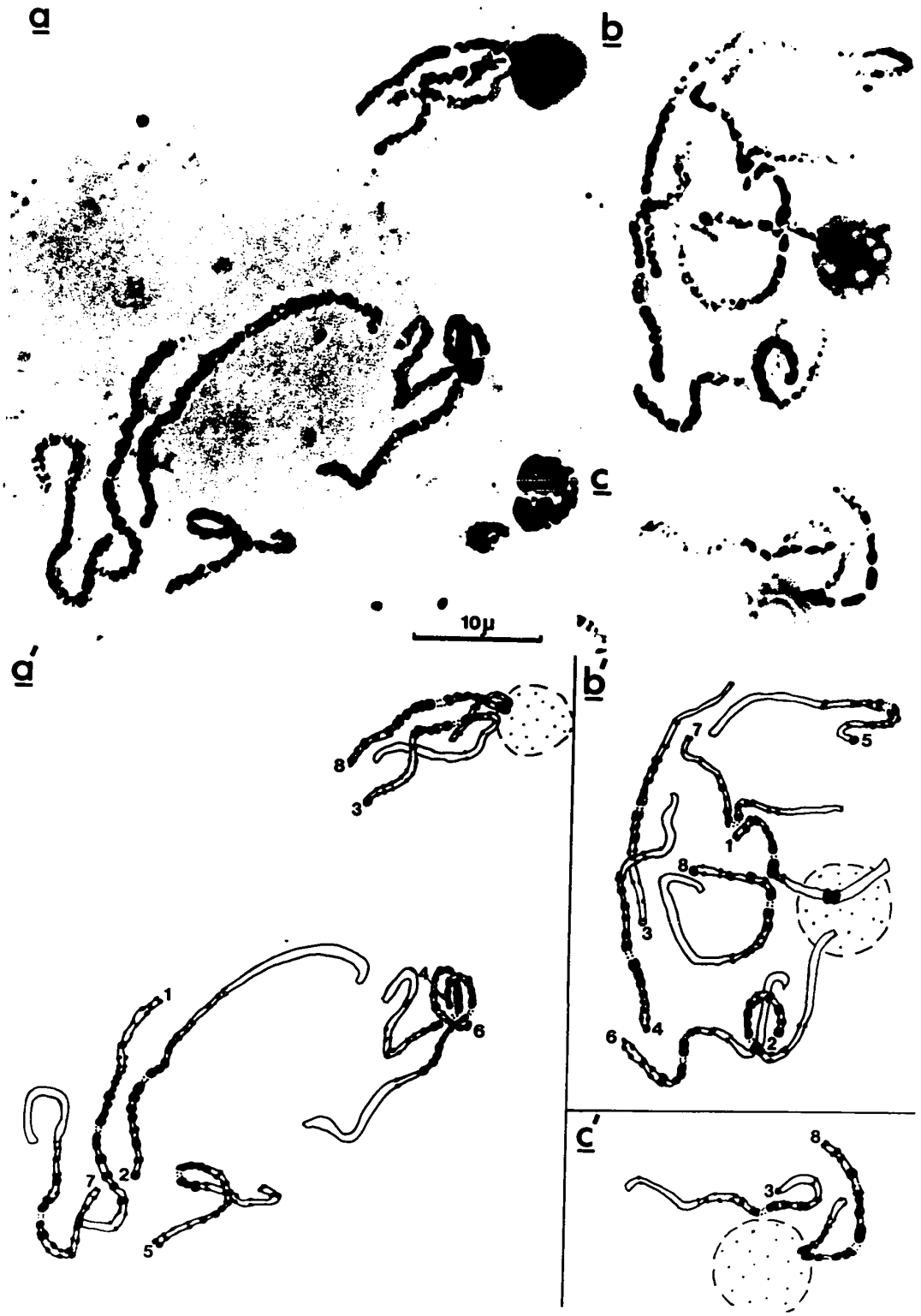

a
b
c
10μ
a'
b'
c'
1
2
3
4
5
6
7
8

TABLE 6. MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF PACHYTENE CHROMOSOMES OF *M. COERULEA*

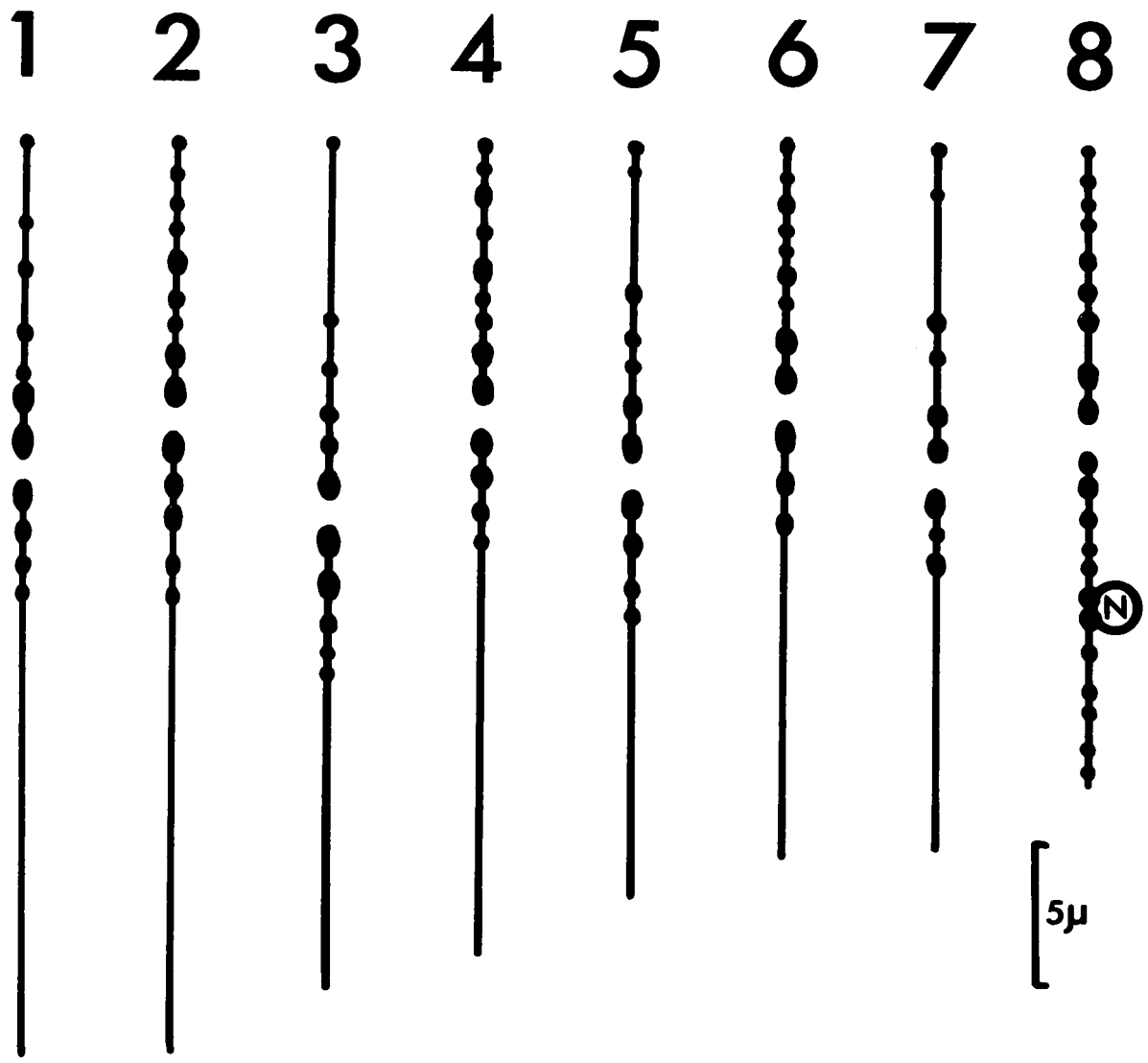
Chromosome		Accession				Mean of First 3	Signif. Diff. (F test)[b]
No.	Item[a]	221	2011	2010	1975		
1	L	37.0 ± 1.03	32.4 ± 1.11	24.9 ± 0.97	27.3	31.9	**
	AR	1.78 ± 0.092	1.91 ± 0.172	1.67 ± 0.141	2.12	1.79	n.s.
	PL	0.145	0.147	0.141	-	0.146	-
	N	14	12	11	2	37	-
2	L	37.6 ± 1.38	32.2 ± 1.29	25.0 ± 0.84	28.6	31.8	**
	AR	2.28 ± 0.173	2.53 ± 0.133	1.99 ± 0.068	2.08	2.30	*
	PL	0.147	0.146	0.142	-	0.146	-
	N	11	15	10	6	36	-
3	L	34.7 ± 1.70	29.8 ± 1.20	23.6 ± 0.84	25.5	29.5	**
	AR	1.28 ± 0.076	1.21 ± 0.054	1.40 ± 0.176	1.27	1.29	n.s.
	PL	0.136	0.136	0.134	-	0.135	-
	N	12	14	11	2	37	-
4	L	34.1 ± 1.38	29.0 ± 1.36	23.0 ± 0.52	25.9	28.2	**
	AR	1.96 ± 0.154	2.19 ± 0.172	1.77 ± 0.124	1.89	1.97	n.s.
	PL	0.133	0.132	0.131	-	0.129	-
	N	10	12	13	7	35	-
5	L	30.3 ± 0.97	27.1 ± 0.88	21.5 ± 0.53	22.4	26.1	**
	AR	1.25 ± 0.081	1.36 ± 0.117	1.15 ± 0.069	1.23	1.26	n.s.
	PL	0.118	0.123	0.122	-	0.119	-
	N	13	18	16	4	47	-
6	L	28.6 ± 1.36	24.8 ± 0.82	20.1 ± 0.57	23.5	24.6	**
	AR	1.68 ± 0.108	1.93 ± 0.092	1.47 ± 0.069	1.83	1.72	**
	PL	0.112	0.113	0.114	-	0.113	-
	N	11	14	10	5	35	-
7	L	27.7 ± 1.03	24.4 ± 1.07	19.7 ± 0.87	21.4	24.4	**
	AR	1.03 ± 0.055	1.16 ± 0.053	1.25 ± 0.087	1.14	1.14	n.s.
	PL	0.108	0.111	0.112	-	0.111	-
	N	14	15	10	5	39	-
8	L	25.9 ± 1.58	20.3 ± 1.07	18.2 ± 0.55	19.0	22.0	**
	AR	1.14 ± 0.055	1.14 ± 0.042	1.30 ± 0.049	1.24	1.20	n.s.
	PL	0.101	0.092	0.103	-	0.101	-
	N	14	8	11	1	33	-
Sum of Mean Lengths		255.9	220.0	176.0	193.6	218.5	-
Satellite (chrom.8)	L	7.0 ± 0.41	6.0 ± 0.38	5.8 ± 0.21	6.0	6.4	*
	N	14	8	11	1	33	-
Haploid Complement	L	261.8 ± 14.39	197.7 ± 6.41	170.6 ± 4.91	184.0	206.5	**
	N	6	5	8	1	19	-

a L - Mean length ± S_E (in μ), AR - mean arm ratio ± S_E, PL - proportional length, N - sample size

b n.s., *, ** - not significant, significant at 5% level, significant at 1% level respectively

Figure 9

Idiogram of pachytene chromosomes of *M. coerulea*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3800.


1
2
3
4
5
6
7
8
N
5μ

available for a valid analysis, so the results for this accession were not used in statistical procedures and are included for comparison only. Twenty-three cells of No. 221, 17 cells of No. 2010 and 26 cells of No. 2011 were analysed, and a total of 19 complete cells were found. Examples of cells from the four accessions are given in Figs. 7 and 8. The mean chromosome lengths and arm ratios are given in Table 6. The means of lengths of equivalent chromosomes from the three accessions were used to determine the order of chromosomes.

F tests were carried out to determine if there was a significant effect of accession on chromosome length and arm ratio. Although the mean lengths of equivalent chromosomes and mean complement lengths were significantly different in the three accessions, only two chromosomes (2 and 6) had significant arm ratio differences. The proportional lengths of equivalent chromosomes in the accessions were almost identical and chromatic patterns were also similar. Hence it was felt that the data from the three accessions could be pooled since length differences probably were a result of differential chromosome contraction. These mean lengths and arm ratios are also given in Table 6.

The idiogram based on the mean lengths and arm ratios for all three accessions is shown in Fig. 9. The chromosomes are about only four-fifths the length of those of *M. sativa* in Figure 2, but resemble them in chromatic pattern and arm ratio. The *M. coerulea* chromosomes appear to be more chromatic, probably as a result of their more contracted nature. The *M. coerulea* idiogram differs from that for *M. sativa* in the order of the first three chromosomes, chromosomes 1, 2 and 3 of *M. coerulea* being equivalent to chromosomes 2, 3 and 1 of *M. sativa*

The descriptions of *M. sativa* chromosomes given in section a. can be applied to *M. coerulea* generally.

As one of the synonyms for *M. coerulea* is *M. sativa* f. *xerophila* (Lesins, 1952) it is not surprising that its pachytene idiogram is almost identical with that of *M. sativa*. Gupta and Lesins (1969) found that *M. coerulea* had anthocyanins identical to those of *M. sativa* and was therefore a member of the *M. sativa* complex. The mean haploid complement length of Accession No. 221 is very close to that of *M. sativa* reported here, while that of No. 2010 is much shorter and approaches the value given by Ho (1969) for the *M. sativa* he studied. This suggests that there exists genetic variability for chromosome length at pachytene among the accessions of *M. coerulea* studied. The greater chromaticity of Nos. 2010 and 2011 compared with No. 221 supports the idea that chromosomes of the former two are shorter because of a greater degree of contraction.

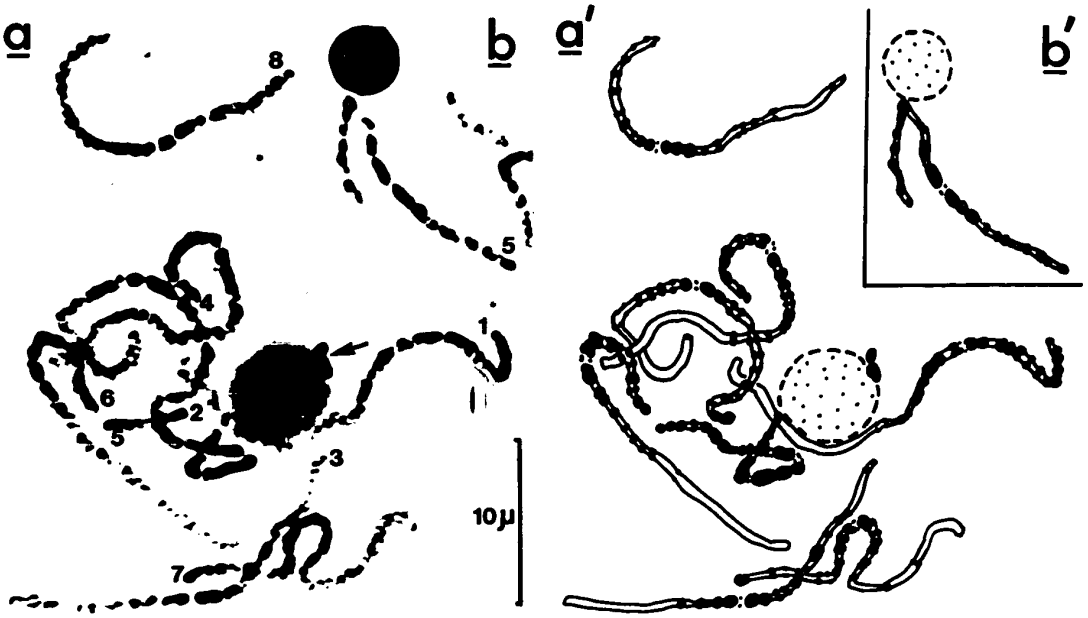
e. *M. glandulosa* David.

M. glandulosa was represented by one accession (No.80) and 16 pachytene cells were examined, only one of which had all eight chromosomes measurable. Fig. 10a, b shows a cell of *M. glandulosa* with a nucleolar chromosome from another cell shown in an inset. The mean lengths and arm ratios of the chromosomes are given in Table 7 and the idiogram constructed for the species is given in Fig. 10c.

M. glandulosa has often been classified as either *M. falcata* or *M. glutinosa*. It is obvious from Table 7 that the chromosomes of *M. glandulosa* are about only three fifths the length of *M. sativa* and

Figure 10

a. Pachytene cell of *M. glandulosa* with chromosomes numbered at their short arm telomeres. Arrow indicates portion of nucleolar chromosome still attached to nucleolus. b. Nucleolar chromosome of *M. glandulosa*. a' and b'. Interpretative drawings of a. and b. with centromeres represented by dotted gaps. X2250. c. Idiogram of pachytene chromosomes of *M. glandulosa*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X2900.


a
b
a'
b'
8
5
4
1
6
2
5
3
7
10μ

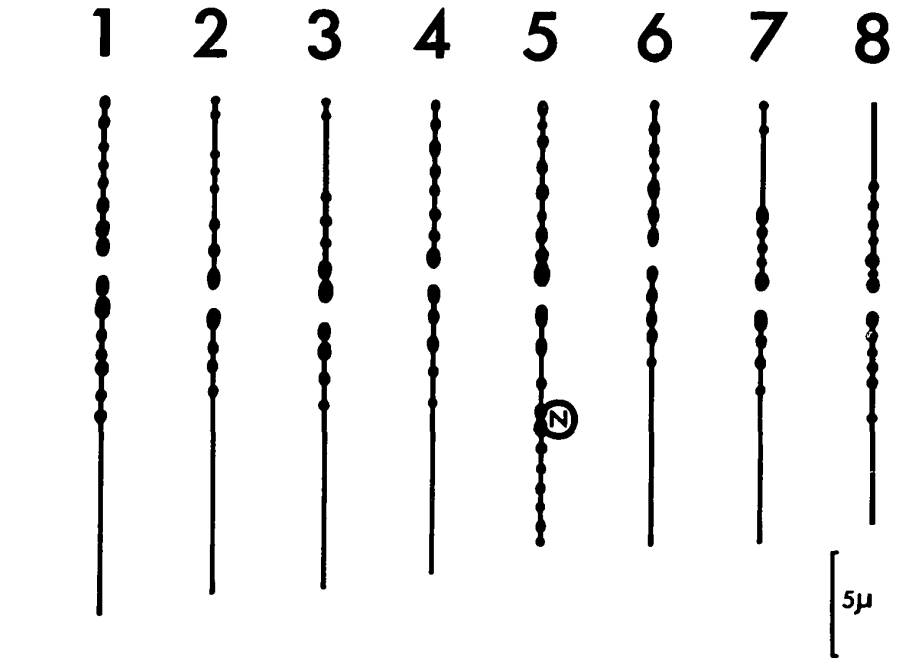

c
1
2
3
4
5
6
7
8
N
5μ

TABLE 7. MEAN LENGTHS AND ARM RATIOS OF PACHYTENE CHROMOSOMES OF *M. GLANDULOSA*

Chromosome	Mean Length $\pm S_E$ (in μ)	Mean Arm Ratio $\pm S_E$	Number Measured
1	24.4 $\pm$ 1.06	2.13 $\pm$ 0.074	11
2	23.4 $\pm$ 1.18	1.48 $\pm$ 0.154	6
3	23.0 $\pm$ 0.44	1.30 $\pm$ 0.098	8
4	22.3 $\pm$ 0.88	1.77 $\pm$ 0.106	11
5	20.8 $\pm$ 0.95	1.30 $\pm$ 0.094	6
6	20.8 $\pm$ 0.89	1.95 $\pm$ 0.109	11
7	20.6 $\pm$ 0.71	1.25 $\pm$ 0.120	11
8	19.7 $\pm$ 0.81	1.11 $\pm$ 0.053	10
Sum of Mean Lengths	175.0	-	-
Satellite (chromosome 5)	6.1 $\pm$ 0.27	-	6
Haploid Complement	142.5	-	1

M. falcata chromosomes and they are much more chromatic than the chromosomes of *M. sativa,* resembling those of the shorter *M. coerulea* accession No. 2010. Arm ratios of chromosomes 1, 2 and 4 are generally lower than those of comparable *M. sativa* chromosomes. The *M. sativa* chromosomes equivalent to *M. glandulosa* chromosomes 1 to 8 are chromosomes 3, 2, 1, 4, 8, 6, 5 and 7 respectively.

The change in position of the nucleolar chromosome from shortest to fifth longest is of interest. However, the length difference between chromosomes 5 and 8 is just over one micron and is not significant, possibly because of the small sample size. It is possible that the N.O. chromosome, being more chromatic initially, does not contract as rapidly as the other chromosomes and hence becomes proportionally longer at later stages of pachytene. The higher arm ratio of this chromosome in *M. glandulosa* compared with other species, although not significantly different, further suggests that contraction rates of chromosome arms may differ as meiosis proceeds, thus altering the arm ratio. Ho (1969) found that among chromosomes 1 to 5 in his study, the less contracted chromosomes had the largest arm ratios. He suggested that this was due to the less chromatic arms contracting faster than the more chromatic short arms, thus reducing arm ratios as pachytene proceeded. In the present case, the satellite may not contract as fast as the short arm, thus causing the arm ratio of chromosome 5 to increase. Buss (1967) found that the arm ratio of the satellited chromosome in somatic cells of *M. sativa* was much higher than it was in pachytene cells.

In spite of this difference it is obvious that the idiogram of *M. glandulosa* is of the same basic type as those of other members of

Figure 11

a. and b. Pachytene cell of *M. glutinosa*. Chromosomes are numbered at their short arm telomeres on the basis of their similarity to *M. sativa* L. chromosomes (Fig. 2). Arrow heads indicate centromere positions. X1850.


a
3IV
7
1
8?
5
1
2
4
2
10µ
b
1
7
5IV
6
3

the *M. sativa - falcata* complex shown above.

f. *M. glutinosa* M.B.

Some buds of a tetraploid *M. glutinosa* were examined and a number of cells at pachytene stage were analysed. No cells were found in which all chromosomes were measurable. Cells typical of those found are shown in Fig. 11. Both of these have quadrivalents present but they apparently involve different pairs of bivalents. The quadrivalent in Fig.11a involves chromosomes resembling *M. sativa* chromosomes 3 or 4 while those in the quadrivalent in Fig. 11b resemble *M. sativa* chromosomes 1 and 5. The lengths of measurable chromosomes ranged from approximately 20 microns to 40 microns but most were between 25 and 35 microns long. Chromosomes were noted similar in arm ratio and chromomere pattern to all *M. sativa* chromosomes, except that no nucleolar chromosomes were measured. The absence of any cells in which these chromosomes were discernable was due mainly to clumping of chromosomes around the nucleolus.

Similarity between the chromosomes of *M. glutinosa* and the chromosomes of the *M. sativa - falcata* complex is to be expected, since some taxonomists have placed them in one species, and *M. glutinosa* is also known as *M. sativa* f. *glutinosa* (M.B.) Urb. Armstrong and Gibson (1941) had found *M. glutinosa* behaved normally in crosses with tetraploid 'Grimm' alfalfa, and Lesins (1952) also found *M. glutinosa* to be cross fertile with *M. sativa*, so it probably has the same basic karyotype as has been described for all the other species of this group.

The finding of quadrivalents at pachytene is evidence for an

autotetraploid origin of *M. glutinosa*. The possibility must be considered that *M. glutinosa* is a polyploid derivative of diploid *M. glomerata*, with which it shares such morphological characters as yellow flowers and coiled pods with glandular hairs. However, the existence of both cream and yellow petals in single flowers, and the slightly looser coiling of the *M. glutinosa* seed pods (Lesins, personal communications) are characters which set *M. glutinosa* slightly apart from the *M. glomerata* phenotype.

g. Hybrids involving *M. glomerata*

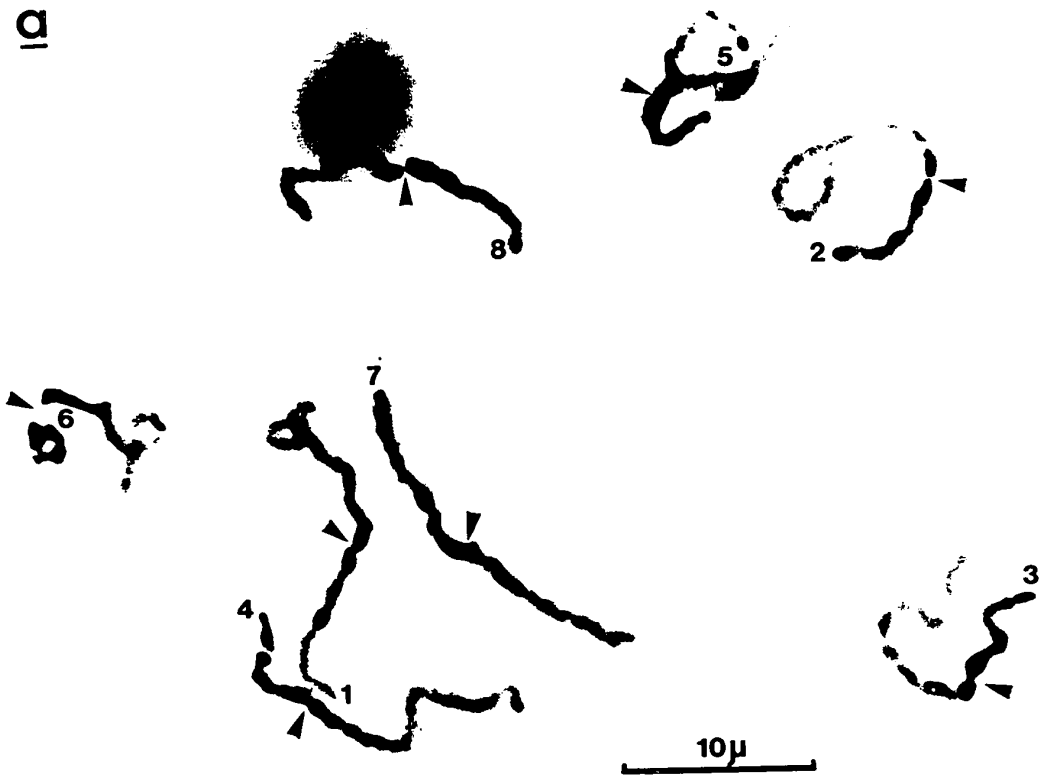
(i) *M. sativa* ('Saskatchewan White') X *M. glomerata* 1529

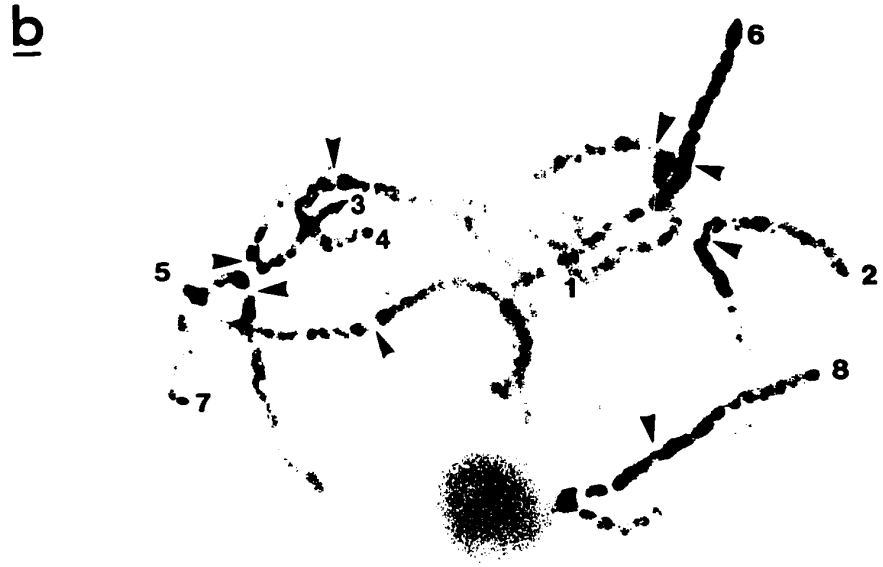
Five complete pachytene cells, and nine pachytene cells with fewer than eight chromosomes discernable were analysed. No gross pairing problems were noted, although in several cells the distal portions of the long arms of one or two chromosomes were unpaired for up to half the length of the long arm. This has been noted, however, in plants not of a hybrid origin, and was also noted by Buss (1967) in diploid *M. sativa*. An example of the complete pachytene pairing in this hybrid is given in Fig. 12a. Chromosomes were very contracted and ranged in length from 16 to 24 microns.

A number of cells at metaphase I were encountered and the pairing of chromosomes in them was analysed. Twenty-four cells had 8 bivalents, seven had 7 bivalents and 2 univalents, and three cells had 6 bivalents and 4 univalents. This is a lower percentage of normal pairing than found by Lesins (1968) but the sample size is smaller in this study.

Figure 12

a. Pachytene cell of *M. sativa* ('Saskatchewan White') X *M. glomerata* 1529. b. Pachytene cell of *M. glomerata* 1529 X *M. sativa* 506 (206-10). Chromosomes are numbered using the *M. sativa* L. idiogram (Fig.2) and arrow heads indicate centromere positions. X2650.


a
5
8
2
6
7
4
1
3
10μ


b
6
3
4
5
1
2
7
8

(ii) *M. glomerata* 1529 X *M. sativa* 506 (206-10)

Two plants of this hybrid composition were examined cytologically. Neither showed any abnormalities in chromosome pairing at pachytene and all diakinesis and metaphase I cells examined had eight bivalents. Two cells were found in which all eight chromosomes could be measured; one is shown in Fig. 12b. Chromosome length ranged from 31.5 microns for chromosome 1 to 20 microns for chromosome 8.

(iii) *M. lavrenkoi* 256 X *M. glomerata* 1529

M. lavrenkoi is a species very similar in appearance to *M. sativa* and it has been considered a part of *M. sativa*. Only a few buds of this plant were available. Cells were obtained in which only some of the chromosomes were discernable, but these all appeared to have normal pachytene pairing (Fig. 13a, b). A few cells at metaphase I were also examined and found to have eight bivalents (Fig. 13c).

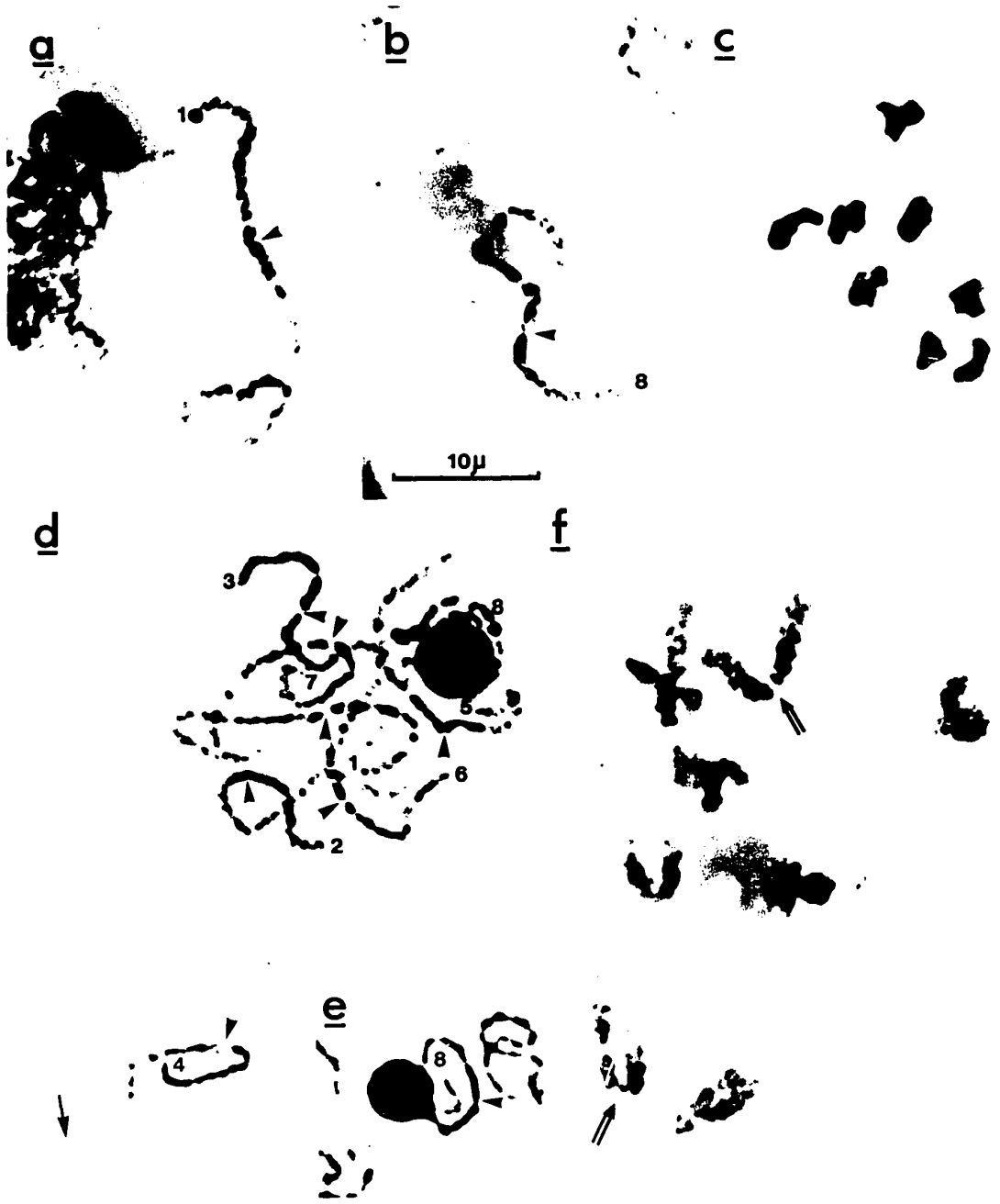
(iv) *M. glomerata* 1529 X *M. falcata* 1830

Pachytene pairing in this hybrid was normal in most cells although a few unpaired portions of bivalents were noted (e.g. terminal portion of chromosome 4 in Fig. 13d). Chromosome length in seven incomplete pachytene cells ranged from 20 to 33 microns. Chromosomes were paired at diakinesis and metaphase I to form eight bivalents. Inequality in the sizes of members of bivalents was noted in some diakinesis cells (Fig. 13 f), possibly an expression of unequal contraction of chromosomes in bivalents.

None of the above four hybrids showed any signs of gross disturbances of pachytene pairing, and the diakinesis and metaphase pairing

Figure 13

a, b and c. Chromosomes of *M. lavrenkoi* 256 X *M. glomerata* 1529. a. and b. Pachytene chromosomes numbered according to *M. sativa* L. idiogram (Fig.2) with arrow heads indicating centromere positions. c. Metaphase I cell with eight bivalents. d, e and f. Chromosomes of *M. glomerata* 1529 X *M. falcata* 1930. d. and e. Pachytene chromosomes numbered according to *M. sativa* L. idiogram (Fig.2) with arrow heads indicating centromere positions. Arrow indicates unpaired portion of chromosome 4. f. Diakinesis cell. Double arrows indicate bivalents with unequal sized members. All X 2200.


a
b
c
1
8
10μ
d
f
3
8
7
5
1
6
2
e
4
8

is close to that expected in plants of a pure species, as reported by Lesins (1968). The few achromatic portions of chromosomes unpaired at pachytene in some cells may or may not be important as indices of non-homology. As mentioned above, the same phenomenon has been noted in pure species, and may simply be a result of environmental influences. Maguire (1960) suggested that such lack of pairing might be due to complement unbalance rather than to non-homology of the chromosomes. Sadasivaiah and Magoon (1965) found that unpaired pachytene chromosome segments did not appear to reduce the fertility in *Sorghum*. Alternatively, the reduced pairing could be a genetically controlled feature introduced by one of the parent species. Genetic variations in the degree of pachytene pairing were reported in maize by Miller (1963).

On the basis of the pachytene pairing in these hybrids and the breeding and genetic studies of Lesins (1968), it must be concluded that the *M. glomerata* karyotype is completely homologous with those of the *M. sativa - falcata* complex.

h. Discussion of species relationships

As has been noted above, all of the species thus far studied have chromosomes whose pachytene morphology is similar, although differences in length and small differences in arm ratio do occur. In Table 8 the mean lengths, arm ratios and proportional lengths of means are given for the diploid species studied. *M. coerulea* is represented by Accession No. 221, since the three accessions of *M. coerulea* were significantly different in length and No. 221 had the longest chromosomes. t tests were conducted to compare the length and arm ratio

TABLE 8. COMPARISON OF THE MEAN LENGTHS, PROPORTIONAL LENGTHS AND MEAN ARM RATIOS OF EQUIVALENT CHROMOSOMES IN SPECIES OF *M. SATIVA s.l.*

Chromosome		Species					Species Significantly Diff. from *M. sativa*[c]
No.[a]	Item[b]	*M. sativa* 505	*M. falcata* (b)	*M. glomerata* (c)	*M. coerulea* 221 (d)	*M. glandulosa* (e)	
1	L	37.4	36.1	37.6	37.6	24.4	e
	PL	0.135	0.144	0.136	0.147	0.140	- - - -
	AR	2.48	2.82	3.01	2.28	2.13	c
2	L	38.2	34.8	39.4	37.0	23.4	e
	PL	0.138	0.139	0.143	0.145	0.134	- - - -
	AR	1.80	1.71	1.62	1.78	1.48	
3	L	39.6	37.4	37.2	34.7	23.0	d e
	PL	0.143	0.149	0.135	0.136	0.131	- - - -
	AR	1.40	1.41	1.47	1.28	1.30	
4	L	35.7	31.1	37.2	34.1	22.3	b e
	PL	0.129	0.124	0.135	0.133	0.127	- - - -
	AR	2.40	2.55	2.78	1.96	1.77	c d e
5	L	33.8	30.8	34.9	30.3	20.6	b d e
	PL	0.122	0.123	0.126	0.118	0.117	- - - -
	AR	1.33	1.36	1.22	1.25	1.25	
6	L	31.7	28.1	32.7	28.6	20.8	b e
	PL	0.114	0.112	0.118	0.112	0.119	- - - -
	AR	1.76	2.03	2.35	1.68	1.95	c
7	L	31.3	27.3	30.7	27.7	19.7	b d e
	PL	0.113	0.109	0.111	0.108	0.112	- - - -
	AR	1.34	1.07	1.22	1.03	1.11	b d
8	L	29.8	25.2	26.6	25.9	20.8	b c d e
	PL	0.107	0.100	0.096	0.101	0.119	- - - -
	AR	1.13	1.20	1.24	1.14	1.30	
Sum of Mean Lengths		277.5	250.8	276.3	255.9	175.0	- - - -
Satellite (chrom.8)	L	8.4	7.7	8.4	7.0	6.1	b d e
	PL	0.030	0.031	0.030	0.032	0.035	- - - -
Haploid Complement	L	284.4	238.8	273.6	261.8	-	b

a For chromosome order and equivalents in each species see Fig. 14

b L - mean length (in μ), PL - proportional length, AR - mean arm ratio

c At the 5% level in t tests

means of each species with those of *M. sativa*, and significant differences are indicated in Table 8. To facilitate comparison a standard order of chromosomes was necessary. This was eventually decided on by using the mean proportional lengths of each chromosome type for the five species, and arranging them in descending order of magnitude. The order decided on is equivalent to chromosomes 3, 2, 1, 4, 5, 6, 7 and 8 of the *M. sativa* idiogram in Fig.2. The equivalent chromosomes in the other species are indicated in Fig. 14.

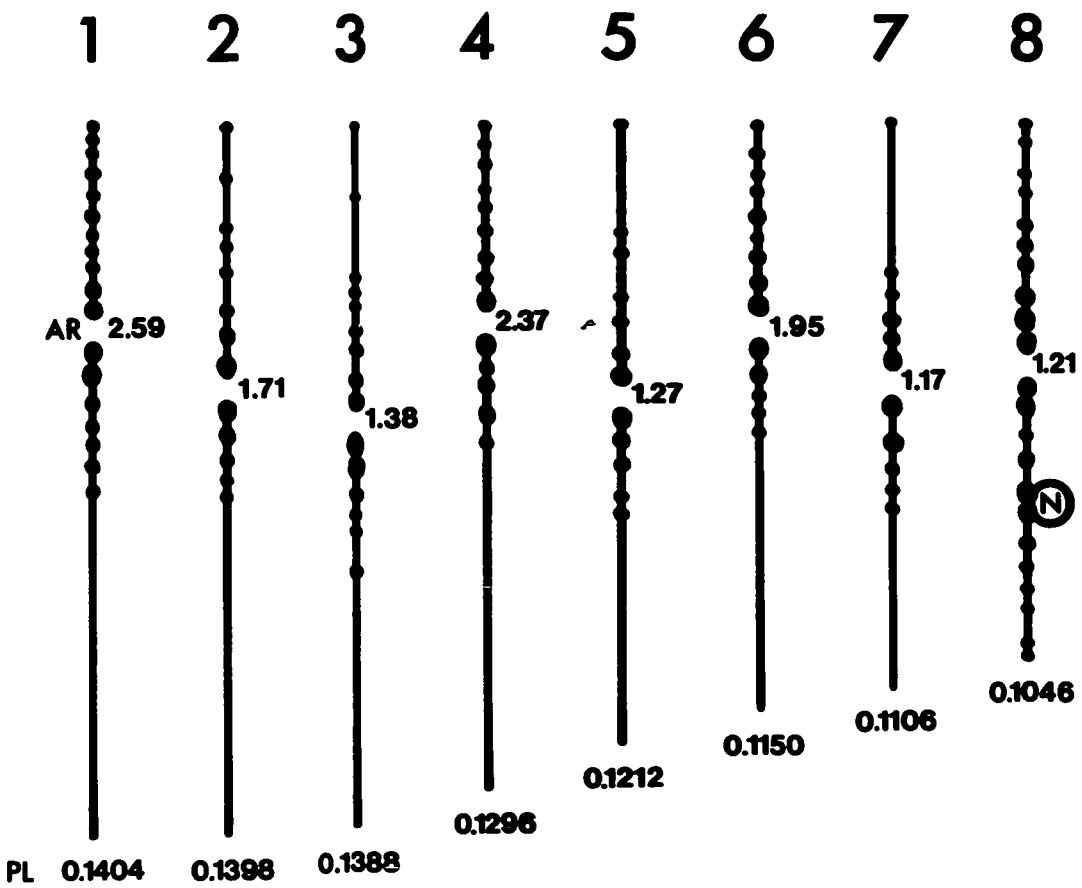
It can be seen from Table 8 that very few species differed from *M. sativa* in the arm ratios of their chromosomes (a maximum of three chromosomes having significantly different arm ratios in *M. glomerata*). *M. glandulosa* had all its chromosomes significantly shorter than those of *M. sativa*. *M. falcata*, *M. glomerata* and *M. coerulea* 221 had 5, 4 and 1 chromosomes respectively, shorter than equivalent chromosomes in *M. sativa*. Except in *M. glomerata*, all species had satellites significantly shorter than in *M. sativa*. *M. falcata* and *M. glandulosa* had significantly shorter complements than *M. sativa*. Only one full complement of *M. glandulosa* was available, however.

Chromosomes 1, 2, 3 and 6 are the most consistent in their length and arm ratios between species. Chromosomes 5 and 8 had consistent arm ratios but varied in length between most species and *M. sativa*.

If the proportional lengths of equivalent chromosomes are compared, it can be seen that most of the species have similar values for the same chromosome, the only exception being chromosome 8 of *M. glandulosa* which is proportionally much larger than the nucleolar chromosomes of the other species. The satellite of chromosome 8 is also very con-

Figure 14

Idiogram of pachytene chromosomes of coenospecies *M. sativa s.l.* based on data from equivalent chromosomes in idiograms of *M. sativa* L. (sat.), *M. falcata* L. (fal.), *M. glomerata* (glom.), *M. coerulea* (coer.), and *M. glandulosa* (glan.). Arm ratios (AR) and proportional lengths (PL) of each chromosome are indicated.



3	2	1	4	5	6	7	8	sat.
2	3	1	4	5	6	7	8	fal.
2	1	4	3	5	6	7	8	glom.
2	1	3	4	5	6	7	8	coer.
1	2	3	4	5	6	7	8	glan.

sistent in proportional length, except in *M. glandulosa*. These similarities in proportional lengths of chromosomes indicate that differences between species in real length of chromosomes may be primarily a result of differences in contraction, as suggested earlier. The chromaticity of the shorter karyotypes of *M. coerulea* and *M. glandulosa* is evidence for this.

As mentioned earlier, Ho (1969) found length differences between species which he explained as the result of differential contraction. He also found that shorter, more chromatic chromosomes had lower arm ratios. This phenomenon is observable in comparisons of *M. sativa* and *M. glandulosa*, although only one *M. glandulosa* chromosome (4) is significantly lower in arm ratio than *M. sativa* and two (chromosomes 6 and 8) have higher (but not significantly different) arm ratios than in *M. sativa*. Variability in proportional length of chromosomes may be due partly to differential contraction rates in achromatic and chromatic areas in the two arms of chromosomes, so that a chromosome with achromatic areas in both arms might shorten faster than one with one arm largely chromatic.

Similar observations have been made in studies of tomato chromosomes by Brown (1949) and Ramanna and Prakken (1967). The latter authors found achromatic parts of pachytene chromosomes were from 25 to 33 times the length of similar parts of somatic metaphase chromosomes, while chromatic parts were only 3 to 6 times as long as comparable somatic parts. They noted differences in contraction between chromatic parts of the long and short arms of chromosomes. Such a possible explanation for the increase in the proportional length of the

nucleolar chromosome of *M. glandulosa* has been suggested above.

In view of the similarities in chromosome morphology between these species, it seems logical to assign them all to one common cytogenetic unit - a coenospecies as suggested by Sinskaya (1940). This is supported by the results of all the workers who have crossed *M. falcata* and *M. sativa* and found a free interchange of genes, by the crosses of *M. coerulea* with *M. sativa* and *M. falcata* (Lesins, 1952), by the crosses of *M. glomerata* with *M. sativa* and *M. falcata* (Lesins, 1968), by the near perfect pachytene pairing exhibited by hybrids between *M. glomerata* and *M. sativa* and *M. falcata* reported here, and by the good pachytene pairing of hybrids involving *M. sativa* and *M. falcata* reported by Sprague (1959), Clement and Stanford (1963), and Ho (1969). The terminology of Lesins (196la), *M. sativa s.l. (sensu lato)*, is suggested as being appropriate for this coenospecies.

The data from Table 8 can be used to produce a common idiogram for the coenospecies. The order of chromosomes and mean proportional lengths can be used, together with arm ratios calculated from all observations in the five species, since there were very few significant differences in arm ratios. A visual comparison of equivalent chromosomes in the idiograms of the five species enables common features of the chromatic patterns of knobs and chromomeres to be determined. The resultant idiogram for *M. sativa s.l.* is presented in Fig. 14 with the arm ratios and proportional lengths of the chromosomes. The equivalent chromosomes in the five *s.s.* idiograms are indicated.

Chromosomes 4, 5, 6, 7 and 8 are all fairly constant in proportional length and hence in their positions in the idiograms, whereas

chromosomes 1, 2 and 3 are more variable and change order in some species.

Using the above idiogram, the chromosomes of all diploid members of the coenospecies can be identified, including those described by Buss and Cleveland (1968b), Ho (1969), and Gillies (1970a). The standard idiogram proposed by Gillies (1970a, b) has arm ratio values which in many cases are not very different from these, and the proportional lengths of chromosomes 6, 7 and 8 are similar. However, the proportional lengths and order of the first five chromosomes vary from those given here. The arm ratios of the pairs of tetraploid *M. sativa* chromosomes suggested as being homologous by Gillies (1970b), agree quite well with those of this common idiogram, with the exception of the two nucleolar chromosomes which in the tetraploid have the N.O. in their short arms.

From these comparisons it can be reasonably stated that this common idiogram is applicable with minor variations to all diploid and tetraploid members of the coenospecies *M. sativa s.l.* that have been studied so far.

i. Diploid *M. prostrata* Jacq.

Two accessions of *M. prostrata*, both from Italy, (Nos. 1682 and 1690) were analysed. A total of 36 cells were analysed and 11 provided complete complements. Fig. 15a shows one of these. The mean chromosome lengths and arm ratios were calculated for each accession (Table 9), and on comparison, only two lengths and no arm ratios were found to be significantly different between the accessions. As the two accessions were from adjacent areas this was not unexpected. The data from the two accessions were pooled and the mean values obtained were used in

TABLE 9. MEAN LENGTHS AND ARM RATIOS OF *M. PROSTRATA* PACHYTENE CHROMOSOMES - ACCESSION NOS. 1682 AND 1690

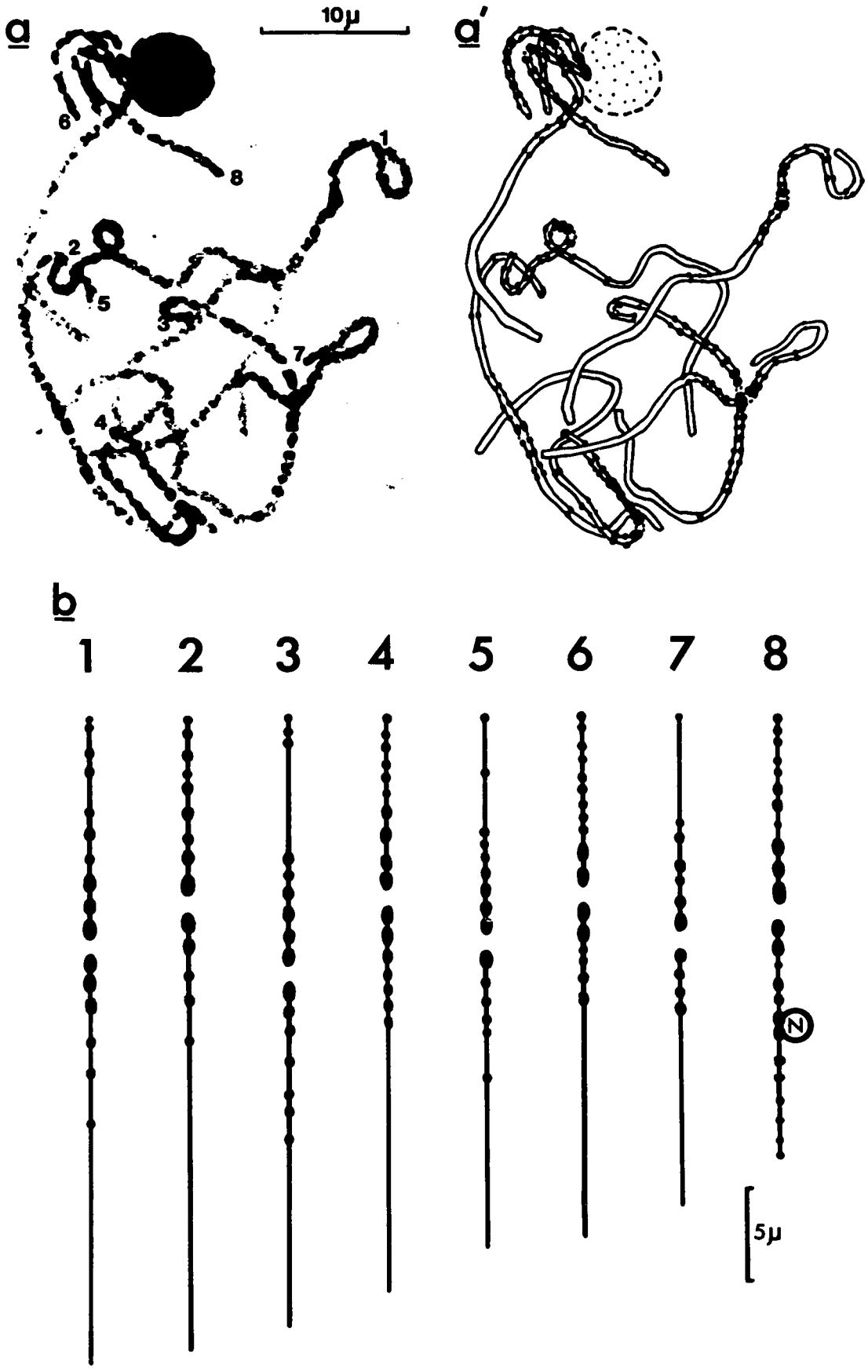
Chromosome		Accession		Significant Difference (t test)+	Mean of Two Accessions
No.	Item	1682	1690		
1	Length ± S_E‡	32.6 ± 1.80	35.5 ± 1.32	n.s.	34.6
	Arm Ratio ± S_E	2.06 ± 0.155	1.75 ± 0.074	n.s.	1.86
	Sample Size	6	13	-	19
2	Length ± S_E‡	33.0 ± 1.45	34.3 ± 0.77	n.s.	33.9
	Arm Ratio ± S_E	2.40 ± 0.135	2.41 ± 0.098	n.s.	2.41
	Sample Size	10	19	-	29
3	Length ± S_E‡	30.1 ± 1.12	33.8 ± 0.67	**	32.5
	Arm Ratio ± S_E	1.27 ± 0.088	1.40 ± 0.068	n.s.	1.35
	Sample Size	8	15	-	23
4	Length ± S_E‡	26.6 ± 1.27	32.2 ± 0.71	**	30.7
	Arm Ratio ± S_E	2.11 ± 0.131	2.28 ± 0.099	n.s.	2.23
	Sample Size	6	17	-	23
5	Lengtn ± S_E‡	26.6 ± 1.12	29.1 ± 0.81	n.s.	28.3
	Arm Ratio ± S_E	1.44 ± 0.167	1.31 ± 0.062	n.s.	1.35
	Sample Size	7	15	-	22
6	Length ± S_E‡	26.9 ± 1.50	28.2 ± 0.70	n.s.	27.7
	Arm Ratio ± S_E	2.05 ± 0.187	1.89 ± 0.082	n.s.	1.94
	Sample Size	8	17	-	25
7	Length ± S_E‡	24.4 ± 1.00	26.7 ± 0.86	n.s.	26.0
	Arm Ratio ± S_E	1.12 ± 0.058	1.22 ± 0.044	n.s.	1.19
	Sample Size	6	14	-	20
8	Length ± S_E‡	23.5 ± 1.90	23.4 ± 0.57	n.s.	23.4
	Arm Ratio ± S_E	1.22 ± 0.165	1.29 ± 0.058	n.s.	1.26
	Sample Size	6	7	-	13
Sum of Mean Lengths		223.7	243.2	-	237.1
Satellite (chrom.8)	Length ± S_E‡	7.0 ± 0.26	7.5 ± 0.29	n.s.	7.3
	Sample Size	6	7	-	13
Haploid Complement	Length ± S_E‡	211.5 ± 12.23	244.9 ± 7.38	*	232.7
	Sample Size	4	7	-	11

+ n.s., *, ** - not significant, significant at 5% level, significant at 1% level respectively.

‡ - in microns.

Figure 15

a. Pachytene cell of diploid *M. prostrata* with chromosomes numbered at their short arm telomeres. a! Interpretative drawing of a. with centromeres indicated by dotted gaps. X2400. b. Idiogram of pachytene chromosomes of diploid *M. prostrata*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer X3000.


a
10μ
a'
6
8
1
2
5
3
7
4
b
1
2
3
4
5
6
7
8
N
5μ

construction of the idiogram in Fig. 15b.

The chromosomes resemble very closely those of the *M. sativa s.l.* common idiogram, with the order of the first two chromosomes reversed. In this respect and in regard to chromosome length, *M. prostrata* is somewhat like *M. coerulea*, as it has chromosomes much shorter than *M. sativa* No. 505 (237.1 microns compared with 277.5 microns in *M. sativa*). However, the *M. prostrata* chromosomes are not as chromatic as those of *M. coerulea* and in this respect are more like *M. falcata* chromosomes in being less heavily stained.

The pooled *M. prostrata* data were compared with *M. sativa* No.505 It was found that, while all the chromosomes of *M. prostrata* except chromosome 1 were significantly shorter than their equivalent *M. sativa* chromosomes, none differed significantly in arm ratio. The haploid complement lengths of the two species were significantly different.

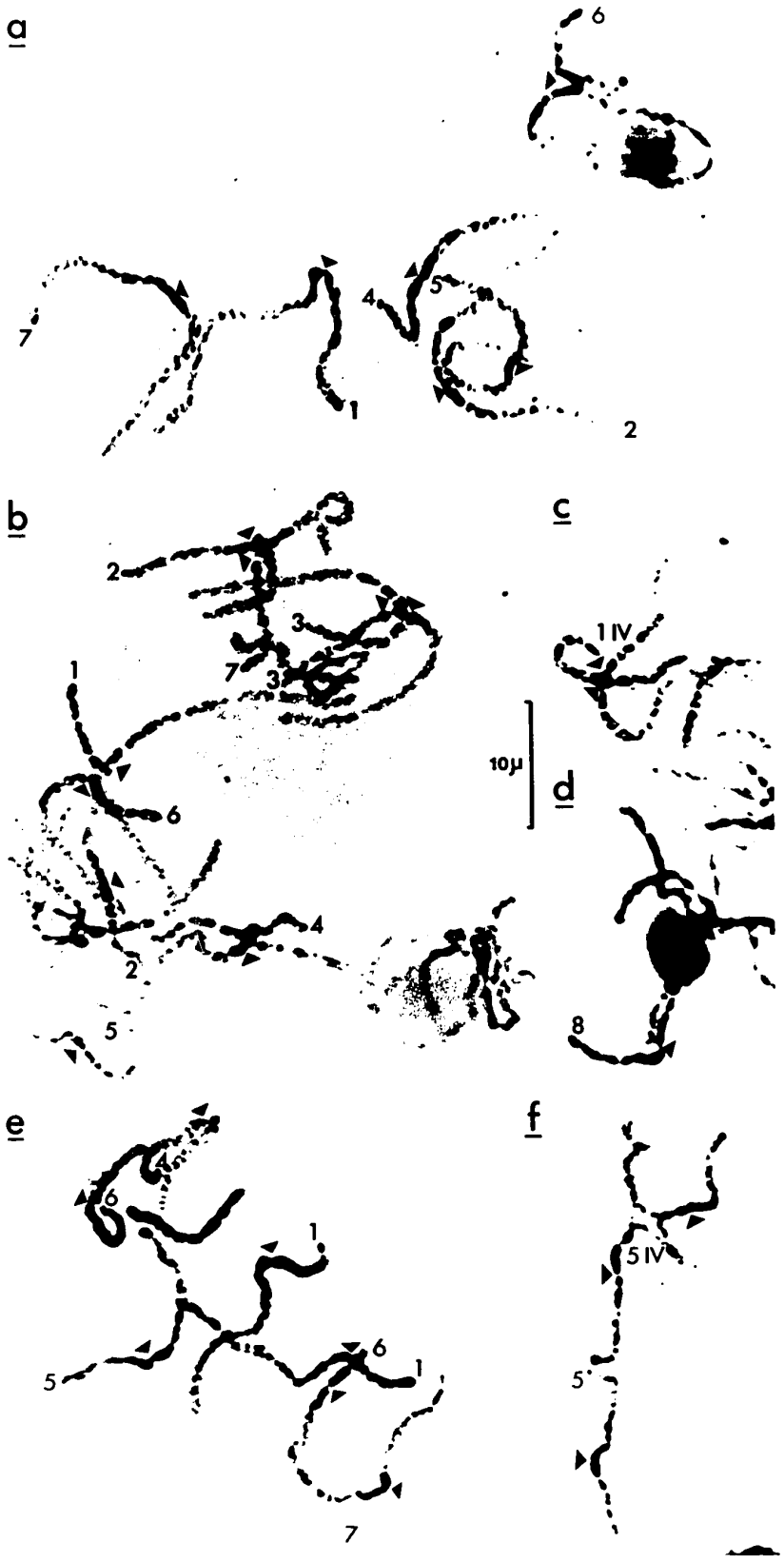
On the basis of the similarities of karyotypes (proportional lengths of chromosomes, arm ratios and chromatic patterns), it would appear that diploid *M. prostrata* should be a member of the *M. sativa s.l.* coenospecies. However, some caution is advised since the data of Lesins (1962) suggests that some barriers do exist between *M. prostrata* and other species of *M. sativa s.l.* and pachytene studies of hybrids of it with *M. sativa*, *M. falcata* and *M. glomerata* are advised before it is considered a member of this group.

3. Pachytene chromosome morphology at different ploidy levels - diploid, tetraploid and hexaploid

The availability of autotetraploid and essentially autohexaploid plants of *M. sativa s.l.* made possible the comparison of pachytene

Figure 16

Pachytene chromosomes of diploid, tetraploid and hexaploid *M. sativa s.l.* a. Diploid *M. falcata* L. Acc. No. 136. b, c and d. Autotetraploid *M. falcata* L. No. 1-5-2. e and f. Hexaploid *M. sativa L.* No. hexa 8-13. Chromosomes are numbered according to the *M. sativa s.l.* idiogram (Fig. 14), and centromeres are indicated by arrow heads. Quadrivalents are present in c. and f. All X1850.


a
6
7
4
5
1
2
b
2
3
7
3
1
6
4
2
5
10µ
c
1 IV
d
8
e
4
6
1
5
6
1
7
f
5 IV
5

chromosomes at three ploidy levels. In this comparison the diploid *M. falcata* No. 136 was used since one of the autotetraploid *M. falcata*s (1-5-2) had been derived from it by colchicine treatment. The hexaploid 8-13 was essentially autoploid since it was obtained from a backcross of auto-octaploid *M. sativa* to the parental tetraploid *M. sativa*. The data used for the diploid is the same as given in section 2b.

From the tetraploid 1-5-2 seventeen pachytene stage cells were analysed and a total of 61 chromosomes were measured. None of the cells had all 16 chromosomes measurable. A number of quadrivalents were among the chromosomes measured. In hexa 8-13 only 7 cells were found with measurable chromosomes and the total number of chromosomes measured was 44. Fig.16 shows diploid, tetraploid and hexaploid pachytene cells. The chromosomes of all three ploidy levels were classified into *M. sativa s.l.* idiogram classes on the basis of arm ratio, chromomere patterns and relative length. The mean lengths and arm ratios (with S_E's) are given in Table 10. F tests were carried out to determine if there was an effect of ploidy on mean length and mean arm ratio in each chromosome class. The results of these tests are also shown in Table 10.

It can be seen that there was a highly significant effect of ploidy on mean length of chromosomes and the satellite. In only two chromosomes, 1 and 4, was there a significant effect of ploidy on arm ratio. The F value for the arm ratio of chromosome 8 was approaching the 5% level. For those classes with significant F values critical differences between means were calculated by Duncan's multiple range method. In all cases the diploid means were significantly different from both tetraploid and hexaploid means, but only in the cases of the

TABLE 10. COMPARISON OF MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF DIPLOID, TETRAPLOID AND HEXAPLOID *M. SATIVA s.l.*

Chromosome		Mean Values			F Value	Significant Difference‡		
No.	Item+	2n *M. falcata* (a)	4n *M. falcata* (b)	6n *M. sativa* (c)		a-b	a-c	b-c
1	L	36.1 ± 0.82	25.3 ± 0.89	21.0 ± 0.55	51.96**	X	X	X
	AR	2.82 ± 0.138	2.26 ± 0.204	2.00 ± 0.117	7.18**	X	X	n.s.
	N	21	7	8				
2	L	34.8 ± 0.88	25.2 ± 0.44	21.3 ± 1.75	41.84**	X	X	n.s.
	AR	1.71 ± 0.064	1.67 ± 0.098	1.58 ± 0.020	0.19	n.s.	n.s.	n.s.
	N	20	12	2				
3	L	37.4 ± 1.15	25.6 ± 0.66	23.7 ± 2.17	31.94**	X	X	n.s.
	AR	1.41 ± 0.079	1.57 ± 0.154	1.17 ± 0.186	1.58	n.s.	n.s.	n.s.
	N	14	6	3				
4	L	31.1 ± 0.82	20.6 ± 0.71	19.3 ± 0.68	51.10**	X	X	n.s.
	AR	2.55 ± 0.090	2.07 ± 0.176	1.80 ± 0.179	9.25**	X	X	n.s.
	N	17	5	6				
5	L	30.8 ± 0.71	21.6 ± 0.27	18.6 ± 0.69	96.13**	X	X	X
	AR	1.36 ± 0.076	1.26 ± 0.101	1.15 ± 0.091	1.53	n.s.	n.s.	n.s.
	N	19	10	10				
6	L	28.1 ± 1.10	19.4 ± 0.87	17.5 ± 0.98	22.80**	X	X	n.s.
	AR	2.03 ± 0.120	1.98 ± 0.183	1.70 ± 0.145	0.84	n.s.	n.s.	n.s.
	N	15	7	4				
7	L	27.3 ± 0.87	19.2 ± 0.48	17.1 ± 0.44	51.75**	X	X	n.s.
	AR	1.07 ± 0.048	1.12 ± 0.092	1.15 ± 0.052	0.52	n.s.	n.s.	n.s.
	N	15	8	8				
8	L	25.2 ± 0.95	19.8 ± 1.48	13.7 ± 1.20	15.49**	X	X	X
	AR	1.20 ± 0.036	1.24 ± 0.057	1.44 ± 0.117	3.34	n.s.	n.s.	n.s.
	N	15	6	3				
Satellite	L	7.70 ± 0.23	6.25 ± 0.17	4.33 ± 0.33	25.89**	X	X	X
	N	15	6	3				
Sum of Mean Lengths		250.8	176.7	152.2	-	-	-	-

\+ L - length ± S_E (in μ); AR - arm ratio ± S_E; N - number measured.

** significant at 1% level.

‡ Duncan's Multiple Range test:- X - difference between means indicated significant at 5% level; n.s. - difference between means indicated not significant.

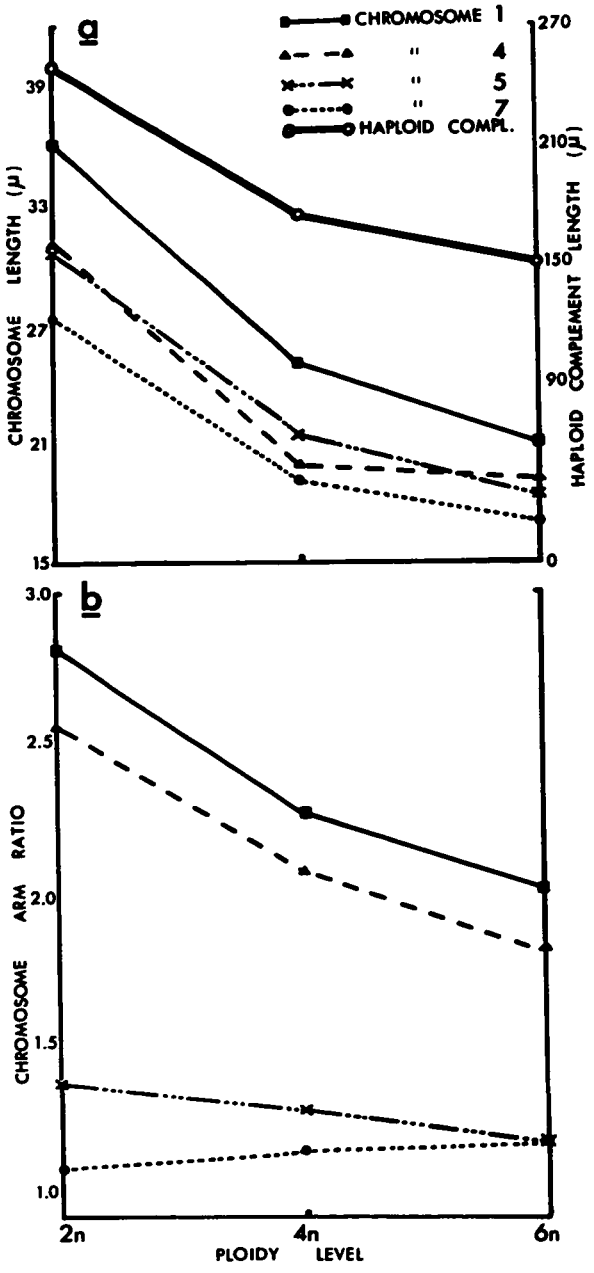
lengths of chromosomes 1, 3, 8 and the satellite was the tetraploid mean significantly different from that of the hexaploid. The small number of hexaploid samples, especially in chromosomes 2, 3, 6 and 8, may be partially to blame for the lack of significant differences between tetraploids and hexaploids.

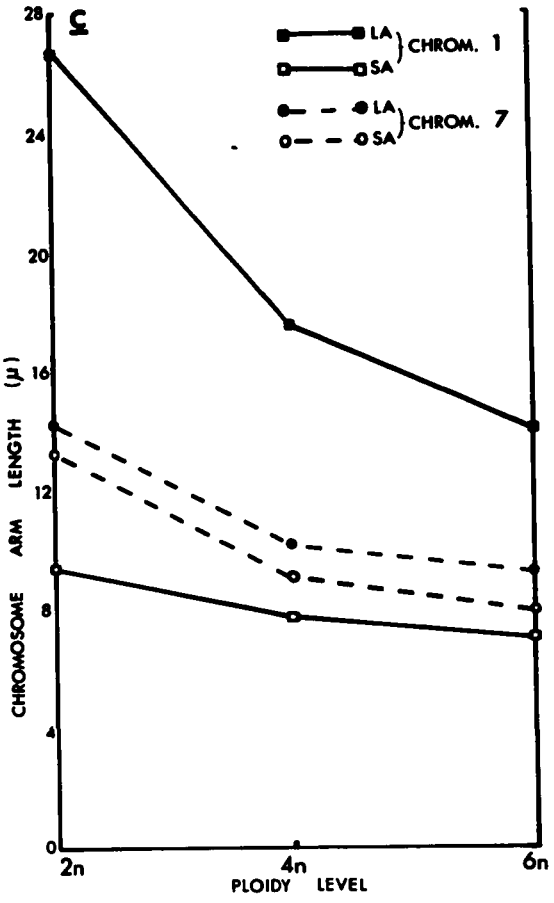
The results from four chromosomes (1, 4, 5 and 7) for which there were reasonable numbers of hexaploid measurements were examined more closely. Two of these (1 and 5) had significant length differences between all ploidy levels; the other two (4 and 7) did not have significant length differences between tetraploid and hexaploid levels. The effect of ploidy on chromosome length in these four is shown graphically in Fig. 17a. It can be seen that the diploid to tetraploid reduction in length is much greater than the tetraploid to hexaploid reduction in all four chromosomes. Included in Fig. 17a. is the sum of the eight mean chromosome lengths (haploid complement length) at each ploidy level. The slope of the diploid to tetraploid line in this instance is also greater than that of the tetraploid to hexaploid, so the reduction in the length of all the chromosomes is greater from diploid to tetraploid than from tetraploid to hexaploid. The slopes of the lines for all four chromosomes are almost the same, showing that the rate of reduction in length is fairly uniform for all chromosomes.

Two of these four chromosomes also had significant arm ratio differences between the diploid level and the other two ploidy levels. The arm ratio changes are illustrated in Fig. 17b. The pronounced significant fall in arm ratios of chromosomes 1 and 4 can be contrasted

Figure 17

Effect of ploidy level on chromosome lengths and arm ratios. a. Total chromosome lengths of chromosomes 1, 4, 5 and 7, and the haploid complement at three ploidy levels. b. Arm ratios of chromosomes 1, 4, 5 and 7 at three ploidy levels. c. Long arm and short arm lengths of chromosomes 1 and 7 at three ploidy levels.


a
CHROMOSOME 1
" 4
" 5
" 7
HAPLOID COMPL.
CHROMOSOME LENGTH (μ)
HAPLOID COMPLEMENT LENGTH (μ)
39
33
27
21
15
270
210
150
90
0
b
CHROMOSOME ARM RATIO
3.0
2.5
2.0
1.5
1.0
2n
4n
6n
PLOIDY LEVEL


c
LA } CHROM. 1
SA
LA } CHROM. 7
SA
CHROMOSOME ARM LENGTH (μ)
28
24
20
16
12
8
4
0
2n
4n
6n
PLOIDY LEVEL

with the non-significant changes in the arm ratios of chromosomes 5 and 7. Chromosome 7 actually had a non-significant increase in arm ratio from diploid to hexaploid. An interesting trend is indicated in the arm ratios of chromosome 8 where the increase in arm ratio from diploid to hexaploid almost reaches significance. The small hexaploid sample could be causing this variation, but the situation is similar to that obtaining in the nucleolar chromosome of *M. glandulosa* where the arm ratio was much higher than in other *M. sativa s.l.* nucleolar chromosomes.

Differences in chromosome size between ploidy levels, and reduction in chromosome size with polyploidy, have been noted before. Manton (1950) found that there was a relationship of smaller chromosome size with higher chromosome number in the *Pteridophyta*, and he suggested this was a nuclear or physiological adjustment to polyploidy. Darlington (1964) states that polyploid species regularly have smaller chromosomes than their diploid relatives, and that it is probably an evolutionary adaption to the increase in chromosome number. It is interesting to note that Fryer (1930) measured the chromosomes at somatic metaphase in a tetraploid root sector of a diploid *M. falcata*, and reported chromosome lengths which were about 15% shorter than those he gave for chromosomes from diploid cells of the same plant.

In the present case it appears that contraction of the chromosomes is the immediate cause of the reduction in chromosome lengths with increase in ploidy level. This is borne out by the fact that the chromosomes are still recognisable by means of relative lengths, arm ratios and chromatic patterns. The more chromatic nature of the higher

ploidy level chromosomes could be a result of the higher staining ability of the chromosome as the DNA - histone complex becomes more compact. The chromosome lengths of the tetraploid and hexaploid are very similar to those found by Ho (1969) in diploid *M. sativa*, by Gillies (1970b) in tetraploid *M. sativa*, and for *M. glandulosa* in this report. The values given for chromosome lengths by Ho (1969) were 24 to 16 microns, and by Gillies (1970b) were 26 to 18 microns. In all these cases the chromosomes were more chromatic than those found in diploids having haploid complement lengths of 230 to 280 microns. Ho (1969) suggested that chromosome contraction might be under genetic control, and the results obtained with *M. coerulea* (section 2d.) tend to support this. Joshi and Swaminathan (1968) found such a control of chromosome condensation in wheat, and using monosomics, located on chromosome 5B of 'Chinese Spring', the genes which appeared to control the condensation.

Differential contraction was suggested by Tobgy (1943) as the cause of chromosome length differences between two species of *Crepis*. He found that in a hybrid the chromosomes of the two species paired and appeared to be the same length, showing that they were homologous. Support for the argument that contraction is the main cause of length differences between ploidy levels is lent by the results found for the arm ratios. The only two chromosomes with significant arm ratio reductions (chromosomes 1 and 4) are the only chromosomes with initially high arm ratios and associated highly chromatic short arms. Since Brown (1949) and Ramanna and Prakken (1967) have shown that in pachytene chromosomes of tomato (which are similar to those in *Medicago*) the achromatic zones contract many times more than the chromatic ones, it is

reasonable to expect the achromatic long arms of chromosomes 1 and 4 to contract more than the chromatic short arms, thus causing reductions in arm ratios. However, in the symmetrical, median centromered chromosomes such as 5 and 7, the contraction in both arms should be similar and little change in arm ratio is to be expected due to contraction. This point is further demonstrated by plotting the lengths of the long arms and short arms of chromosomes 1 and 7 at the three ploidy levels (Fig.17c). It can be seen that there is considerable difference in the slopes of the lines for the long and the short arms of chromosome 1, whereas for chromosome 7 the slopes of the lines for the two arms are almost identical.

The exact cause of the increased contraction of pachytene chromosomes at the higher ploidy levels is difficult to determine. Lewis and John (1963) discussed a number of cases of polyploidy in coccids and aphids in which the tetraploids had smaller chromosomes than the diploids but had the same amount of DNA per cell as the diploids. In these cases the smaller size of chromosomes in the tetraploids was considered to be due to a reduction in the level of polynemy in the chromosomes.

In the present case any attempt to relate reduction in length of chromosomes to reduced polynemy would have to await determination of DNA values in cells at the different ploidy levels. The differences in genetic background of the different specimens, viz. *M. falcata* and *M. sativa*, should not be a problem in the light of the genetical and cytological similarities which have been demonstrated between them. However, in view of descriptions of variations in chromosome length at

one ploidy level (Ho (1969) and *M. coerulea,* section 2d.) it would appear unlikely that differential polynemy is involved in this case.

Gillies (1970b) suggested that the shorter tetraploid *M. sativa* chromosomes were a result either of a slightly later and hence more contracted stage of pachytene being more amenable to study in the tetraploid, or of contraction commencing earlier in the meiotic cycle of the tetraploid. The possibilities of nuclear volumes of higher ploidy level cells not being exact multiples of the nuclear volume of diploid cells was suggested by Gillies as possible reason for this. Ichikawa and Sparrow (1967) found that nuclear volumes of tetraploid and hexaploid wheats were not as large as the expected volumes calculated from the diploid ancestors. Troy and Wimber (1968) also found a decrease in the relative size of chrysanthemum nuclei with increasing polyploidy, so that the volume of a 22n nucleus was only eight times that of a 2n nucleus. The pollen grain diameters given by Bingham and Binek (1969b) result in cell volumes of hexaploid *M. sativa* being as expected, approximately 1.5 times those of tetraploids. However Julen (1944) found pollen diameters in diploid and tetraploid alfalfa which give a volume increase of only 1.9 times with a doubling of ploidy. These two results are inconclusive as the relationship of nuclear volumes to cell volumes is unknown, and the two reports differ in the values of the tetraploid pollen diameters. Thus it would seem that the cause of the ploidy effect on chromosome contraction cannot at present be determined.

In addition to the tetraploid 1-5-2, a second autotetraploid *M. falcata* (No. 10-3-1) was studied at pachytene stage. The values of

TABLE 11. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF AUTOTETRAPLOID *M. FALCATA*

Chromosome No.	Item+	Mean values of lines 1-5-2	Mean values of lines 10-3-1	t value - Signif. Diff. Test	Mean of Two
1	L	25.29 ± 0.89	27.69 ± 1.01	1.76	26.57
	AR	2.26 ± 0.204	2.41 ± 0.178	0.56	2.34
	N	7	8		15
2	L	25.21 ± 0.44	28.00 ± 1.29	2.57*	26.14
	AR	1.67 ± 0.098	1.55 ± 0.096	0.77	1.63
	N	12	6		18
3	L	25.58 ± 0.66	29.38 ± 0.97	2.60*	28.11
	AR	1.57 ± 0.154	1.11 ± 0.087	2.82*	1.26
	N	6	12		18
4	L	20.60 ± 0.71	25.38 ± 1.11	3.78**	22.72
	AR	2.07 ± 0.176	1.98 ± 0.255	0.30	2.03
	N	5	4		9
5	L	21.55 ± 0.27	24.44 ± 1.18	2.22*	22.92
	AR	1.26 ± 0.101	1.07 ± 0.068	1.53	1.17
	N	10	9		19
6	L	19.36 ± 0.87	20.50 ± 0.87	0.78	19.70
	AR	1.98 ± 0.183	2.01 ± 0.142	0.10	1.99
	N	7	3		10
7	L	19.19 ± 0.48	19.50 ± 0.44	0.47	19.33
	AR	1.12 ± 0.092	0.97 ± 0.051	1.37	1.05
	N	8	7		15
8	L	19.75 ± 1.48	20.67 ± 2.42	0.34	20.06
	AR	1.24 ± 0.057	1.06 ± 0.088	1.77	1.18
	N	6	3		9
Satel-lite	L	6.25 ± 0.17	6.00 ± 0.50	0.61	6.17
	N	6	3		9
Sum of Mean Lengths		176.53	195.56		184.55

+ L - length ± S_E (in μ); AR - arm ratio ± S_E; N - number measured

* significantly different at 5% level

** significantly different at 1% level

chromosome lengths and arm ratios obtained for 10-3-1 are given in Table 11 together with the results of 1-5-2 for comparison. The means of the two were compared in t tests and four were found to be significantly different in length, but only one (chromosome 3) had significantly different arm ratios in the two plants. The only highly significant difference was between the lengths of chromosome 4 in the two. The total lengths of the haploid complements of the two plants differed by approximately 19 microns. Mean values of length and arm ratio were calculated for the pooled data of the two plants and these are given in Table 11. As only five of the 17 t tests produced significant differences it was felt that pooling the data was justified. The pooled data for tetraploid *M. falcata* was later used for comparison with data from dihaploid *M. sativa*.

4. Dihaploid *M. sativa*

a. Karyotypes

Plants with 16 chromosomes which have been derived from unfertilized gametes of tetraploid *M. sativa* have been variously called polyhaploids (Lesins, 1957), dihaploids (Clement and Stanford, 1963, Clement and Lehman, 1963) and haploids (Stanford and Clement, 1958, Bingham, 1969). "Dihaploid" appears to be correctly used (Rieger, Michaelis and Green, 1968) and will be used here to avoid confusion with "haploid" (n = 8), which has been used in this study in connection with complement lengths to refer to the sum of the eight different chromosomes of the basic set.

Six dihaploids and a hybrid between H-7 and a diploid *M. falcata* (D_{48}) (Table 2) were examined, and sufficient pachytene cells were found

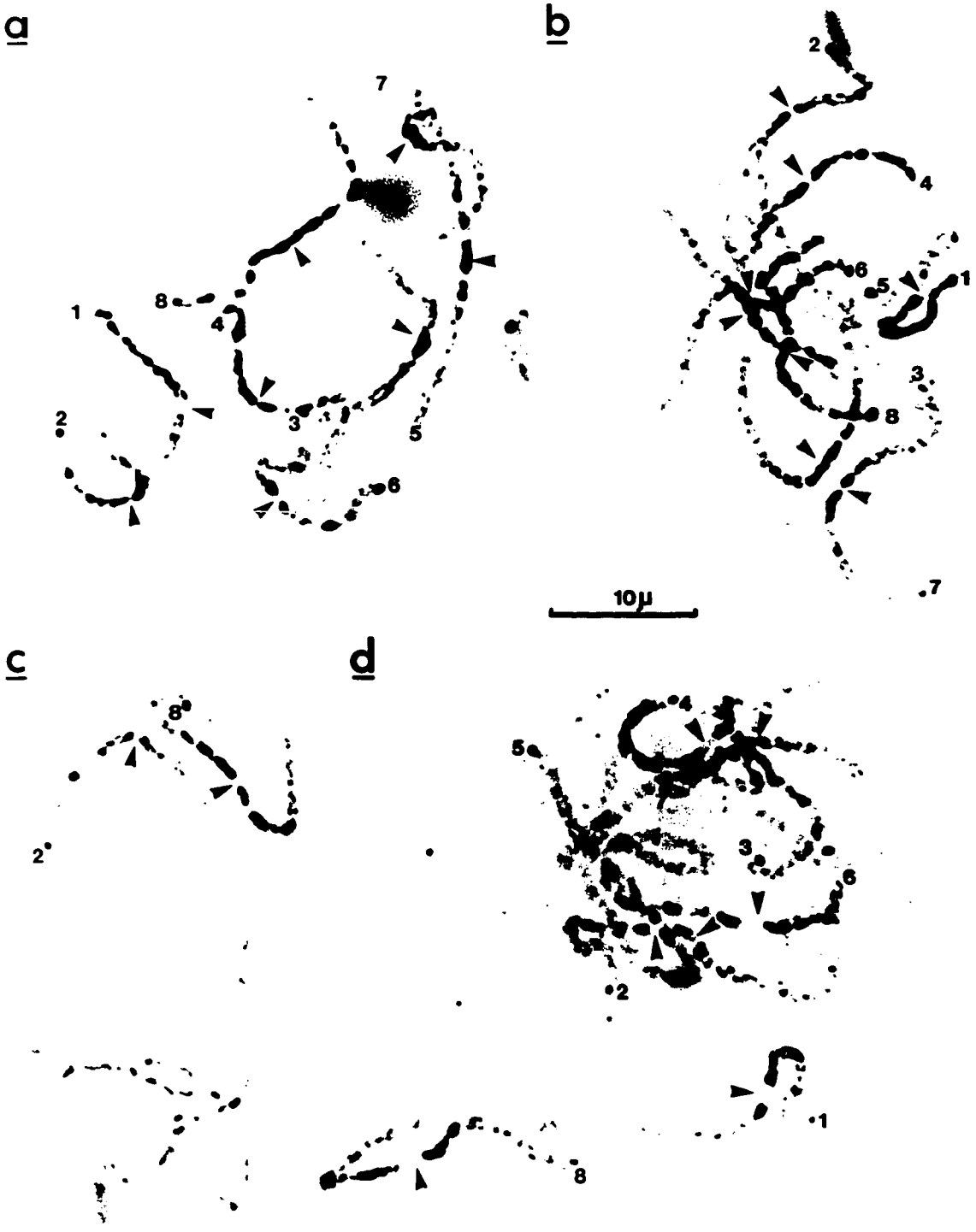
Figure 18

Pachytene cells of dihaploid *M. sativa*. a. Dihaploid H-1. b. Dihaploid H-2. c. Dihaploid H-6. Broken lines and arrows in a. indicate breaks in chromosomes. Numbering is according to the *M. sativa s.l.* idiogram and arrow heads indicate centromere positions. X2150.


a
b
c
10μ

Figure 19

Pachytene cells of dihaploid *M. sativa* a. Dihaploid H-3. b. and c. Dihaploid H-7. d. Dihaploid H-7 X *M. falcata* D48. Numbering is according to the *M. sativa s.l.* idiogram and arrow heads indicate centromere positions. X2150.


a
b
c
d
10μ

for idiograms to be constructed for five of the dihaploids and the hybrid. H-8 proved to be more difficult to prepare due to greater stickiness and clumping of the chromosomes, and insufficient pachytene cells were found for analysis. Typical cells from each plant are shown in Figs. 18 and 19. It can be seen that all have similar chromosomes with the main variations being in length and degree of chromaticity. The chromosomes were measured and mean lengths and arm ratios for the eight chromosomes of each were calculated. These are given in Table 12, the order of chromosomes being the same as that of the *M. sativa s.l.* common idiogram. F tests were carried out on lengths and arm ratios of each chromosome class and it was found that there were significant effects of haploid line on five chromosome lengths, satellite length, complement length and arm ratios of two of the chromosomes (Table 12).

A limited number of observations were made of other meiotic stages such as diakinesis, metaphase I and anaphase I and II. Few deviations from normal eight bivalent pairing and 8-8 disjunction were found. In H-2 some cells had seven bivalents and two univalents at metaphase I, and this plant also had several cells with 16 chromosomes at metaphase II, seemingly the result of restitution at anaphase I. It was noted that one cell in this plant had a bridge at anaphase I.

b. Relationship of dihaploid *M. sativa* to diploid and tetraploid *M. sativa s.l.*

Although morphologically the chromosomes of the dihaploids closely resemble those of diploid *M. sativa s.s.*, it can be seen that

TABLE 12. MEAN CHROMOSOME LENGTHS AND ARM RATIOS OF DIHAPLOIDS AND A DIHAPLOID X *N. FALCATA* HYBRID

Chromosome No.	Item*	H-1	H-2	H-3	H-6	H-7	H-7 X D48	F test
1	L	30.7 ± 1.08	32.9 ± 0.94	26.9 ± 0.96	35.1 ± 1.58	33.0 ± 1.26	33.0 ± 0.95	6.38**
	AR	2.91 ± 0.184	2.51 ± 0.094	2.17 ± 0.086	2.80 ± 0.252	2.59 ± 0.160	3.17 ± 0.215	5.18**
	N	11	20	14	8	11	11	
2	L	30.8 ± 1.99	30.1 ± 0.90	27.7 ± 1.50	31.8 ± 1.84	33.8 ± 1.44	31.2 ± 1.27	2.05
	AR	2.04 ± 0.154	1.70 ± 0.113	1.83 ± 0.121	1.91 ± 0.168	2.12 ± 0.082	1.80 ± 0.096	1.76
	N	7	17	10	8	11	14	
3	L	34.3 ± 1.87	32.6 ± 1.27	28.8 ± 1.32	34.0 ± 1.70	33.7 ± 1.21	35.4 ± 2.13	2.34
	AR	1.43 ± 0.120	1.40 ± 0.098	1.45 ± 0.100	1.30 ± 0.133	1.29 ± 0.125	1.13 ± 0.170	0.84
	N	7	18	12	6	9	7	
4	L	27.1 ± 0.92	28.8 ± 0.89	24.5 ± 0.76	28.8 ± 1.35	29.4 ± 1.22	27.7 ± 1.24	3.21*
	AR	2.94 ± 0.200	2.42 ± 0.130	2.11 ± 0.121	2.19 ± 0.161	2.23 ± 0.167	2.45 ± 0.115	3.32**
	N	8	19	15	9	8	13	
5	L	25.1 ± 1.46	27.5 ± 0.67	23.8 ± 0.74	28.4 ± 1.07	29.2 ± 1.02	27.2 ± 0.74	4.47**
	AR	1.05 ± 0.079	1.18 ± 0.053	1.15 ± 0.082	1.07 ± 0.072	1.18 ± 0.084	1.11 ± 0.050	0.55
	N	8	25	14	14	12	18	
6	L	23.5 ± 0.71	26.1 ± 0.79	22.3 ± 0.82	24.4 ± 1.22	26.1 ± 1.84	24.3 ± 1.08	2.31
	AR	2.11 ± 0.240	2.03 ± 0.089	1.86 ± 0.115	1.93 ± 0.248	1.56 ± 0.274	1.99 ± 0.137	0.81
	N	5	20	14	6	5	14	
7	L	22.6 ± 0.895	24.2 ± 0.71	20.4 ± 0.89	25.7 ± 1.81	24.4 ± 0.97	24.3 ± 1.85	2.95*
	AR	1.12 ± 0.102	1.02 ± 0.048	1.04 ± 0.064	1.16 ± 0.104	1.28 ± 0.194	1.24 ± 0.106	1.43
	N	8	21	13	7	5	7	
8	L	23.3 ± 0.37	24.8 ± 0.98	20.0 ± 0.77	22.9 ± 1.43	20.8 ± 0.75	21.6 ± 0.98	3.43**
	AR	1.25 ± 0.059	1.15 ± 0.054	1.26 ± 0.078	1.14 ± 0.076	1.20 ± 0.080	1.17 ± 0.067	0.54
	N	9	16	11	9	9	16	
Satellite (Chrom.8)	L	7.7 ± 0.26	7.0 ± 0.36	5.6 ± 0.27	5.9 ± 0.53	6.5 ± 0.50	6.4 ± 0.31	19.17**
	N	9	16	11	9	9	16	
Haploid Complement	L	235.8 ± 4.75	224.1 ± 9.25	200.3 ± 9.40	226.7 ± 21.89	219.5 ± 10.96	208.5 ± 18.24	7.83**
	N	2	12	6	3	4	3	
Sum of Mean Lengths		217.4	227.0	194.4	231.1	230.4	224.7	-

\+ L - length ± S_E (in μ); AR - arm ratio ± S_E; N - number of observations.

*, ** - significantly different at 5% and 1% levels respectively.

all of the dihaploid lines' chromosome complements are considerably shorter than those found in the diploid. A t test was carried out to compare lengths and arm ratios of H-2 (since it had approximately median chromosome lengths) with those of *M. sativa* No. 505 (Table 13). All chromosomes, the satellite, and the complement differed significantly in length, but only one chromosome (chromosome 7) was significantly different in arm ratio between the two.

The shortness of the dihaploid chromosomes suggested that they might be more similar in length to the chromosomes of the tetraploid measured earlier. To test this the chromosome lengths and arm ratios of the shortest dihaploid (H-3) were compared with those of the autotetraploid *M. falcata* in t tests (Table 13). It was found that only chromosome 6 had a significantly different length, and no chromosomes differed significantly in arm ratios between the two. Thus it appears that H-3 is similar in degree of chromosome contraction to the autotetraploids. Chromosome lengths of H-3 also differ very little from the values given by Gillies (1970b) for a tetraploid alfalfa (*M. sativa*) - H-3 ranges from 28.95 to 20 microns while the tetraploid *M. sativa* ranged from 26 to 18.1 microns.

The fact that the tetraploid *M. sativa* described by Gillies (1970b) and the autotetraploid *M. falcatas* described above have chromosomes of similar lengths to those of the dihaploids, suggests that the contraction is a property of the tetraploid state and it is still present in the dihaploid progeny which are derived from the tetraploids. The occurrence of highly contracted chromosomes in diploid *M. sativa* (Ho, 1969), diploid *M. glandulosa* and diploid *M. coerulea* (above),

TABLE 13. COMPARISON OF DIHAPLOID CHROMOSOME LENGTHS AND ARM RATIOS WITH OTHER DIHAPLOIDS, DIPLOIDS AND TETRAPLOIDS

Chromosome No.	Item[1]	H-2 vs 2n *M. sativa* #505[2]	H-3 vs 4n *M. falcata*[2]	H-2 vs H-3[3]	H-6 vs H-7[3]	H-7 vs H-7 X *M. falcata*[3]
1	L	*	n.s.	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	*
2	L	**	n.s.	n.s.	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
3	L	**	n.s.	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
4	L	**	n.s.	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
5	L	**	n.s.	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
6	L	**	*	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
7	L	**	n.s.	*	n.s.	n.s.
	AR	**	n.s.	n.s.	n.s.	n.s.
8	L	**	n.s.	*	n.s.	n.s.
	AR	n.s.	n.s.	n.s.	n.s.	n.s.
Satellite	L	**	n.s.	*	n.s.	n.s.
Haploid Complement	L	**	-	*	n.s.	n.s.

1. L - length; AR - arm ratio.
2. t tests: *, ** - significant difference at 5% and 1% level respectively; n.s. - not significantly different.
3. Critical differences by Duncan's Multiple Range Test: * - significantly different at 5% level; n.s. - not significantly different.

shows that this contraction is not unique to tetraploid plants and their recent diploid progeny. It is possible that genetic factors causing this contraction may be found on the chromosomes, and the higher dosage present in tetraploids causes greater contraction. If this is so, it should be possible to find tetraploid plants lacking the dominant alleles and having chromosomes as long as the diploids'. Some support for this idea comes from Ho (1969) who crossed *M. sativa* with contracted chromosomes with *M. falcata* with longer chromosomes, and found that the hybrid's chromosome were almost as contracted as those of the *M. sativa* parent.

To determine if there was any variability between plants with the same parentage, critical differences between means were calculated by Duncan's multiple range method for H-2 and H-3 and for H-6 and H-7 (Table 13). It was found that H-6 and H-7 (both derived from tetraploid T-9) were not significantly different for chromosome lengths or arm ratios, but H-2 and H-3 (derived from tetraploid T-27) had significantly different means for lengths of chromosomes 1, 3, 4, 5, 6, 7 and 8, the satellite and the haploid complement, but were not different for arm ratios. From Table 12 it can be seen that all the dihaploids, with the exception of H-3, were remarkably uniform in chromosome length, despite their different tetraploid parents. An F test of chromosome lengths of H-1, H-2, H-6, H-7 and H-7 X D_{48} showed that only the satellite length was significantly different among these plants.

The hybrid H-7 X D_{48} appeared to have normal pachytene and metaphase I pairing, and the chromosome lengths were not significantly different from those of H-7. This is not surprising since the haploid complement of H-7 (230 microns) is not very different from that given above

for diploid *M. falcata* No. 136 (250 microns). Of course, the *M. falcata* used in the cross (D_{48}) could possibly be closer in complement length to the *M. falcatas* described by Gillies(1970a) and Ho (1969), but Ho (1969) has shown that differences in length were reconciled in hybrids.

The dihaploids described here are remarkably free of cytological abnormalities and have been shown also to be quite fertile in crosses with both diploid and tetraploid alfalfa (Bingham, personal communication), to yield fertile normal hybrids. The H-7 X D_{48} hybrid studied here is an example. In this they differ from the dihaploids of Lesins (1957) and Stanford and Clement (1958), which were found to have some abnormalities (30% metaphase I abnormalities: Lesins, 1959; translocation: Stanford and Clement, 1958). Bingham (personal communication) reveals that some trisomics and pentaploids are produced in crosses with diploids and tetraploids respectively, so in this respect these dihaploids are similar to that of Clement and Stanford (1961a). The slightly abnormal meiotic behaviour of H-2 gives an idea of how occasional aneuploid and polyploid gametes might be produced by the dihaploids.

From the similarity of the dihaploid complements to those of diploid *M. sativa* and *M. falcata*, and from the perfect pairing in the hybrid H-7 X D_{48}, it is evident that the tetraploid *M. sativas* from which the dihaploids originated, have chromosome complements which are essentially autotetraploid versions of those in diploid *M. sativa s.l.* This evidence, together with the numerous genetic studies which have demonstrated tetrasomic inheritance in tetraploid *M. sativa* (Barnes

and Hanson, 1967), and the reports of the similarities of chromosome complements of species in *M. sativa s.l.*, leads one to conclude that tetraploid *M. sativa* is indeed an autotetraploid in all facets of its behaviour, even though its origin may not have been strictly autotetraploid in the precise definition of the word.

IV. TRISOMICS OF DIPLOID *M. SATIVA S.L.*

1. Review of Literature

Individuals in which three copies of a particular chromosome occur instead of the normal two, and which therefore have the chromosome number of 2n + 1, are referred to as trisomics. They are found occasionally among the offspring of diploid organisms but usually result from the crossing of a triploid with a diploid. The study made by Blakeslee of trisomics in the Jimson weed (*Datura stramonium*) has been a model for all subsequent investigations. Blakeslee (1921) reported the occurrence of twelve different morphological classes of plants corresponding to the twelve haploid chromosomes, and Belling and Blakeslee (1924) described the trivalents formed by the trisomic chromosomes at metaphase I of meiosis. In 1927 Blakeslee reported on 'Nubbin', a complex tertiary trisomic.

McClintock (1929) found trisomics were produced in 3n x 2n crosses in maize. The different trisomics were not identifiable morphologically, but all showed a decrease in size and vigour. McClintock and Hill (1931) subsequently used trisomics to identify the chromosome on which the R-G linkage group was located. Trisomics have been a valuable tool in gene mapping in the tomato ever since Lesley (1932) identified eleven of the twelve trisomics. Rick and Barton (1954) extended this work to the identification of all 12 trisomics, although Rick, Dempsey and Khush (1964) subsequently found that one of these was a tertiary trisomic. Rick and Barton (1954) found that the degree of trivalent formation in a trisomic was correlated with the pachytene length of the chromosome involved. Rick, Dempsey and Khush (1964)

listed 30 genes which had been assigned to eleven of the tomato chromosomes. The twelfth chromosome had been shown not to carry any of 60 known genes. They explained this as a consequence of the highly chromatic nature of this chromosome (chromosome 12). Subsequently Khush and Rick (1969, 1966, 1968) have used secondary, tertiary and telo-trisomics in cytogenetic analysis of the tomato.

The trisomics of many other plants have been produced since Blakeslee's work and they can be divided into those morphologically identifiable and those which are not. For example, Sen (1965) produced the twelve primary trisomics of rice but could identify only five of them on the basis of morphology, while Chen and Grant (1968) found they could morphologically identify all of the primary trisomics of *Lotus pedunculatis*.

There has been interest in the production and use of trisomics among workers in *Medicago* for considerable time but the tetraploid nature of commercial alfalfa (*M. sativa*) has proven something of an obstacle until recently. The discovery of diploid *M. sativa* and the demonstration of the probable autotetraploidy of alfalfa have renewed interest in this field, now that it has become evident that diploid lines can be used to gain an insight into gene-chromosome relationships in tetraploid *M. sativa*.

The first reported trisomics in *M. sativa s.l.* were the result of crosses by Ledingham (1940) of triploid *M. sativa* - *M. falcata* hybrids with diploid *M. falcata*. He did not report any attempts to identify or use these trisomics, however. Little was done with trisomics in alfalfa until Stanford (1959b) used trisomic tetraploids (2n = 4x-1

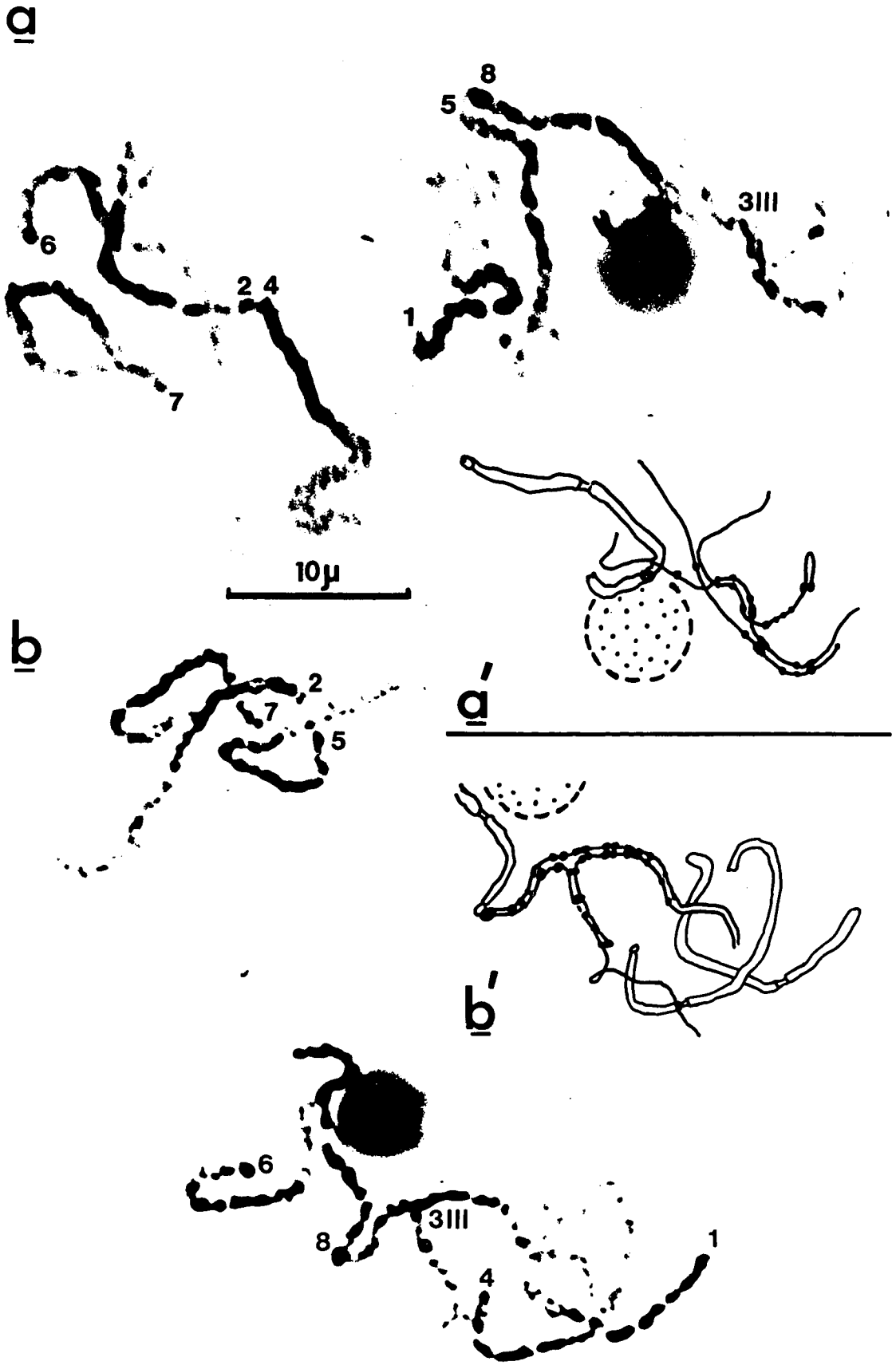
= 31) to try to locate three genetic characters on particular chromosomes. One character, sticky leaf, was found to be associated with a particular trisomic tetraploid, but inability to identify chromosomes other than the satellited one resulted in incomplete specification of the trisomic. Bingham (1968b) found that 31 and 33 chromosome aneuploids occurred at low frequency in tetraploid lines of *M. sativa* but concluded that they would have little effect on segregation ratios.

Clement and Stanford (1963), in their pachytene study of diploid *M. sativa*, speculated that pachytene analysis might be a useful tool for identifying trisomics. They suggested that if identification of trisomic tetraploids was too difficult, trisomic diploids might be identified by this means and then used in crosses with tetraploids to locate genes on the tetraploid chromosomes. In 1967 Kasha and McLennan reported their production of a number of trisomic lines by crossing triploid hybrids (originating from 4n x 2n crosses) with diploid *M. sativa* and *M. falcata*. Fertility and trisomic transmission were quite variable in the different trisomics, but none could be identified morphologically. In crosses with diploids up to 25% transmission of the extra chromosome occurred if the trisomic was the female parent, but pollen transmission of the extra chromosome was, with two exceptions, below 10%. Only one line, a trisomic for the satellited chromosome, was identified cytologically.

Buss and Cleveland (1968b) made some suggestions for identifying trisomics by means of pachytene analysis. They felt that cells with most or all of the chromosomes analysable would be needed, since relative length played an important part in the identification of chromosomes

Figure 20

a. and b. Pachytene cells of TR 6-3. Chromosomes are numbered according to the *M. sativa s.l.* idiogram. a' and b'. Interpretative drawings of trivalents involving chromosome 3 in a. and b. X2900.


a
8
5
3III
6
2 4
1
7
10μ
a′
b
2
7
5
b′
6
3III
8
1
4

at pachytene. They also concluded that identification of trisomic chromosomes should be best based on analysis of trivalent figures since univalents would be difficult to distinguish because of the light staining of the achromatic long arms.

2. Identification of trisomic chromosomes

a. Trisomic TR 6-3

Two pachytene cells of this trisomic are illustrated in Fig.20. The trivalent occurred quite frequently in pachytene cells and ten analysable cells were found. Tentative identification was made of the trisomic chromosome as chromosome 3*. The following is the reasoning for this decision:

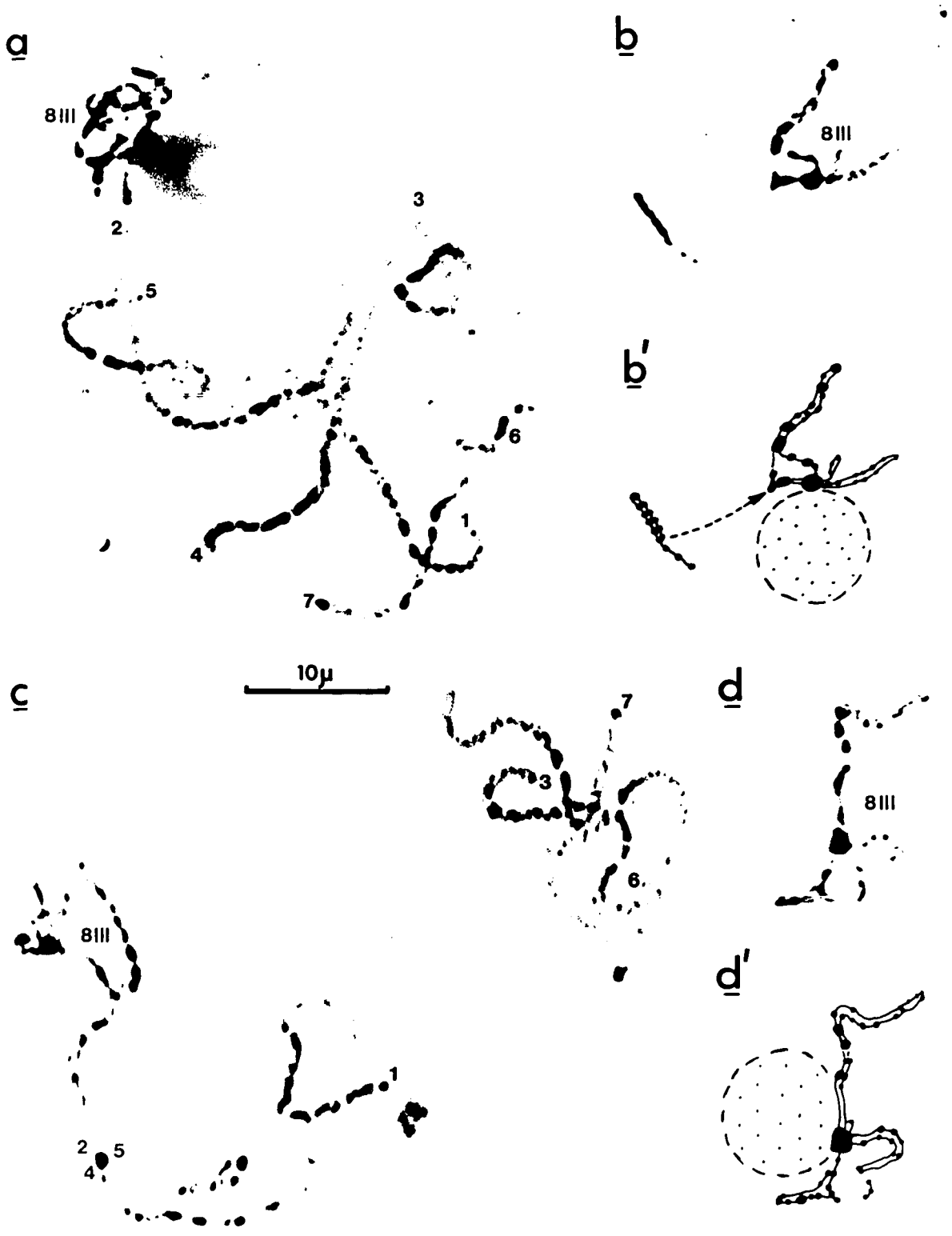
1. all four submetacentric, asymmetrical chromosomes are present (chromosomes 1, 2, 4 and 6).
2. the trivalent has a fairly median centromere and symmetrical chromatin distribution.
3. no metacentric bivalent as long as chromosome 1 or 2 appears to be present.
4. two short metacentrics (chromosomes 5 and 7) are present.
5. the nucleolar chromosome (chromosome 8) is not involved.

Pairing in the trivalent was often almost complete with some non homologous and fold-back pairing obviously occurring. Chromosome lengths ranged from 14 to 32 microns with the majority of cells having

* All chromosome numbers referred to are those of the *M. sativa s.l.* common idiogram developed in section III 2 h.

Figure 21

a. Pachytene cell of TR 19-3 X Du Puit. Chromosomes are numbered according to the *M. sativa s.l.* idiogram. b. Trivalent involving chromosome 8 in TR 19-3 X Du Puit. b' Interpretative drawing of b. Portion of the trivalent is separated from the main part (broken line and arrow). c. Pachytene cell of TR33^{L}1. Chromosomes are numbered according to the *M. sativa s.l.* idiogram. d. Trivalent involving chromosome 8 in TR33^{L}1. d' Interpretative drawing of d. All X 2250.


a
8III
2
3
5
6
1
4
7
b
8III
b'
10μ
c
7
3
6
8III
1
2
5
4
d
8III
d'

chromosomes in the range 15 to 21 microns.

b. Trisomic TR 19-3 x Du Puit

This trisomic was received from Kasha with the information that it was trisomic for the satellited chromosome. Pachytene studies confirmed that it was trisomic 8 (Fig. 21a, b). Pairing of the three chromosome 8's to form a trivalent was usual and many diakinesis cells with a trivalent attached to the nucleolus were seen. All three chromosomes appear to be associated with the nucleolus at their nucleolus organizers, while pairing in the rest of the trivalent involves only two of the three chromosomes at any one point.

c. Trisomic TR 33 $^{L}1$

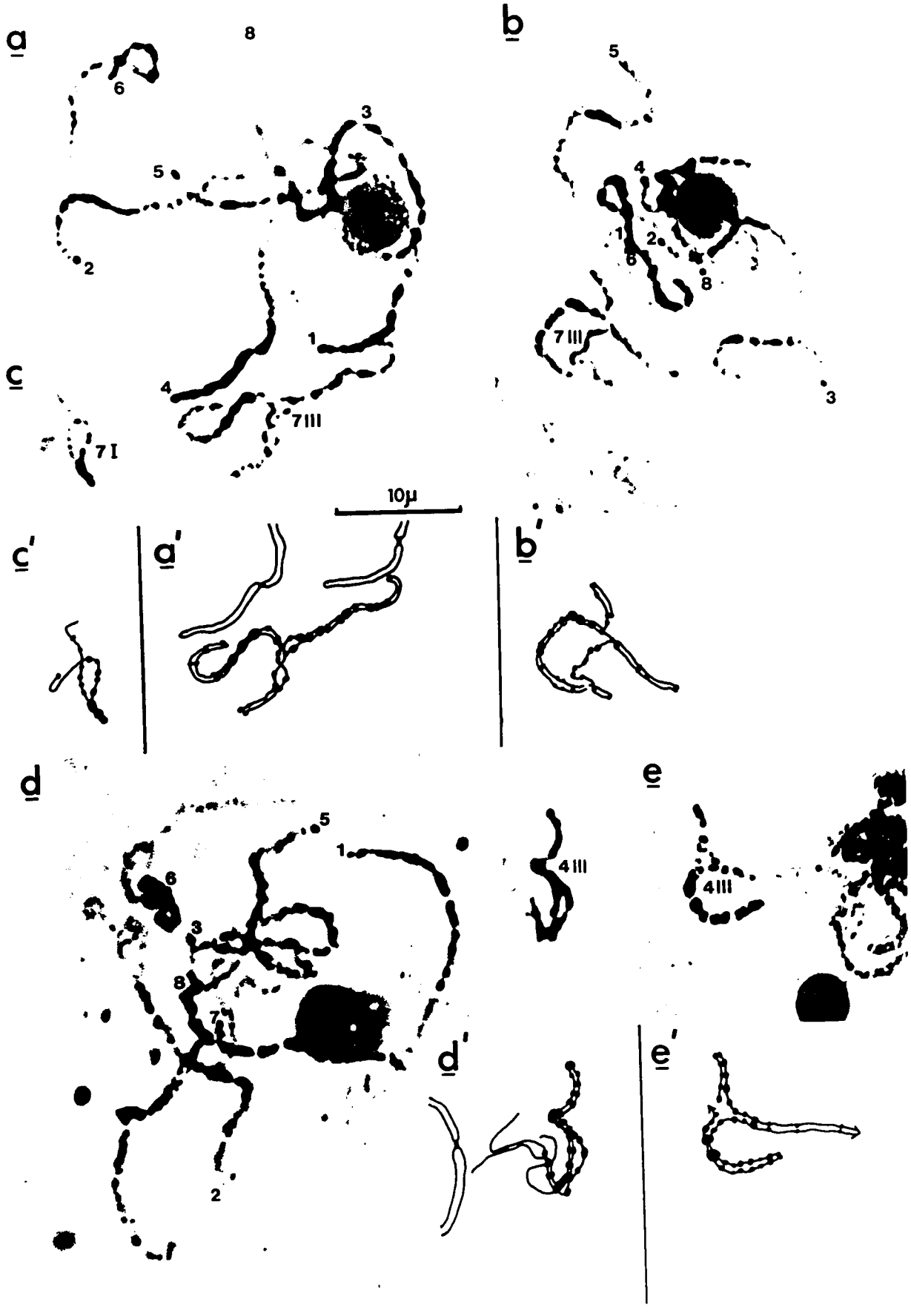
Kasha (1968) suggested that this trisomic might be linked with purple flower colour. On examination of pachytene cells it was found to be trisomic for chromosome 8, the nucleolar chromosome (Fig. 21c, d). The trivalents formed were mostly well paired (Fig. 21c, d) in spite of the apparent non homologous pairing involved. Again the three chromosomes seemed to be associated at the N.O., often forming a large heavily stained knob (Fig. 21d).

d. Trisomic TR 34 $^{S}3$

This plant was described by Kasha as semi-dwarf with short internodes and dark green leaves. Twelve cells were analysed and it was tentatively identified as trisomic 7 (although it could possibly be trisomic 5). Two cells with trivalents and a univalent are shown in Fig. 22a - c. It appears that the trisomic chromosome is a metacentric as all the submetacentrics are present as bivalents. The trivalent is

Figure 22

a. and b. Pachytene cells of TR34^{S}3. c. Univalent of chromosome 7 from TR 34^{S}3. a', b' and c'. Interpretative drawings of trivalents (a' and b') and univalent (c') involving chromosome 7 in a, b and c. d. Pachytene cell of TR37^{L}12. e. Trivalent involving chromosome 4 in TR37^{L}12. d' and e'. Interpretative drawings of trivalents in d and e. All numbering is according to the *M. sativa s.l.* idiogram. All X2150.


a
b
c
a'
b'
c'
d
d'
e
e'
10μ
1
2
3
4
5
6
7 I
7 III
8
4 III

one of the shorter metacentrics, either 5 or 7, but it is probably 7 since the trisomic chromosome is most often the shortest in the complement. Because of the difficulty of making precise length measurements in trivalents this is not conclusive, however.

The univalent in Fig. 22c is partially paired with itself at the chromatic regions adjacent to the centromere. This non homologous pairing may be a result of the so called "stickiness" of the chromatic parts such as terminal knobs and the centromere flanking areas.

e. Trisomic TR 37 $^{L}12$

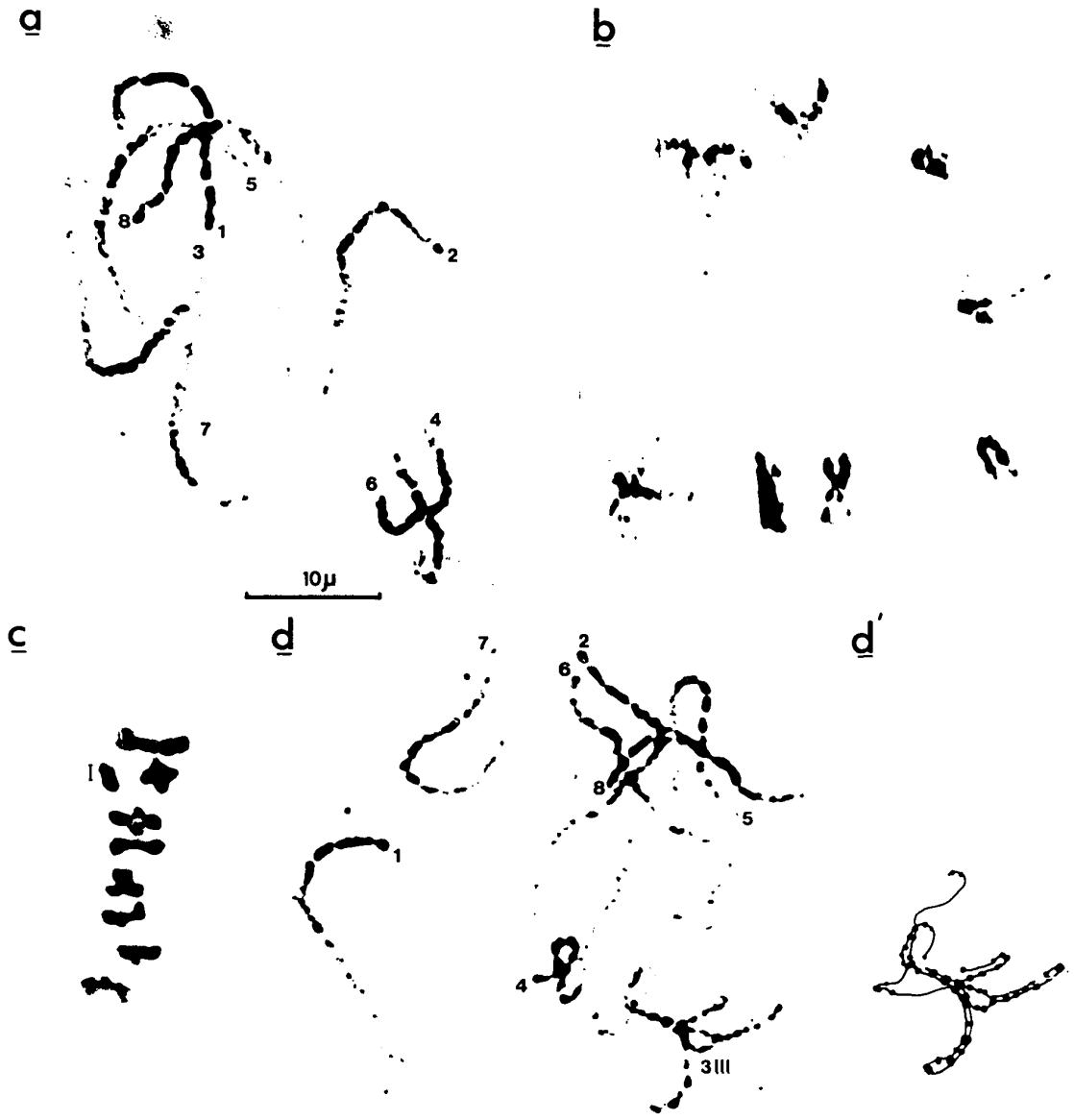
This trisomic has been shown to be linked to the male sterile character by Kasha (1968). Some difficulty was encountered in obtaining good pachytene spreads of this trisomic and six analysable cells only were produced. Several of those are shown in Fig. 22d and e. From the cells analysed it was found that all the metacentrics (chromosomes 3, 5 and 7) were present as bivalents. Chromosomes 1 and 2 were both present as bivalents and the nucleolus chromosomes were not involved in trivalents. Hence the trivalent was either chromosome 4 or 6. The trivalents shown in Fig.22d,e,f have one short arm heavily chromatic and most resemble chromosome 4. Unfortunately in several of the cells studied many of the bivalents had distal portions of their arms unpaired, making accurate measurements and identification difficult. The identity of this trisomic is hence tentatively made as trisomic 4.

f. Trisomic TR 37 $^{L}15$

This had been noted by Kasha as one of the more vigorous trisomics. The two cuttings received showed some differences in vigour,

Figure 23

Chromosomes of TR37^{L}15. a. Pachytene cell of disomic with eight bivalents. b. Diakinesis cell of disomic with eight bivalents. c. Metaphase I cell of trisomic with eight bivalents and a univalent (I). d. Pachytene cell of trisomic. d'. Interpretative drawing of trivalent involving chromosome 3 in d. All numbering according to the *M. sativa s.l.* idiogram. All X1950.


a
b
5
8
1
3
2
7
4
6
10μ
c
d
d'
I
7
2
6
8
5
1
4
3III

one being quite prolific in growth and flowering. Pachytene analysis revealed that this plant was in fact a normal diploid (Fig. 23a), and this was subsequently confirmed by study of metaphase I and diakinesis stages (Fig. 23b). The length of chromosomes ranged from 39.5 microns to 19.5 microns with the average complement length of three cells being 214 microns.

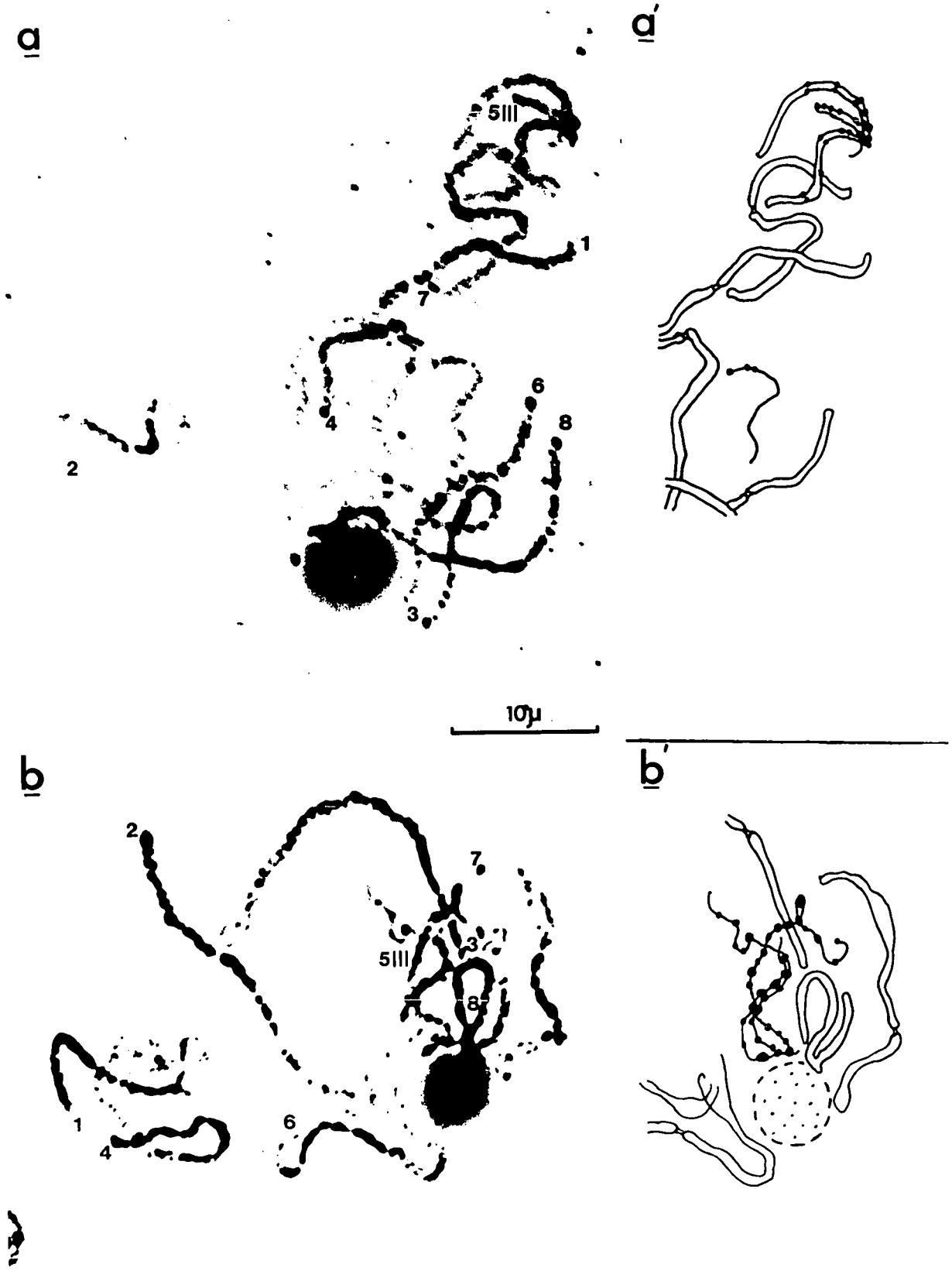
The second cutting however, was definitely a trisomic as indicated by eight bivalents plus a univalent at metaphase I (Fig. 23c). Few pachytene cells were found with easily analysed chromosomes (Fig. 23d). All three asymmetrical submetacentrics (1, 4 and 6) were present as bivalents, and neither the two short metacentrics nor the N.O. chromosome was trisomic. The trisomic chromosome was therefore either 2 or 3. The trivalent in Fig. 23d is fairly chromatic and could be chromosome 2. A univalent in another cell was folded back on itself and hence difficult to analyse, but it appeared to have a reasonably long achromatic portion separating the chromatic region from the terminal knob. In several other cells the trivalent appeared to involve chromosome 3, as indicated by its median centromere and by the presence of a symmetrical submetacentric bivalent (chromosome 2). This trisomic was therefore tentatively identified as trisomic 3.

g. Trisomic TR 37 $^{L}17$

Deviations in the segregation of the male sterile character in this plant, suggested to Kasha (1968) that this trisomic might be linked to the character, but conflicts in reciprocal crosses led him to conclude that it was not. As with the other TR 37 trisomics, difficulty was encountered in obtaining good preparations of this line.

Figure 24

a. and b. Pachytene cells of TR37^{L}17. a' and b'. Interpretative drawings of trivalents involving chromosome 5 in a and b. Numbering according to the *M. sativa s.l.* idiogram. X2400.


a
a'
5III
1
7
6
8
4
2
3
10µ
b
b'
2
7
5III
3
1
8
1
4
6

Two cells are illustrated in Fig. 24. All the submetacentrics, the longest metacentric and the nucleolar chromosome were present as bivalents. The trivalent appeared to be a metacentric (Fig. 24b). The other metacentric present as a bivalent was usually as short as or shorter than the shortest submetacentric, and hence was probably chromosome 7. The plant is therefore possibly trisomic 5.

h. Trisomic 39 $^{s}3$

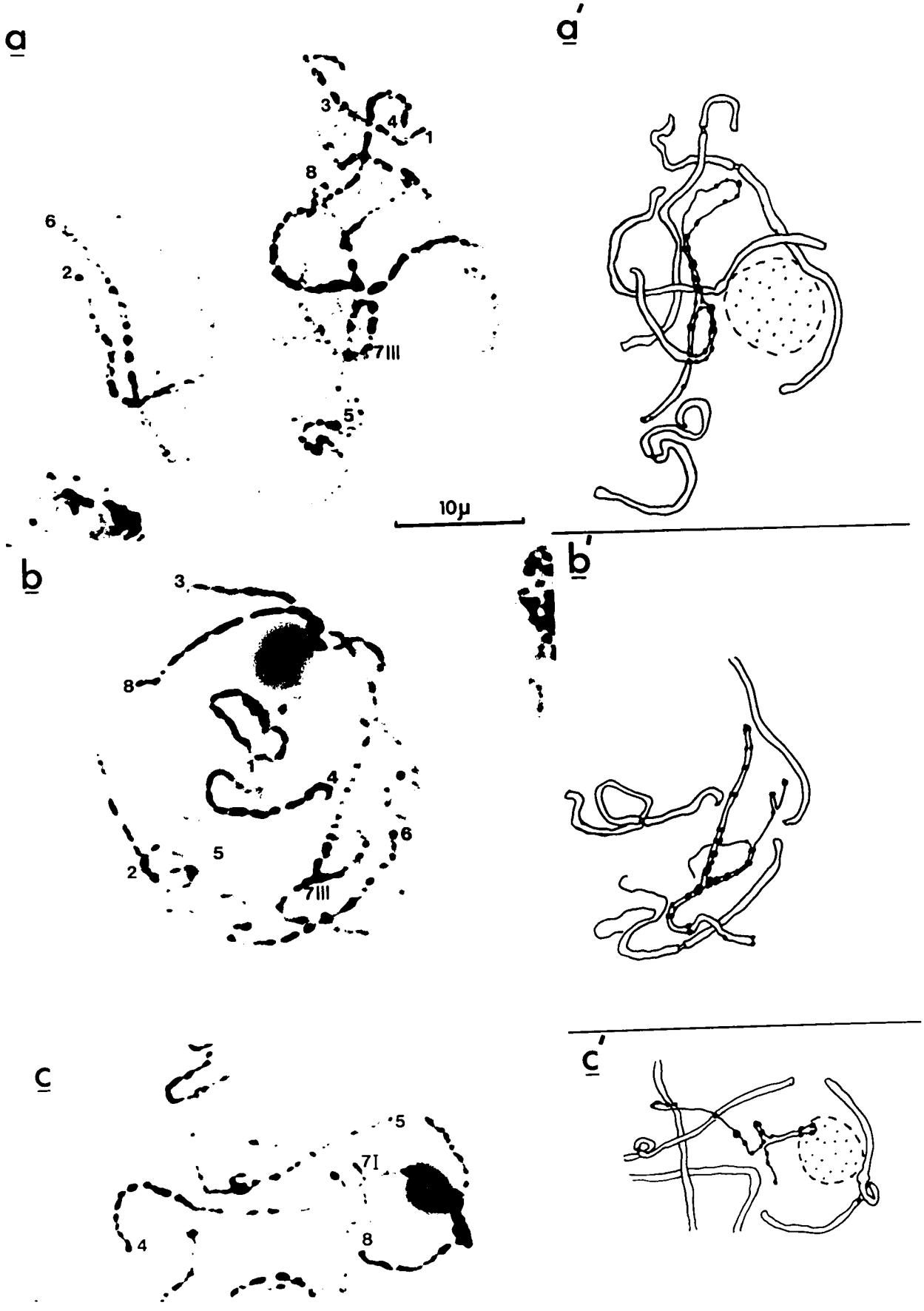
This trisomic was similar to TR 34 $^{s}3$ in having dark green leaves, but it had longer internodes. The trivalents found at pachytene had median centromeres and generally showed good pairing (Fig. 25a, b). The univalent in Fig. 25c has several regions of fold back pairing. The univalents and trivalents were tentatively identified as involving either chromosome 5 or 7. The trivalent was shorter than the shortest metacentric and hence was tentatively described as trisomic 7, but the same caution is advised in accepting this as for TR 34 $^{s}3$.

i. Double Trisomic TR 39 $^{L}2$

The two cuttings of this plant were quite vigorous and more fertile than the other trisomics. Pachytene analysis proved relatively straight forward and cells were found with two trivalents, indicating that two different chromosomes were present in the trisomic condition (Figs. 26a, b). One of the trisomic chromosomes were chromosome 8, the N.O. carrying chromosome. The second trivalent was metacentric, and in length lay between the largest metacentric (chromosome 3) and the shortest metacentric (chromosome 7). It was therefore tentatively identified as trisomic for chromosome 5. This plant is therefore a

Figure 25

a. and b. Pachytene cells of TR $39^{S}3$. c. Univalent of chromosome 7 in $TR39^{S}3$. a', b' and c'. Interpretative drawings of trivalents (a' and b') and univalent (c') involving chromosome 7 in $TR39^{S}3$. Numbering according to the *M. sativa s.l.* idiogram. All X 2200.


a
a'
3
4
1
8
6
2
7III
5
10μ
b
b'
3
8
1
4
6
5
2
7III
c
c'
5
7I
4
8

double trisomic, trisomic for both 5 and 8.

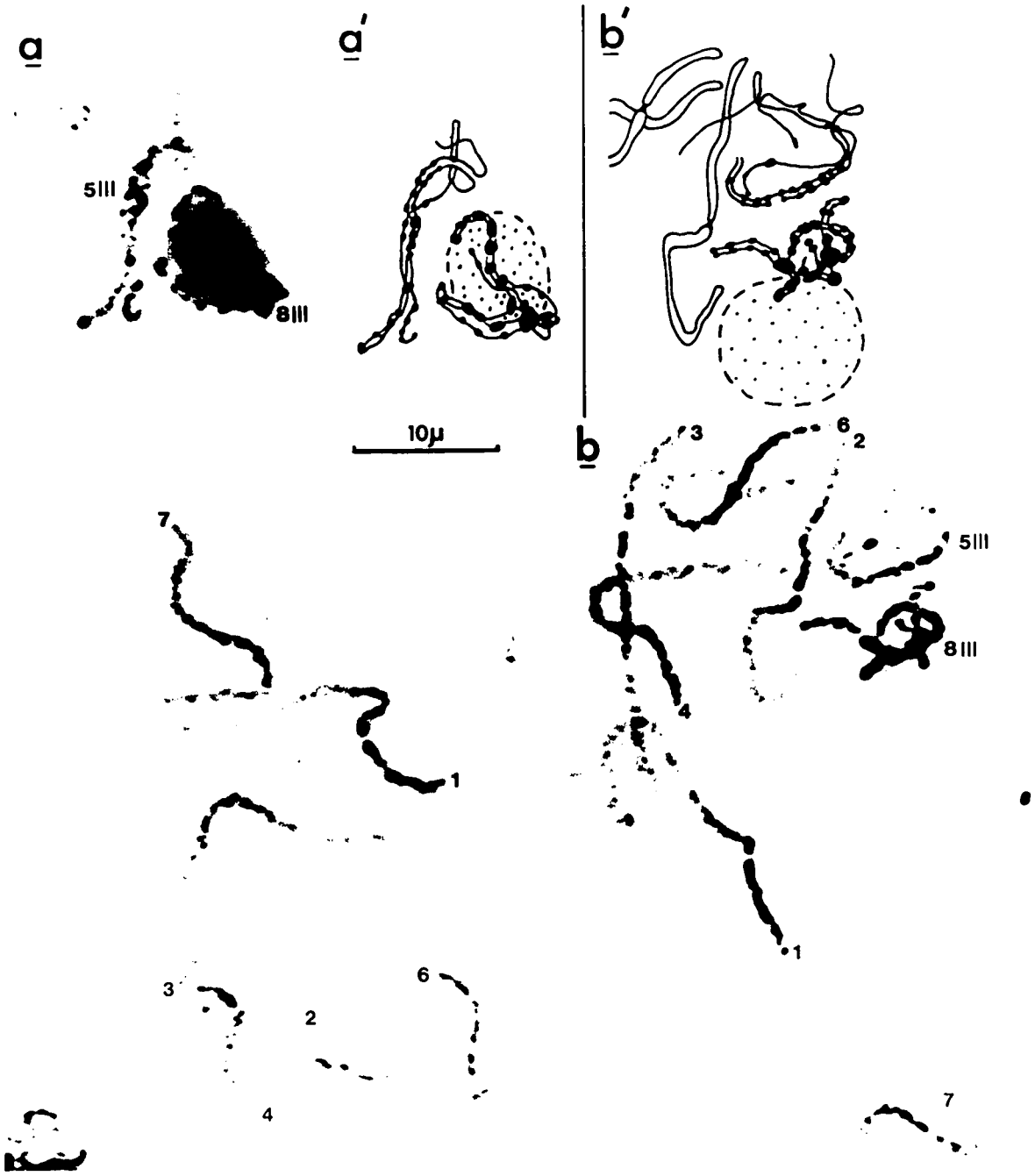
3. Discussion

An interesting point is that only one (possibly two) of the trisomics studied was trisomic for a submetacentric asymmetrical chromosome. If this sample of trisomics is representative of the whole collection of trisomics obtained by Kasha, then the frequency of submetacentric trisomics is much below that expected if all trisomics occur with the same frequency. A possible explanation for this is offered by Clement and Stanford's (1961b) finding that there is a reduction in the chiasma frequency in the shorter chromatic arms of chromosomes of *M. sativa*. This could lead to a lower frequency of trivalents of the chromosomes with largely chromatic short arms (chromosomes 1, 4, 6 and possibly 2) in the triploid plants from which the trisomics were derived, and hence to fewer gametes with extra chromosomes of this type being formed.

The pairing of chromosomes in trivalents and univalents of many of the trisomics frequently involved parts of different chromosomes or of the same chromosome which by all indications should not be homologous. While the presence of duplicated regions of chromosomes cannot be ruled out, the pairing sometimes involved regions which appeared cytologically different. Such non-homologous pairing has been demonstrated by McClintock (1933) in maize pachytene chromosomes heterozygous for various structural rearrangements, and Gottschalk and Peters (1956) found non-homologous pairing in the chromatic portions of chromosomes of a hybrid between *Solanum* species with different ploidy levels. The involvement of the "stickiness" of the chromatic regions of chromosomes in causing this pairing is suggested in the present case, as much of the pairing in univalents involved chromatic regions. Some role of

Figure 26

a. and b. Pachytene cells of double trisomic TR39^{L}2. a' and b'. Interpretative drawings of the two trivalents involving chromosomes 5 and 8 in a. and b. Numbering according to the *M. sativa s.l.* idiogram. X2100.


a
a'
b'
b
5III
8III
10μ
7
1
3
2
6
4
5III
8III
6
2
3
4
1
7

the nucleolus organizer in the pairing of the nucleolar chromosomes is indicated by the fact that all three chromosomes of the chromosome 8 trivalent were always associated with one nucleolus at this point. No univalents of chromosome 8 were seen.

This success in tentatively identifying the trisomic chromosomes by cytological means indicates that the studies which have been undertaken to produce pachytene idiograms of the *M. sativa* complex (Buss and Cleveland, 1968b; Gillies, 1968; Ho, 1969) have been put to practical use. In identifying trisomics, the criteria and techniques found most useful were similar to those suggested by Buss and Cleveland (1968b). In most cases the identification was not definite, as only a small number of cells were studied. A more intensive study of each trisomic, and analysis of more pachytene cells, should result in definite identification of the trisomic chromosomes, particularly if chromosome lengths and arm ratios could be subjected to statistical analysis. Insufficient cells were studied here for any valid statistical tests to be carried out.

The final step in the positive identification of the trisomics will involve the association of linkage groups with particular trisomic chromosomes. This will also involve the comparison of different trisomic plants so that cytologically determined trisomic classes can be verified as being the same in all cases involving the same linkage group. Dr. Kasha is currently involved in linkage studies and it is hoped that the association of linkage groups and trisomic classes will soon be accomplished. The remaining task will then be one of testing many of the characters listed by Barnes and Hanson (1967), for assignment to linkage groups and chromosomes. The genetic knowledge

of alfalfa available to plant breeders will then be closer to the state which has existed for some years in other crops such as maize and tomatoes.

V. KARYOTYPE STUDIES OF PERENNIAL SPECIES OTHER THAN *M. SATIVA S.L.*

1. Review of literature

In the genus *Medicago* the importance of alfalfa (*M. sativa L.*) has led to a neglect of other perennial species, even though these may be valuable sources of breeding material in alfalfa improvement. This neglect has been the result partly of a scarcity of specimens of some of the rarer species; however, the collections made by Lesins have made possible some of the first cytological investigations of such species. Lesins and Lesins (1961) describe, among other species, *M. daghestanica* Rupr. and *M. pironae* Vis., giving the chromosome number of both as 2n = 16. The former is the only species outside the *M. sativa* group which has purple (anthocyanin containing) flowers.

Lesins and Gillies (1968) noted that Urban (1873) had placed *M. daghestanica* and *M. pironae* in different sections of the genus otherwise containing only annual species, raising doubts about the validity of Urban's classification. As the two species were morphologically similar, Lesins and Gillies (1968) attempted to cross them and found hybrids were easily obtained. Attempts to cross both species with *M. sativa s.l.* were unsuccessful. The *M. daghestanica* X *M. pironae* hybrids were sterile and had meiotic irregularities such as univalents at metaphase I and bridges at anaphases I and II. Although limited pachytene studies indicated some structural differences between the chromosomes of the two species, the authors concluded that the two species belonged to one group but had diverged under spatial isolation. The findings of Gupta and Lesins (1969) that *M. daghestanica* flowers

contained a number of anthocyanins not present in *M. sativa s.l.* supported Lesins and Gillies' suggestion that the relationship between the *M. daghestanica - pironae* group and the *M. sativa s.l.* group was remote.

The chromosome number of the species *M. rhodopaea* Velen. is given by Nicoloff (1961) as 2n = 16. This species is endemic to Bulgaria. Nicoloff mentions that it is a member of the same section of the genus as *M. rupestris, M. prostrata* and *M. cancellata,* and the similarity of *M. rhodopaea* to the latter species led Nicoloff to suggest that they had a common origin. Lesins (1961b) too noted this similarity and suggested that four of the genomes of hexaploid *M. cancellata* might be derived from *M. rhodopaea,* while two genomes could have come from the *M. sativa s.l.* group. In reporting the occurrence of hexaploid *M. saxatilis M.B.,* Lesins and Lesins (1963b) noted that three perennial species had similar spiny pods - *M. pironae, M. daghestanica* and *M. rhodopaea,* and that the latter was morphologically most similar to *M. saxatilis.* Pollen from an artificial tetraploid *M. rhodopaea* produced 20% pod set in crosses with *M. saxatilis,* indicating that the two taxa were related.

In 1966 Lesins and Lesins reported the chromosome number of an accession of *M. rupestris M.B.* from Crimea as 2n = 16. This plant was small, and in pod and other characters resembled *M. rhodopaea,* supporting the idea that the two taxa were closely related. As a result of a series of interspecific crosses involving *M. saxatilis, M. sativa, M. rhodopaea* and *M. cancellata,* Lesins (1970) concluded that *M. saxatilis* was probably of genomic constitution S^1S^1XXXX, where the S^1 genome was closely related to *M. sativa s.l.,* and the X genome possibly was from *M. rhodopaea.* He suggested also that *M. cancellata* had a genomic con-

stitution of SSCCCC, where the C genome possibly was from *M. rupestris* and the S genome from *M. sativa s.l.*

Lesins and Lesins (1966) described a collection of *M. hybrida* Trautv. from the French Pyrenees. They mentioned that its chromosome number was 16 and that the chromosomes were larger than those of any other *Medicago* species investigated. *M. hybrida* could not be crossed with members of the *M. sativa s.l.* group, and these authors noted that the position of the species in the genus was in some doubt. Lesins (1969) discussed this problem and also presented evidence that *M. suffruticosa* is related to *M. hybrida*, which has similar vegetative characters. The hybrids between the two species were highly fertile and segregated normally for two characters. *M. suffruticosa* could not be crossed with diploid *M. sativa s.l.* Lesins (1969) noted that while the genus of *M. suffruticosa* has never been questioned, because of its coiled pods, *M. hybrida* and *M.suffruticosa* should obviously be in the same genus. However, he did not resolve the problem of whether the two taxa should belong in the genus *Medicago* or the genus *Trigonella*.

Baum (1968), in an extensive study of the characters used to delimit the genera *Medicago* and *Trigonella*, found that Urban's (1873) classification using cotyledons was the only one supported by other floral character differences. On this basis he found that *Medicago hybrida* was correctly placed in the genus *Medicago*.

2. *M. daghestanica* and *M. pironae*

a. Karyotype of *M. daghestanica* Rupr.

A number of plants of accession No. 67 were used to supply material for pachytene analysis. Only two complete cells were produced, although a number of cells were found in which only the nucleolar

Figure 27

a. Pachytene cell *M. daghestanica.* b. Nucleolar chromosome of *M. daghestanica.* a' and b'. Interpretative drawings of a and b. Chromosomes are numbered at their short arm telomeres, and centromeres are indicated by dotted gaps. Only portion of the nucleolar chromosome is present in a (6F). X2200.

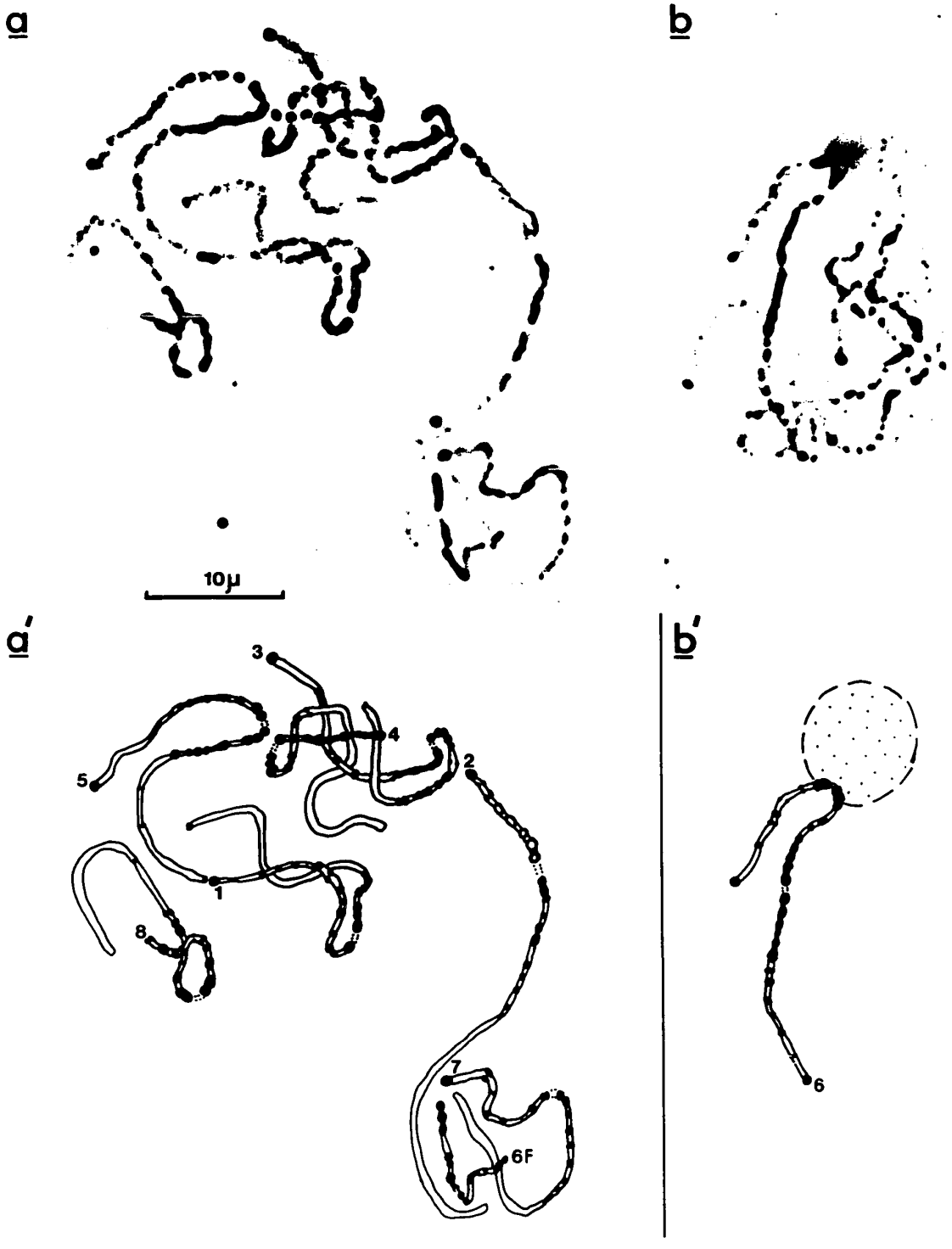

a
b
10μ
a'
b'
3
4
2
5
1
8
7
6F
6

TABLE 14. MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF CHROMOSOMES OF *H. DAGHESTANICA*, *H. PIRONAE* AND THEIR HYBRID

Chromosome No.	Item[+]	*H. daghestanica* (a) Values	Chrom. No. in Idiogram	*H. pironae* (b) Values	Chrom. No. in Idiogram	*H. daghestanica* X *H. pironae* (c) Values	Chrom. No. in Idiogram	Significant Difference[‡] (Length and Arm Ratio) a-b	a-c	b-c
1	L	45.6 ± 2.03	1	33.7 ± 2.55	1	47.8 ± 4.39	1	*	n.s.	*
	AR	1.49 ± 0.061		1.42 ± 0.142		1.97 ± 0.248		n.s.	*	*
	PL	0.160		0.152		0.168		-	-	-
	N	22		10		7				
2	L	43.5 ± 1.89	2	31.9 ± 2.56	2	41.3 ± 2.45	2	*	n.s.	n.s.
	AR	2.70 ± 0.105		2.31 ± 0.299		3.26 ± 0.560		n.s.	n.s.	n.s.
	PL	0.153		0.144		0.145		-	-	-
	N	20		7		11				
3	L	41.5 ± 1.54	3	29.8 ± 3.01	3	38.2 ± 2.25	3	*	n.s.	*
	AR	1.17 ± 0.053		1.42 ± 0.156		1.45 ± 0.106		n.s.	*	n.s.
	PL	0.146		0.135		0.134		-	-	-
	N	21		7		13				
4	L	34.3 ± 3.43	4	27.3 ± 1.12	5	35.6 ± 1.61	4	*	n.s.	*
	AR	2.30 ± 0.190		1.96 ± 0.100		2.29 ± 0.190		n.s.	n.s.	n.s.
	PL	0.120		0.124		0.125		-	-	-
	N	10		11		13				
5	L	34.3 ± 2.15	5	28.3 ± 2.39	4	34.0 ± 1.70	5	n.s.	n.s.	n.s.
	AR	1.14 ± 0.054		1.39 ± 0.126		1.37 ± 0.092		n.s.	*	n.s.
	PL	0.120		0.128		0.119		-	-	-
	N	14		7		13				
6	L	28.4 ± 1.97	7	24.3 ± 2.38	6	30.0 ± 1.31	6	n.s.	n.s.	n.s.
	AR	1.29 ± 0.113		1.27 ± 0.087		1.28 ± 0.089		n.s.	n.s.	n.s.
	PL	0.100		0.110		0.105		-	-	-
	N	9		6		13				
7	L	28.0 ± 2.23	8	24.0 ± 2.16	7	28.9 ± 1.73	8	n.s.	n.s.	n.s.
	AR	1.83 ± 0.278		1.88 ± 0.186		2.19 ± 0.159		n.s.	n.s.	n.s.
	PL	0.098		0.109		0.101		-	-	-
	N	7		9		12				
8	L	29.6 ± 1.63	6	21.7 ± 1.13	8	29.3 ± 3.23	7	*	n.s.	*
	AR	1.14 ± 0.036		1.30 ± 0.114		1.16 ± 0.132		n.s.	n.s.	n.s.
	PL	0.104		0.098		0.103		-	-	-
	N	10		7		4				
Satellite	L	9.3 ± 0.35	6	6.4 ± 0.32	8	7.2 ± 0.91	7	*	*	n.s.
	PL	0.033		0.029		0.025		-	-	-
	N	10		7		4				
Sum of Mean Lengths		285.2	-	221.0	-	285.1	-	-	-	-
Haploid Complement	L	343.3 ± 11.75	-	244.0 ± 17.80	-	316.4 ± 33.06	-	n.s.	n.s.	n.s.
	N	2		4		4				

+ L - length ± S_E (in μ); AR - arm ratio ± S_E; PL - proportional length; N - number of observations.

‡ F test and Duncan's Multiple Range Test; * - significant difference at 5% level; n.s. - not significantly different.

chromosome could not be analysed. The chromosomes are illustrated in Fig. 27. The chromosomes ranged in length from 45.6 microns to 29.6 microns, and the mean length of the haploid complement in the two complete cells was 343.25 microns, considerably more than the sum of the eight mean lengths (285.2 microns). The nucleolus carrying chromosome was sixth longest in the complement.

From the data in Table 14 an idiogram was compiled for the species, showing also the position of chromatic areas and chromomeres. This is shown in Fig. 31a. The chromosomes conform to the same general pattern of chromatic area distribution noted for *M. sativa s.l.* The submetacentric chromosomes (2, 4 and 8) have largely chromatic short arms, while the metacentrics, with the exception of the nucleolar chromosome, have symmetrical chromatin distribution on proximal parts of both arms and achromatic distal regions. The shorter arm of chromosome 1 is more chromatic than the longer arm but it is still achromatic in its distal third.

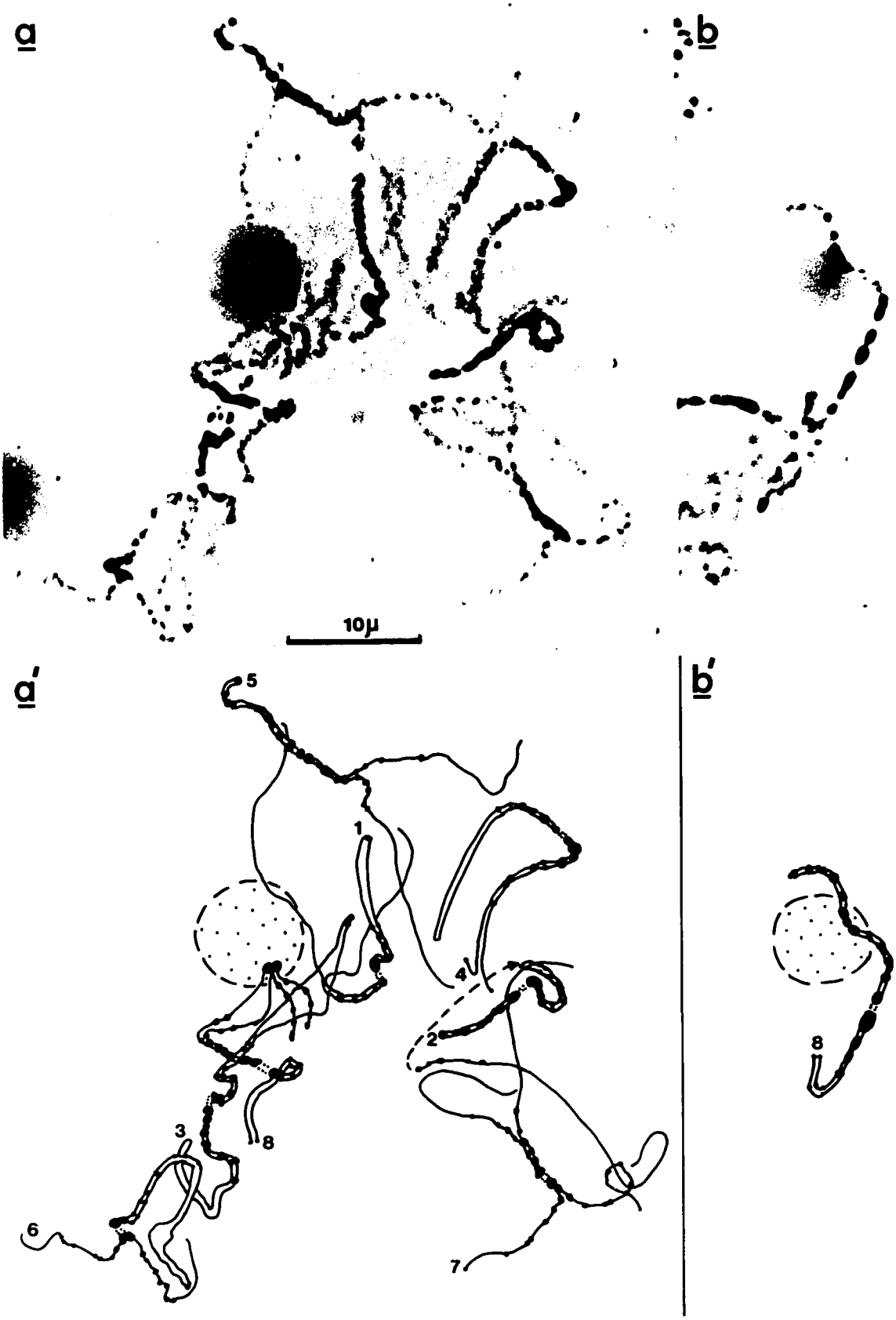
b. Karyotype of *M. pironae* Vis.

Only one accession of this species was available for study and considerable difficulty was encountered in obtaining good pachytene preparations. Many of the cells were clumped, and problems in squashing made it difficult to obtain photographs completely in focus. For this reason the number of cells analysed was only 19, and of these only four had all eight chromosomes measurable.

In some of the cells the chromosomes were unpaired in the achromatic parts of their arms. This could be a result of the inclusion of late zygotene stages with incomplete synapsis among the pachytene cells analysed. However, the fact that some chromosomes in a particular cell

Figure 28

a. Pachytene cell of *M. pironae*. b. Nucleolar chromosome of *M. pironae*. a' and b' Interpretative drawings of a and b. Chromosomes are numbered at their short arm telomeres, and centromeres are indicated by dotted gaps. The non chromatic portions of chromosome arms in a are unpaired and chromosome 2 has a break in its long arm (broken line and arrow). X2200.


a
b
10μ
a'
b'
1
2
3
4
5
6
7
8
8

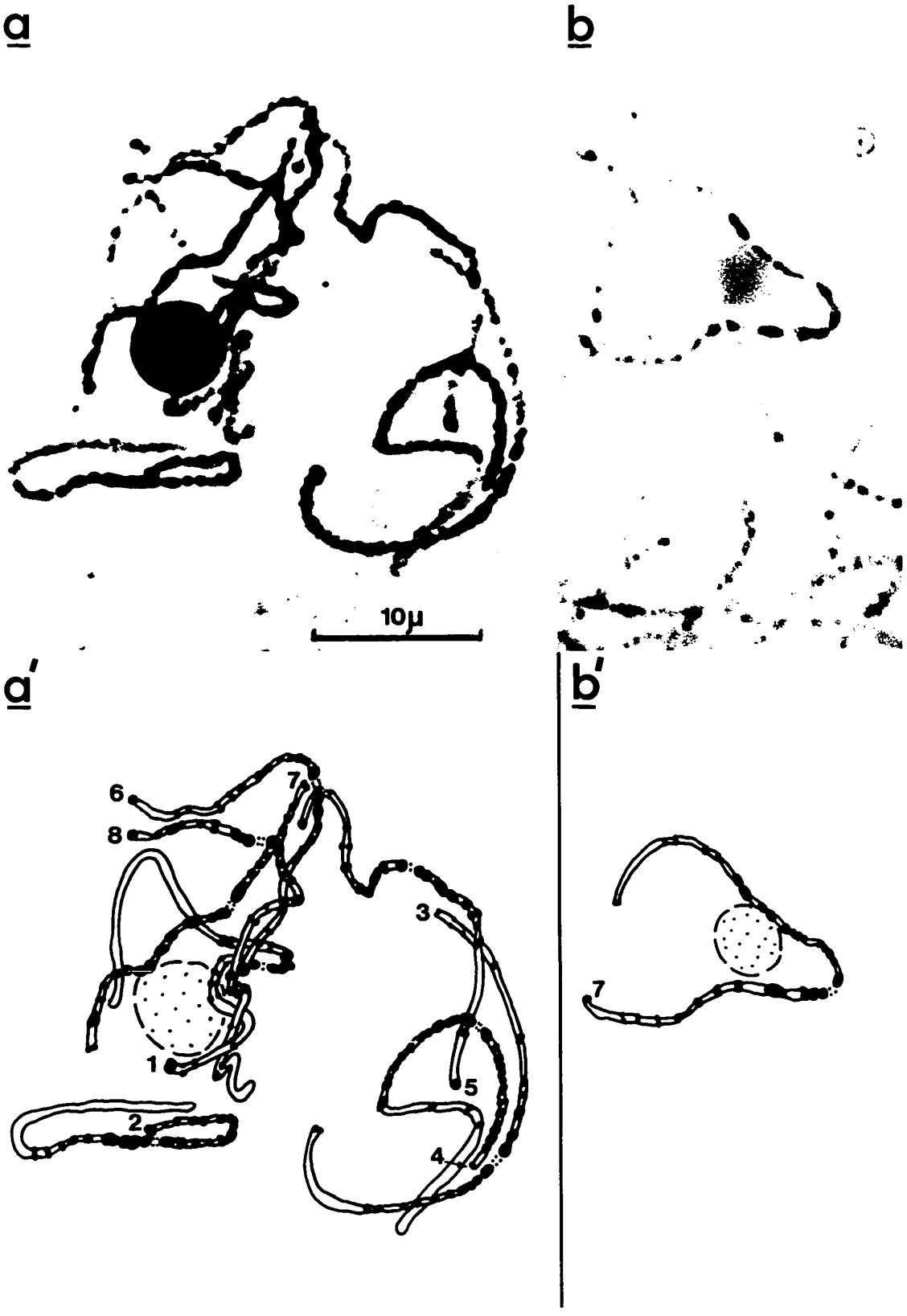
were completely synapsed while others had one or both arms unpaired, tends to rule out this explanation. It is more likely that the pairing failure or delay is a result of some environmental or genetic factor. A cell with incomplete synapsis in some chromosomes is shown in Fig. 28a. A completely synapsed chromosome 8 is shown in Fig. 28b. From the available cells the data for *M. pironae* given in Table 14 was compiled. The haploid chromosome complement length of 244 microns is much shorter than that of *M. daghestanica*. The idiogram constructed (Fig. 30b) is very similar to that of *M. daghestanica*, with the order of chromosomes 4 and 5 reversed, and the nucleolar chromosome shortest in the complement, compared with sixth in length in *M. daghestanica*. The chromatic patterns of the chromosomes are similar to those of *M. daghestanica*, although *M. pironae* does not have pronounced chromatic knobs on the ends of the chromosomes except in the submetacentrics.

c. Karyotype of *M. daghestanica* X *M. pironae* and the relationship of the two species

Both *M. daghestanica* X *M. pironae* and the reciprocals were studied but no differences in pachytene behaviour were detected. All hybrids had completely aborted pollen. Pachytene pairing was, however, quite complete, although occasional unpaired portions of chromosomes were noted. Their importance may have been overstressed by Lesins and Gillies (1968) in view of the results found above in *M. pironae*, and results from other pure species which have shown partial pairing failure. No evidence of gross pairing difficulties was encountered although there were a few instances of differences in chromomere pattern between short portions of the paired members of the bivalents. A cell with eight

Figure 29

a. Pachytene cell of *M. daghestanica* X *M. pironae*. b. Nucleolar chromosome of *M. daghestanica* X *M. pironae*. a' and b'. Interpretative drawings of a and b. Chromosomes are numbered at their short arm telomeres, and centromeres are indicated by dotted gaps. X2900.


a
b
10µ
a'
b'
6
7
8
3
1
5
2
4
7

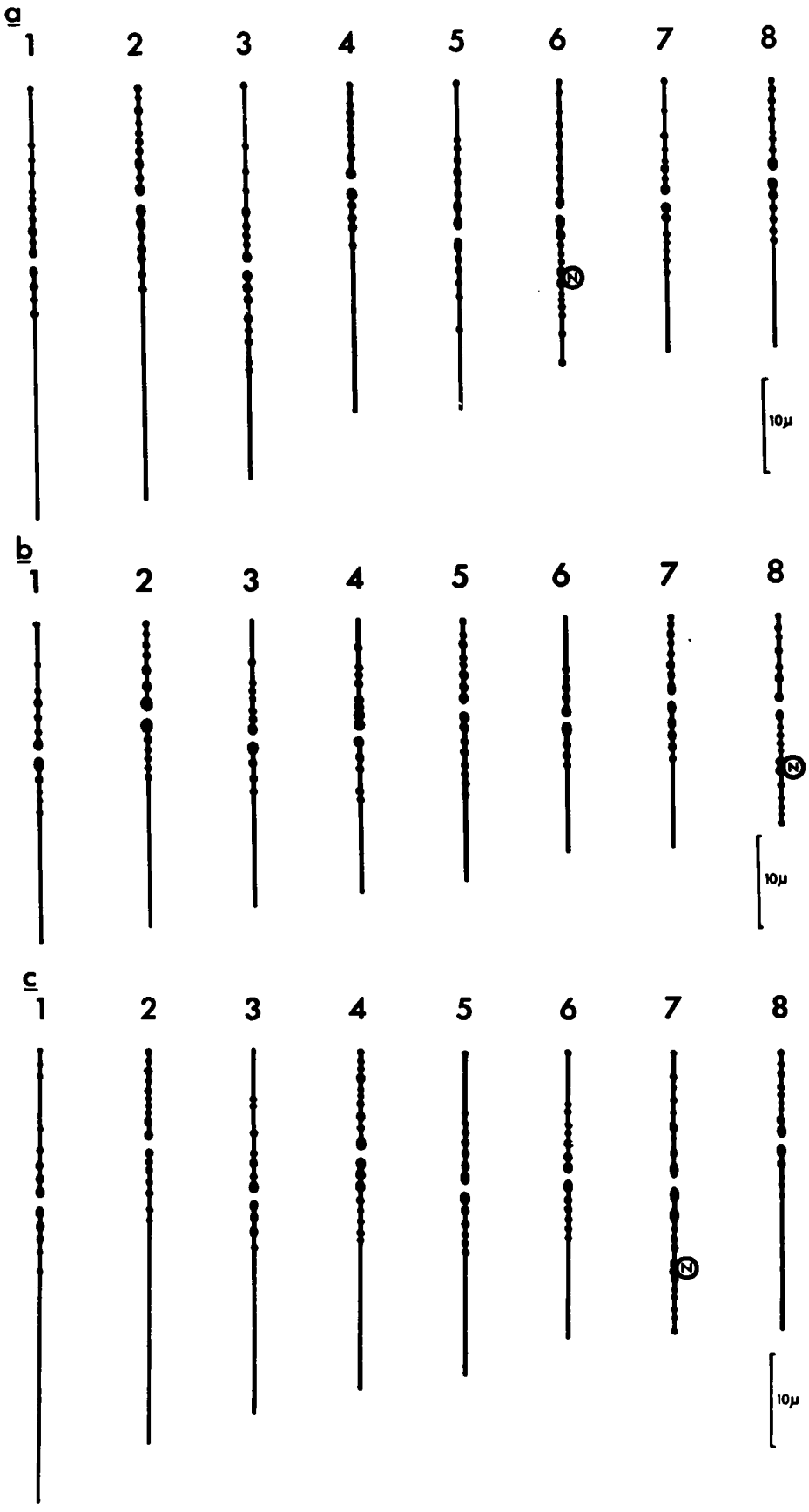
bivalents is shown in Fig. 29a and a well paired nucleolar bivalent is shown in Fig. 29b.

From a total of 24 cells the mean lengths and arm ratios of the eight chromosomes of the hybrid were calculated, and these are given in Table 14. The chromosomes were similar in length to *M. daghestanica* chromosomes but in some cases the arm ratios of the hybrid chromosomes were higher than those of equivalent *M. daghestanica* chromosomes. The idiogram which was constructed (Fig. 30c) shows that the order of chromosomes is the same as in *M. daghestanica* except that the nucleolar chromosome is seventh in order of length.

The chromosomes which were considered to be equivalent in the idiograms of these two species and their hybrid are shown in Table 14. These were tested by F test and Duncan's multiple range method, for significant differences between their mean lengths and arm ratios. Five chromosomes were significantly different in length between the two species, but none differed significantly in arm ratio between the two species. None of the lengths of hybrid chromosomes were significantly different from the equivalent *M. daghestanica* chromosomes, but three hybrid chromosomes had arm ratios significantly different from the *M. daghestanica* equivalent chromosomes. Four chromosome lengths and only one arm ratio were significantly different between the hybrid and *M. pironae*. No significant differences were found between haploid complement lengths, probably because of the small sample size. Quite possibly with larger samples all of the *M. pironae* - *M. daghestanica* length differences would have been statistically significant, as the differences involved are relatively large.

Figure 30

a. Idiogram of pachytene chromosomes of *M. daghestanica*. b. Idiogram of pachytene chromosomes of *M. pironae*. c. Idiogram of pachytene chromosomes of *M. daghestanica* X *M. pironae*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organiser. All X1400.


a
1
2
3
4
5
6
7
8
N
10μ
b
1
2
3
4
5
6
7
8
N
10μ
c
1
2
3
4
5
6
7
8
N
10μ

In spite of these length differences, it can be seen that the idiograms of the two species are extremely similar, as indicated by the absence of significant differences between arm ratios. Comparison of the proportional lengths of chromosomes in the two species shows some similarities. The longest three *M. daghestanica* chromosomes are proportionally longer than the longest three *M. pironae* chromosomes, while the next four chromosomes of *M. pironae* (Table 14) are proportionally longer than the equivalent *M. daghestanica* chromosomes. These differences may be the result of differential contraction rates which cause the longer chromosomes of *M. pironae* to contract faster than the shorter ones. However, no indication of this was found earlier in the comparison of different ploidy levels of *M. sativa s.l.* and the proportional lengths of the hybrid chromosomes do not appear to fit this hypothesis, as all are much longer than those of *M. pironae*, but several (e.g. chromosomes 2 and 3) have proportional lengths almost identical to *M. pironae's*. Unfortunately, insufficient complete cells were available for a statistical comparison of the similarity of proportional lengths of chromosomes in the two species. However, the good pairing of the chromosomes in the hybrid suggests that differences in proportional lengths between the two species, if real, are probably the result of differences in the control of chromosome behaviour (as found in *Crepis* by Tobgy, 1943), and are not the result of structural changes in the chromosomes.

The completeness of pachytene pairing in some hybrid cells, and the absence of any evidence of large structural rearrangements in chromosomes may mean that the abnormalities observed by Lesins and Gillies

(1968) at diakinesis, metaphase I and anaphases I and II manifest themselves after pachytene and are a consequence of either chiasma formation or failure. Lesins and Gillies (1968) suggested that univalents originated during diplotene, possibly due to failure of chiasma formation. The anaphase I and II bridges observed by these authors must be the result of either small structural rearrangements not detected at pachytene, or of some form of breakage and fusion of chromatid arms as suggested by Newman (1966) in *Podophyllum*. If the inversion differences were small enough to remain undetected, it would seem unlikely that sufficient pairing loops containing crossovers would occur to cause the high proportion of bridges observed by Lesins and Gillies (1968). Although in the present study a few loops were seen in pachytene cells, they appeared to be unpaired chromosomes, and not the result of inversion loop pairing.

Although differences occurred in the lengths and the arm ratios of the nucleolar chromosomes of *M. daghestanica* and *M. pironae*, they seem to be the result mainly of contraction differences. No immediate explanation emerges for the abnormalities which Lesins and Gillies (1968) found associated with the nucleolar chromosomes of the hybrid. The quadrivalents associated with the nucleolus observed by these authors, could be the result of fortuitous association of a second bivalent with the nucleolar bivalent near the "sticky" centromeric chromatic regions. The occurrence of two nucleoli in one cell has occasionally been found to be a result of fragmentation and separation of the nucleolus in the preparation of squashes. A second bivalent could have been erroneously associated with such a nucleolar fragment. The low

frequency of these pachytene abnormalities suggests that they might have been errors of interpretation, but the higher frequency of later stage abnormalities confirms that some differences do exist between the nucleolar chromosomes of the two species.

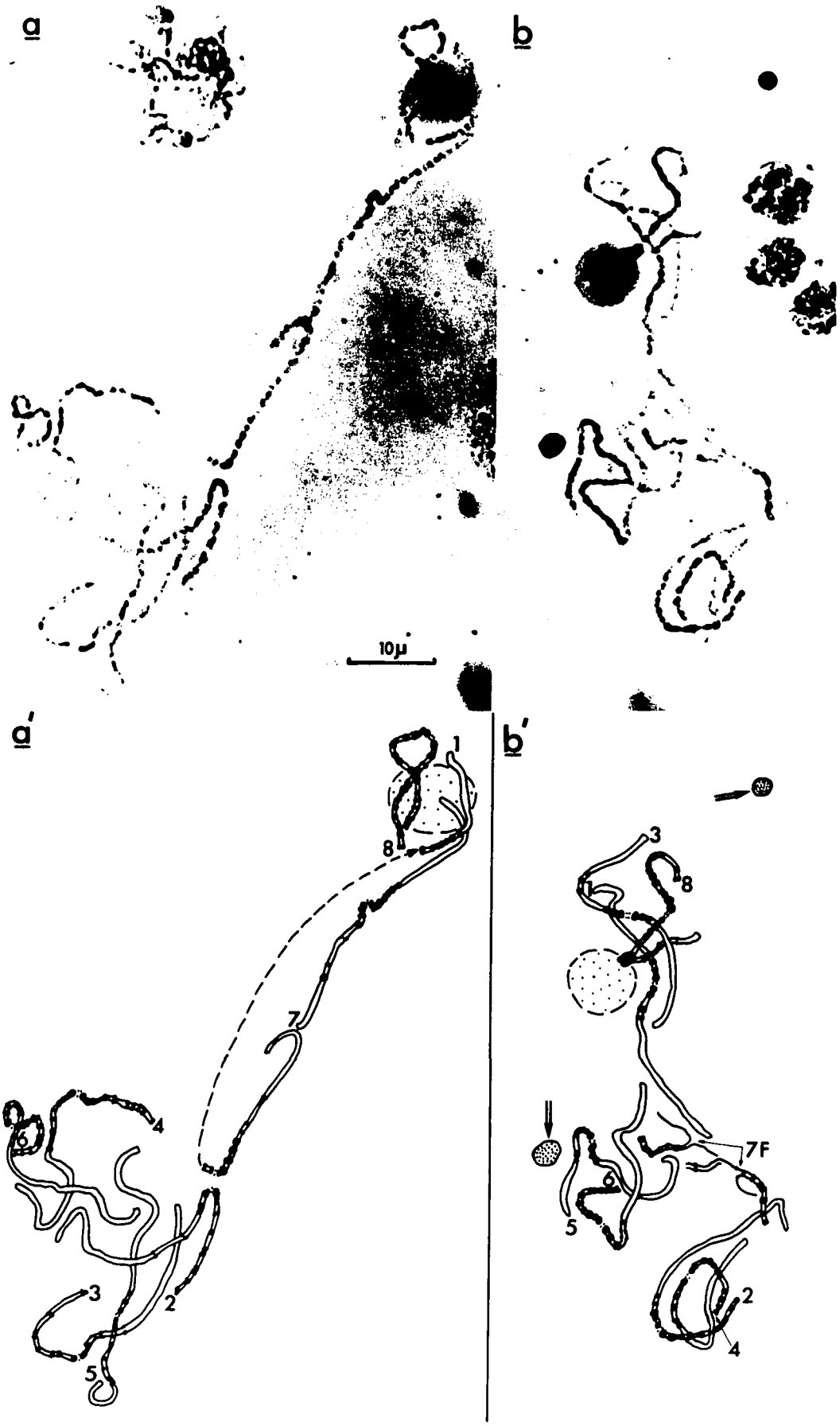
Stages beyond meiosis in the microsporocytes of hybrids were studied, in an attempt to further determine the causes of pollen sterility. Microspores were formed after telophase II, although in some cells chromosomes or chromosome fragments were excluded. In two of the hybrids, abnormally large and extremely small microspores were seen. The first pollen mitosis proceeded normally up to late prophase, but no cells beyond this stage were seen. Thereafter, only empty pollen grains were found. Thus it appears that abortion is the result of imbalance in the meiotic products, due to either irregular anaphase segregation or to interspecific chromosome incompatibilities, but the effect is not manifested until the first pollen mitosis.

The complete sterility of the hybrids was explained by Lesins and Gillies (1968) as the result of many small differences in the genomic backgrounds of the two species. The similarities found here in the two idiograms support the idea that the two species are closely related in origin but have diverged more recently and built up a barrier to interspecific hybridization.

Lesins and Gillies (1968) had found that neither *M. daghestanica* nor *M. pironae* could be crossed with *M. sativa s.l.*. Possible reasons for this failure may be found by comparing the idiograms of the two groups. The *M. sativa s.l.* chromosomes (Fig. 14) equivalent in morphology to chromosomes 1 to 8 of the *M. daghestanica - M. pironae* group

Figure 31

a and b. Pachytene cells of *M. rhodopaea*. a' and b'. Interpretative drawings of a and b. Chromosomes are numbered at their short arm telomeres, and centromeres are indicated by dotted gaps. Chromosome 1 in a has a break in the short arm (broken line and arrow) and chromosome 7 in b has several breaks (7F). The heavily stained secondary nucleoli are indicated by double arrows in b'. X1400.


a
b
10μ
a'
b'
1
8
7
4
6
3
2
5
3
8
7F
6
5
2
4

listed in Table 14, are chromosomes 2, 1, 3, 4, 5, 7, 6 and 8. It can be seen that there are some differences in proportional length, the most extreme being between chromosomes 1, 2 and 7 of the *M. daghestanica - M. pironae* group and their equivalents in *M. sativa s.l.*. Real lengths also differ considerably, *M. daghestanica* chromosomes being longer, and *M. pironae* chromosomes being shorter than those of *M. sativa* No. 505, but the problem of contraction differences negates the usefulness of these comparisons. The two groups are, however, very similar in chromosome arm ratios and chromatic patterns. This could be a reflection of a common ancestry of the *M. sativa s.l.* group and the *M. daghestanica - M. pironae* group, but at a stage prior to the divergence of *M. daghestanica* and *M. pironae* into separate species.

3. *M. rhodopaea* and *M. rupestris*

Accessions of both of these species were available for study, as well as hybrids between them, and between a colchicine-induced tetraploid *M. rhodopaea* and diploid *M. sativa*. Unfortunately the conditions necessary for the induction of flowering in *M. rupestris* are extremely exacting, and under glasshouse conditions these plants flowered too sparsely to enable sufficient buds of the right stage to be obtained for pachytene analysis of the species. Hence studies have been confined to *M. rhodopaea* and the hybrids with it.

a. Karyotype of *M. rhodopaea* Velen.

Twenty-eight analysable cells at the pachytene stage were produced. Fig. 31 shows two such cells. From these 28 cells the mean lengths and arm ratios for *M. rhodopaea* (Table 15) were calculated,

TABLE 15. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF *M. RHODOPAEA* AND *M. RHODOPAEA* X *M. RUPESTRIS*

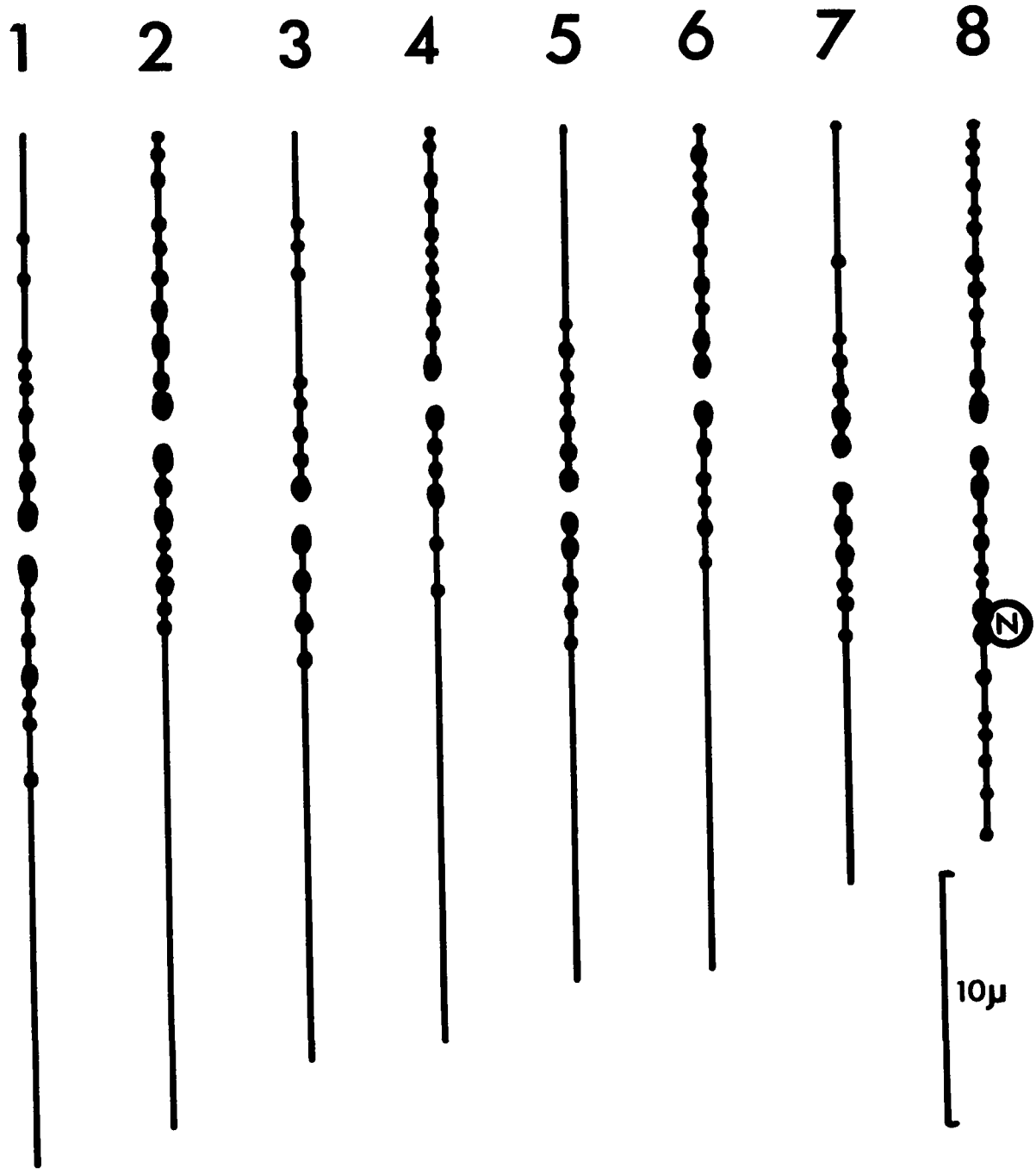
Chromosome No.	Item+	*M. rhodopaea* 493	*M. rhodopaea* X *M. rupestris*	Mean	t Test - Mean Vs. *M. sativa* #505 ‡
1	L	40.2 ± 1.31	41.0 ± 1.82	40.5	n.s.
	AR	1.54 ± 0.067	1.43 ± 0.088	1.50	*
	PL	-	-	0.149	-
	N	20	13	33	
2	L	38.7 ± 1.55	40.3 ± 1.38	39.4	n.s.
	AR	2.40 ± 0.094	2.73 ± 0.147	2.55	n.s.
	PL	-	-	0.145	-
	N	21	17	38	
3	L	36.0 ± 1.03	36.8 ± 0.91	36.4	*
	AR	1.43 ± 0.066	1.42 ± 0.072	1.42	n.s.
	PL	-	-	0.134	-
	N	22	27	49	
4	L	35.4 ± 1.40	34.5 ± 0.98	35.0	n.s.
	AR	2.49 ± 0.125	2.15 ± 0.103	2.31	n.s.
	PL	-	-	0.129	-
	N	19	21	40	
5	L	33.1 ± 0.98	32.7 ± 1.02	32.9	n.s.
	AR	1.29 ± 0.060	1.31 ± 0.069	1.30	n.s.
	PL	-	-	0.121	-
	N	22	20	42	
6	L	32.8 ± 1.21	29.4 ± 0.96	31.0	n.s.
	AR	2.26 ± 0.138	2.08 ± 0.181	2.16	**
	PL	-	-	0.114	-
	N	17	19	36	
7	L	29.4 ± 1.19	28.4 ± 1.14	28.9	n.s.
	AR	1.19 ± 0.037	1.17 ± 0.035	1.18	*
	PL	-	-	0.107	-
	N	17	18	35	
8	L	27.7 ± 1.18	26.1 ± 1.04	27.0	*
	AR	1.29 ± 0.045	1.28 ± 0.048	1.29	**
	PL	-	-	0.100	-
	N	19	16	35	
Satellite	L	8.66 ± 0.37	8.78 ± 0.36	8.69	n.s.
	PL	-	-	0.032	-
	N	19	16	35	
Complement	L	273.3 ± 12.11	263.75 ± 15.19	270.1	n.s.
	N	12	6	18	
Sum of Mean Lengths		273.3	269.2	271.1	-

\+ L - length ± S_E (in μ); AR - arm ratio ± S_E; PL - proportional length; N - number of observations.

‡ * - significantly different at 5% level; ** - significantly different at 1% level; n.s. - not significantly different.

Figure 32

Idiogram of pachytene chromosomes of *M. rhodopaea*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3750.


1
2
3
4
5
6
7
8
N
10μ

including the mean haploid complement length of twelve complete cells. The idiogram drawn from the data is presented in Fig. 32.

The general pattern of arm ratios and chromatin distribution, and the chromosome lengths, are very similar to those of *M. sativa s.l.*. This will be further discussed below. An interesting feature of the cell in Fig. 31b is the presence of a darkly staining, roughly circular body, lying close to the chromosomes, but not attached to any one of them. This was present is almost all pachytene cells of the accession of *M. rhodopaea* studied.

b. Karyotype of *M. rhodopea* X *M. rupestris* and the relationship of the two species

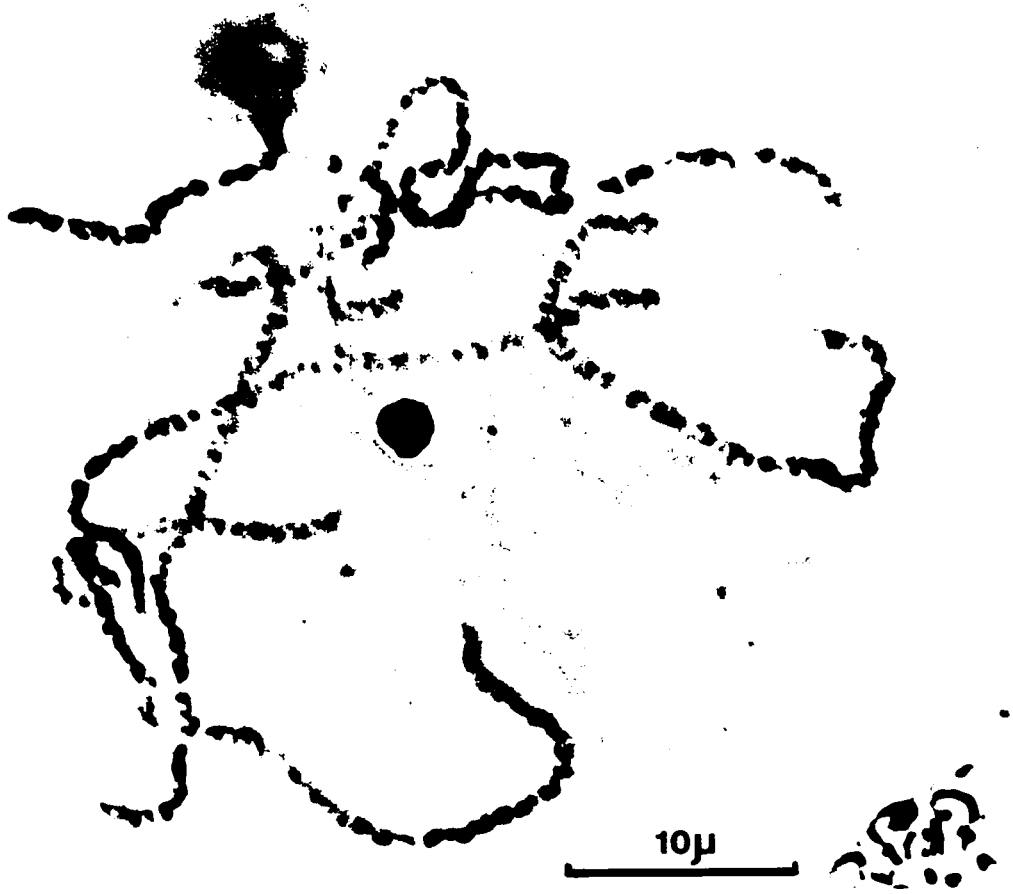
Sporocytes from four hybrid plants were examined at pachytene and several other meiotic stages. A total of 38 pachytene cells were analysed, including six complete cells. A cell from one of the hybrids is illustrated in Fig. 33. The chromosome lengths and arm ratios which are given in Table 15 were found to be almost identical with those of *M. rhodopaea*, only the mean lengths of the two chromosome 6's and the arm ratios in the chromosome 4's differing significantly in t tests. This fact, the absence of any abnormalities in chromosome pairing in the hybrid, and the similarities of chromatic patterns in the two karyotypes, lead one to conclude that *M. rupestris* must have an idiogram extremely similar in all respects to that of *M. rhodopaea*.

This supports the suggestion made by Lesins and Lesins (1966), based on the great morphological similarity between the species, that they are closely related. Their occurrence in two relatively closely situated areas, Crimea and Bulgaria, suggests that they may have had

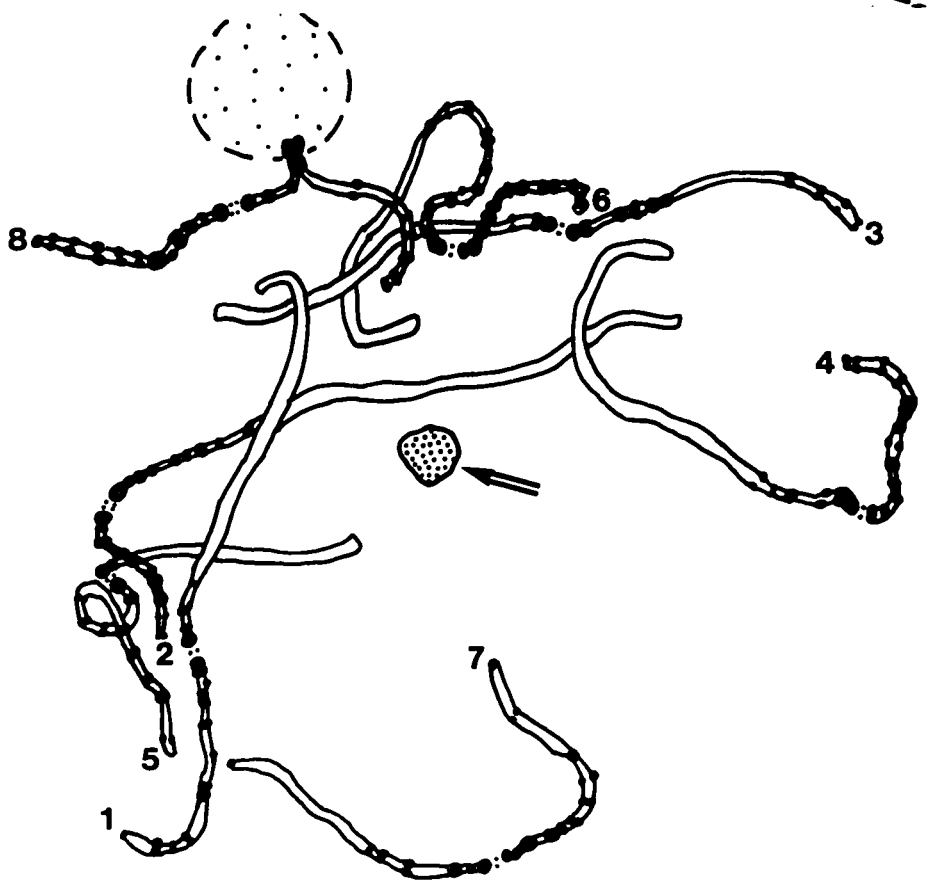
Figure 33

a. Pachytene cell of *M. rhodopaea* X *M. rupestris* No.3. a'. Interpretative drawing of a with chromosomes numbered at the short arm telomeres and centromeres indicated by dotted gaps. The heavily stained secondary nucleolus is indicated by the double arrow. X2850.

a



a'



a common origin and have diverged only recently. The possibility exists too, that they are in fact only geographic variants of the one species.

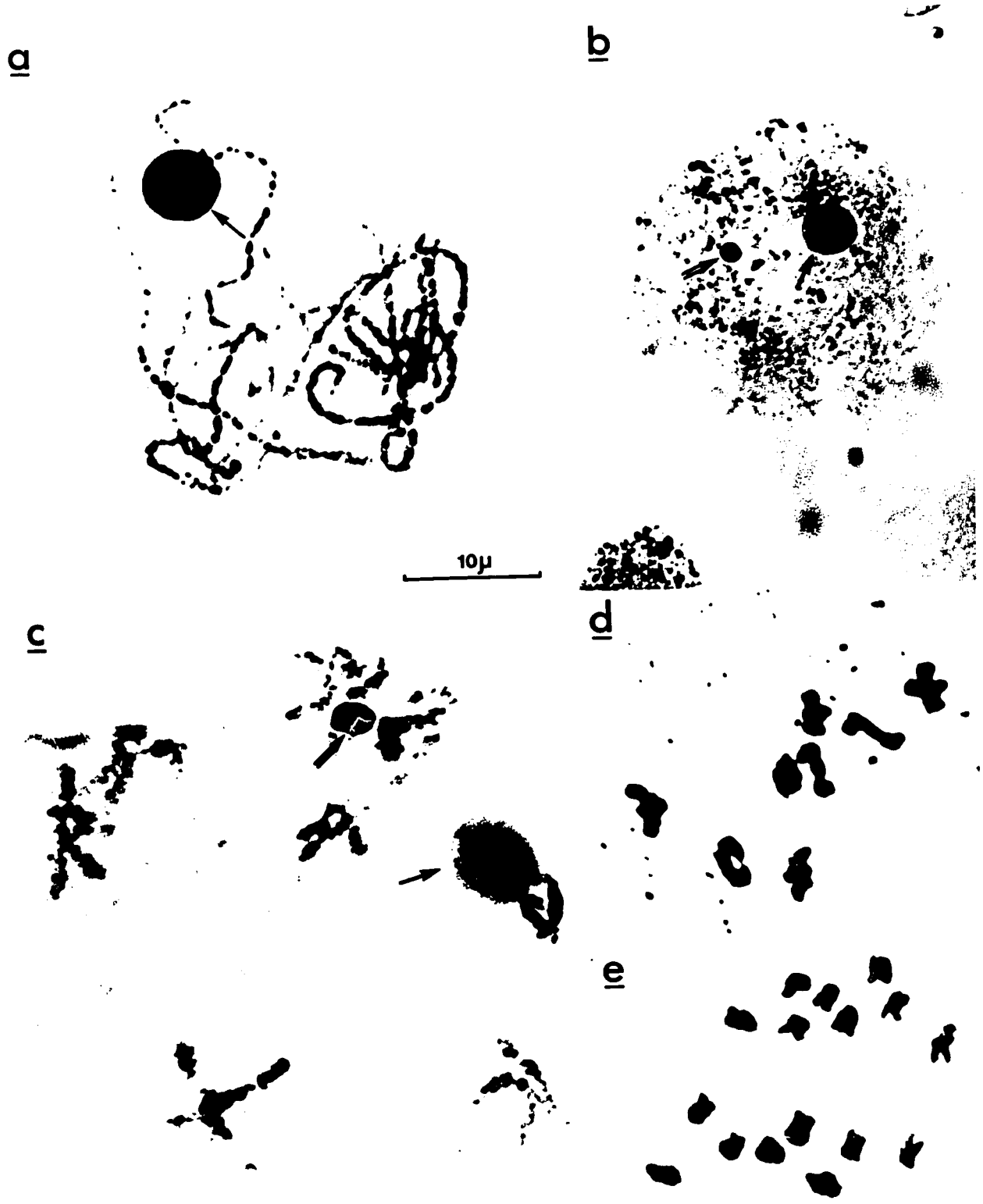
The presence of darkly staining bodies in the pachytene cells was noted in two of the hybrids, but they were absent from the other two (Fig. 34a). This led to a further investigation of these bodies in an attempt to determine their origin and nature. Other meiotic stages were examined and the bodies were found to be present in pre-meiotic interphase nuclei (Fig. 34b) and at diakinesis (Fig. 34c) but to have disappeared at metaphase I and anaphase I (Figs. 34d and e). In this behaviour they resembled the nucleolus, but their staining (in the carmine stain used) was much heavier than that of the nucleolus, and they were only one third to one half the diameter of the nucleolus. In addition, they sometimes had irregular edges and were uniformly dark, whereas the nucleolus was a lighter and often mottled colour.

In order to determine whether this similarity to nucleoli was paralleled by a similarity in chemical composition, pyronine/methyl green staining of sporocytes from a hybrid plant with the body, was undertaken. Staining was according to the schedule of Jordan and Baker (1955), which was adapted for bulk staining of buds. After fixation the buds were softened in a 5% pectinase solution (Pandey and Henry, 1959) for several hours, washed, and stained in a vial for two days at room temperature. They were then rinsed in a mixture of absolute alcohol and tertiary butyl alcohol (1:3) and anthers were squashed in acetate buffer (pH 4.8). Buds from *M. sativa* No. 505 were used as a control.

It was found that two nucleolus-like bodies were present in each

Figure 34

Chromosomes of *M. rhodopaea* X *M. rupestris*. a. Pachytene cell of No.6. Arrow indicates nucleolus. b, c, d and e. Cells of No.3. b. Premeiotic interphase cell c. Diakinesis cell. d. Metaphase I cell. e. Anaphase I cell. Single arrow indicates primary nucleolus, double arrow indicates the secondary nucleolus. All X2200.


a
b
10μ
c
d
e

cell of the *M. rhodopaea* X *M. rupestris* interphase/prophase sporocytes compared with one in each *M. sativa* sporocyte cell. Both "nucleoli" stained a translucent red compared with the blue-green colour of the surrounding chromosomal DNA. The cytoplasm was a granular pink-red. The larger nucleolus in each cell was about the same size as the nucleoli in the *M. sativa* sporocytes but the second nucleolus-like body was smaller, about equal in diameter to one of the two nucleoli present in each somatic anther cell. Because of the similarity in staining of the two "nucleoli" in the hybrid sporocytes (both apparently RNA - containing), the small darkly staining body was termed a secondary nucleolus.

The fact that this secondary nucleolus had not been observed attached to any chromosome makes it difficult to explain. The presence of DNA in it could not be determined by the pyronine/methyl green method. The finding of secondary nucleoli in only two of the four progeny of the *M. rhodopaea* line suggests that segregation has occurred for the presence of the secondary nucleolus. Possibly the secondary nucleolus is produced by accessory ribosomal DNA, similar to the situation in *Xenopus* oocytes (Brown and Dawid, 1968). Dickinson and Heslop-Harrison (1970) mentioned that supernumerary nucleoli occur unattached to chromosomes in prophase I of *Lilium* sporocytes. Gates (1942) had earlier reported similar bodies in other species. Whether the extra nucleolus is present in somatic cell interphase and prophase nuclei of *M. rhodopaea* has not been determined. If it is, it could be a result of minute B chromosomes carrying accessory ribosomal DNA. Loss of these in cell division could account for the apparent segregation which has occurred.

Walters (1968) has demonstrated secondary nucleoli somewhat

similar to these in maize and *Bromus* sporocytes. These "nebbennucleoli", as she called them, were explained as being the result of the high RNA synthetic activity of the diplotene and diakinesis chromosomes, similar to the activity in lampbrush chromosomes. Walters suggested that the RNA in the nebbennucleoli was for later use in the synthetic activities associated with meiotic chromosome division.

c. *M. sativa* X *M. rhodopaea* (4n) and the relationship of the two species

Both Lesins (1961b) and Nicoloff (1961) remarked on the apparent close relationship of *M. rhodopaea* to *M. cancellata*, and Lesins (1961b) suggested that *M. rhodopaea* and *M. sativa s.l.* might be involved in the origin of hexaploid *M. cancellata*. Later, Lesins (1970) decided *M. rhodopaea* was more likely involved in the ancestry of *M. saxatilis*, and that *M. rupestris* might be related to *M. cancellata*. The similarity of the *M. rhodopaea* idiogram to that of *M. sativa s.l.* has been mentioned above, and to quantify this similarity the mean values of chromosome lengths and arm ratios for the *M. rhodopaea* - *M. rupestris* group were compared with those of *M. sativa* No. 505 in a t test (Table 15). It was found that only two chromosome classes differed significantly in length (chromosomes 3 and 8 of *M. rhodopaea*), and four differed in arm ratio. The mean *M. rhodopaea* - *M. rupestris* arm ratios are even more similar to those of *M. sativa s.l.*. Hence it would seem that *M. rhodopaea* and *M. sativa s.l.* are very similar and probably closely related.

However, Lesins (personal communication) has had no success in attempts to cross diploid *M. rhodopaea* with diploid *M. sativa*. The possibility that hexaploid *M. saxatilis* contained four genomes of *M.*

Figure 35

Chromosomes of *M. sativa* 506 (206-10) X *M. rhodopaea* (autotetraploid). a. Whole pachytene cell with trivalents. b, c, d and e. Pachytene trivalents. f. Pachytene trivalent of nucleolar chromosomes. g. Diakinesis cell with four univalents (small arrows) and four trivalents (large arrows). h. Anaphase II separation with misdivision and lagging chromosomes. All X2200.


a
10µ
b
c
d
e
f
g
h

rhodopaea and two genomes of *M. sativa s.l.* (Lesins, 1970), led him to try crossing an artificial tetraploid *M. rhodopaea* with diploid *M. sativa*. This cross produced sterile triploid hybrids.

Several meiotic stages have been studied in these triploid hybrids. Pachytene was not easy to study because of the large number of unpaired chromosome arms, which made analysis difficult. Many trivalent configurations were present but these could be clearly followed only when one was isolated from the main nuclear mass. Examples of pachytene in the triploid hybrid are given in Fig. 35, a-f. In many of the trivalents the association of the third chromosome with the other two involves only a few short segments, while in several (Fig.35, c and d) the pairing between the three chromosomes was more intimate. The three nucleolar chromosomes were all associated at the N.O., as had been found in plants trisomic for the nucleolar chromosome.(Fig.35f)

A few cells of diakinesis and metaphase I were observed (Fig.35g) and these showed as many as four trivalents and varying numbers of univalents up to a maximum of seven. At anaphases I and II varying numbers of laggard and misdividing chromosomes were seen (Fig. 35h), and some were excluded from the microspores at telophase II.

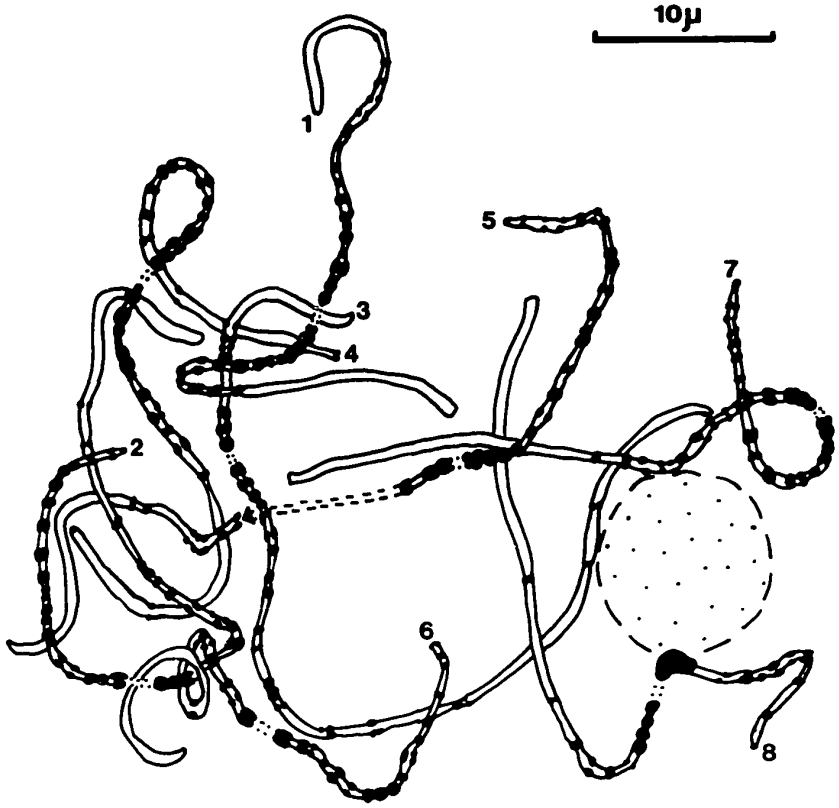
Even from this limited study of the *M. rhodopaea* X *M. sativa* hybrid it is apparent that sufficient homology must exist between some of the chromosomes of the two species for pairing to occur. The failure of crosses at the diploid level may be due to genetic differences operating at the genomic level, and these are overcome if one of the genomes is doubled. This is a similar situation to that found operating in crosses between *M. dzhawakhetica* and *M. sativa* by Lesins (1961a), although

Figure 36

a. Pachytene cell of *M. hybrida*. a! Interpretative drawing of a with chromosomes numbered at short arm telemeres and centromeres indicated by dotted gaps. A break in chromosome 5 is indicated by the double broken line and arrow in a! X2400.

a

a'



Clement (1963) subsequently found he could cross these two species at the diploid level. As both workers used the same *M. dzhawakhetica* accessions, this raises the question of possible differences in the crossability of the *M. sativa* lines they used. Hence it may also be possible to cross *M. rhodopaea* and *M. sativa s.l.* at the diploid level if several different accessions of each are used in all possible combinations. Closely related species such as *M. prostrata* could also be included in these attempts.

4. *M. hybrida* and *M. suffruticosa*

a. Karyotype of *M. hybrida* Trautv.

The sporocytes of this species, and also those of *M. suffruticosa*, proved to be easily prepared for pachytene study and were adequately stained in about two weeks in Snow's stain. No cells of *M. hybrida* with all eight chromosomes measurable were found, although several with seven chromosomes plus a broken chromosome were measured (Fig. 36). From a total of 26 cells with measurable chromosomes, the mean lengths and arm ratios given in Table 16 were calculated, and these were used to construct the idiogram in Fig. 39a. The karyotype consisted of three symmetrical chromosomes with median centromeres, and five asymmetrical chromosomes, only one of which has a median centromere, the remainder having submedian centromeres. Chromosomes 1 and 4 have median centromeres, and about half of both arms proximal to their centromeres is chromatic. There are no telomeric knobs. Chromosomes 2, 3, 6 and 7 have submedian centromeres and most of their short arms are chromatic, although the distal portions have only small chromomeres. The long

TABLE 16. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF *N. HYBRIDA*, *N. SUFFRUTICOSA* AND THEIR HYBRID

Chromosome		*N. hybrida* (a)		*N. suffruticosa* (b)		*N. hybrida* X *N. suffruticosa* (c)		Significant Difference (Length and Arm Ratio)‡		
No.	Item*	Values	Chrom. No. in Idiogram	Values	Chrom. No. in Idiogram	Values	Chrom. No. in Idiogram	a-b	a-c	b-c
1	L	52.6 ± 1.51	1	48.2 ± 1.41	2	46.5 ± 1.43	1	*	*	n.s.
	AR	1.16 ± 0.038		1.15 ± 0.077		1.12 ± 0.019		n.s.	n.s.	n.s.
	N	16		17		14				
2	L	50.4 ± 2.08	2	48.6 ± 1.55	1	46.1 ± 3.17	3	n.s.	n.s.	n.s.
	AR	2.02 ± 0.094		2.51 ± 0.152		2.24 ± 0.357		n.s.	n.s.	n.s.
	N	8		9		5				
3	L	49.1 ± 1.61	3	47.6 ± 0.67	3	46.4 ± 2.21	2	n.s.	n.s.	n.s.
	AR	1.79 ± 0.136		1.91 ± 0.076		1.56 ± 0.057		n.s.	n.s.	n.s.
	N	14		14		9				
4	L	45.4 ± 1.27	4	43.4 ± 1.38	4	40.7 ± 1.46	4	n.s.	n.s.	n.s.
	AR	1.25 ± 0.054		1.19 ± 0.043		1.16 ± 0.049		n.s.	n.s.	n.s.
	N	16				13				
5	L	42.9 ± 1.22	7	42.7 ± 1.24	5	40.3 ± 1.98	5	n.s.	n.s.	n.s.
	AR	1.61 ± 0.051		1.99 ± 0.162		1.75 ± 0.144		n.s.	n.s.	n.s.
	N	11		9		8				
6	L	43.3 ± 1.06	6	42.4 ± 1.47	6	37.5 ± 1.25	7	n.s.	n.s.	n.s.
	AR	1.72 ± 0.077		2.08 ± 0.113		1.73 ± 0.126		*	n.s.	n.s.
	N	16		12		5				
7	L	43.3 ± 1.90	5	41.1 ± 1.73	7	38.5 ± 1.00	6	n.s.	n.s.	n.s.
	AR	1.21 ± 0.050		1.46 ± 0.088		1.31 ± 0.050		*	n.s.	n.s.
	N	11		11		13				
8	L	37.3 ± 1.44	8	37.1 ± 1.61	8	34.3 ± 1.94	8	n.s.	n.s.	n.s.
	AR	1.23 ± 0.130		1.73 ± 0.154		1.45 ± 0.107		*	n.s.	n.s.
	N	8		10		7				
Satellite	L	15.0 ± 0.69	8	12.4 ± 0.55	8	12.5 ± 0.90	8	*	*	n.s.
	N	8		10		7				
Complement	L	-	-	341.7 ± 17.4	-	319.7 ± 21.1	-	-	-	n.s.
	N			3		3				
Sum of Mean Lengths		364.3	-	351.2	-	330.4	-	-	-	-

* L - length ± S_E (ln μ), AR - arm ratio ± S_E; N - number of observations.

‡ F test and Duncan's Multiple Range test; * - significant difference at 5% level; n.s. - not significantly different.

arms are chromatic only in the proximal one third to one half, chromosomes 3 and 7 having the longest chromatic portions. Chromosome 5 has a median centromere but has the whole of its shorter arm chromatic, while the longer arm has only a short proximal portion chromatic. Chromosome 8 is the nucleolar chromosome. The centromere is median and the nucleolus organizer is located in the shorter arm adjacent to the centromere. The chromatin is arranged fairly symetrically about the centromere, and distal parts of both arms are achromatic. In this respect it differs from the nucleolar chromosomes of other perennial *Medicago* species where the N.O. chromosome is usually more chromatic than the other chromosomes of the complement.

Table 16 shows that the chromosomes are considerably longer than those found previously in other perennial species. The sum of the eight mean chromosome lengths is 364.3 microns, almost 100 microns more than that found for most of the *M. sativa s.l.* species. The shortest chromosome is almost as long as the longest chromosome of *M. sativa* No. 505. The chromosomes are also obviously different from those of other perennial species in their arm ratios and chromatic patterns. They are more chromatic, giving the impression that the chromosomes are wider, particularly in the chromatic areas surrounding the centromere. The chromatic knobs are quite large close to the centromere and decrease in size in more distal regions until they merge into the small chromomeres of the achromatic portions. The chromomere size gradient is similar to that described by Lima-de-Faria and Sarvella (1962) in tomato pachytene chromosomes.

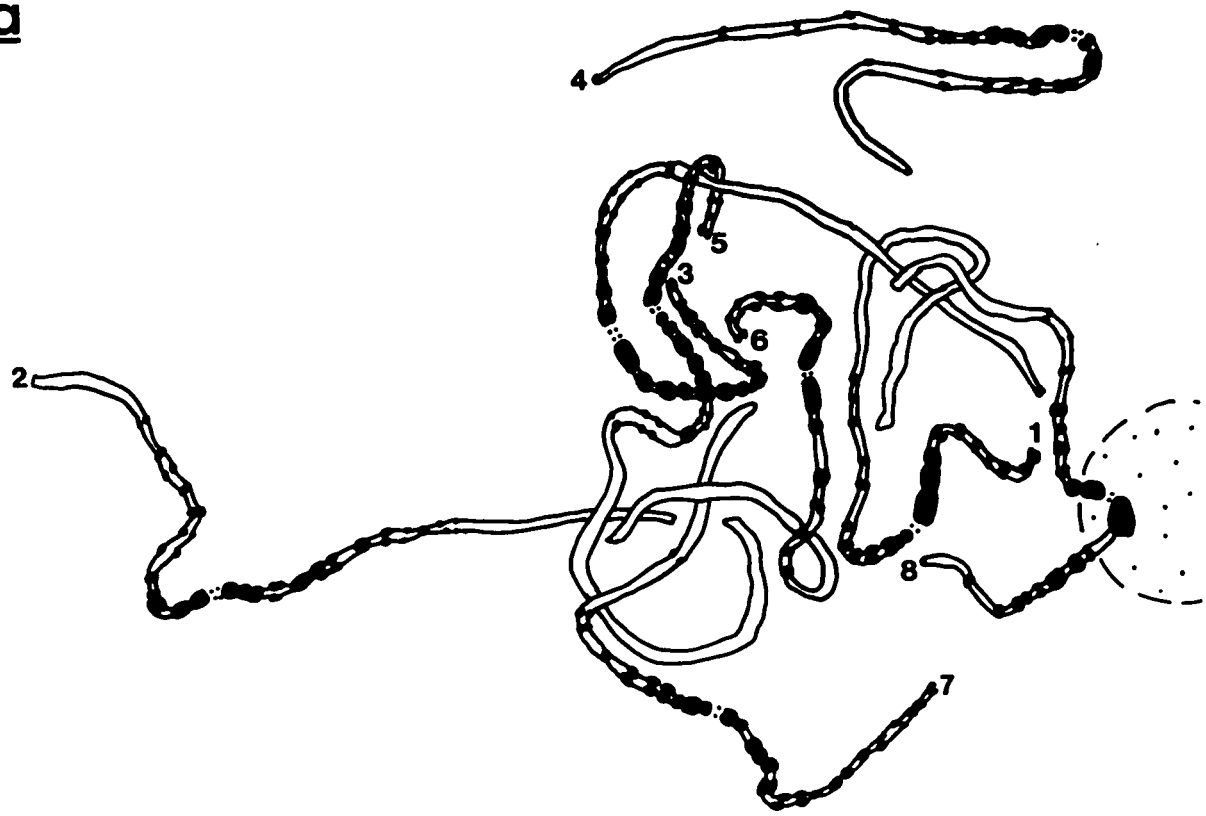
The great length and width of *M. hybrida* chromosomes compared

Figure 37

a. Pachytene cell of *M. suffruticosa*. a' Interpretative drawing of a with chromosomes numbered at short arm telomeres and centromeres indicated by dotted gaps. X2600.

a

a'



with those of other perennial species agrees with the findings of Lesins and Lesins (1966), who reported that somatic chromosomes of this species were the largest they had encountered in the genus. It would be interesting to determine to what extent these size differences are reflected in the DNA contents of *M. hybrida* and other species. The obvious difference in pachytene karyotype of *M. hybrida* from the standard type found previously in all other perennial species sets *M. hybrida* apart, and may partly explain the difficulties that taxonomists have complained of in classifying the species. In some respects the karyotype resembles that of a species of *Trigonella* in which Coutinho and Santos (1943) had studied somatic mitoses. Three of the lines they studied had five acrocentrics, two metacentrics and a satellited chromosome.

b. Karyotype of *M. suffruticosa* Ramond

Two accessions were studied, Nos. 1544 and 1549, but most of the sporocytes used were from the latter. No visible differences in chromosome morphology between the two were noticed. Chromosomes from 27 cells were measured and three complete cells were found. Fig. 37 is such a cell. The mean lengths and arm ratios of the chromosomes are given in Table 16. The idiogram based on these data is shown in Fig. 39b.

The karyotype is extremely similar to *M. hybrida's*, there being three metacentrics, two symetrical and one asymetrical, and four asymetrical submetacentrics. The nucleolar chromosome in this species has a submedian centromere but the length and chromomere arrangement is similar to that in *M. hybrida*. The mean lengths of the chromosomes

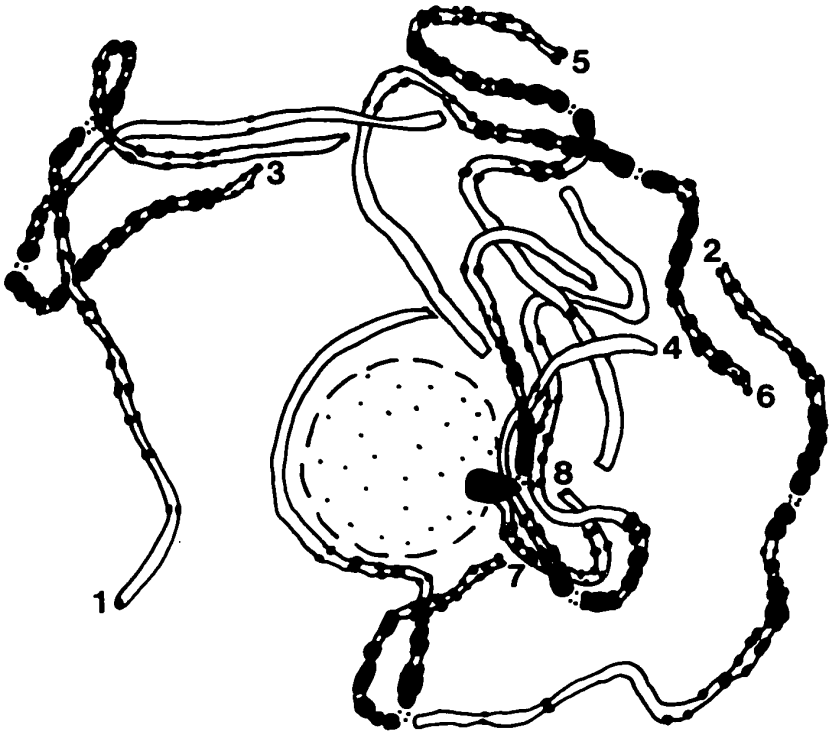
Figure 38

a. Pachytene cell of *M. hybrida* X *M. suffruticosa*. a! Interpretative drawing of a with chromosomes numbered at short arm telomeres and centromeres indicated by dotted gaps. X3200.

a

10μ

a'



were very similar to those in *M. hybrida*, measuring usually one or two microns less. Arm ratios of the asymetrical chromosomes were all slightly higher than those in *M. hybrida*. The equivalent chromosomes in the two species are given in Table 16. The highly chromatic nature of the chromosomes, and the gradient in chromomere size were features similar to *M. hybrida*.

c. Karyotypes of *M. hybrida* X *M. suffruticosa* and the relationship of the species

Two plants of the hybrid between *M. hybrida* No. 2028 and *M. suffruticosa* No. 1544 were used. The 21 cells in which chromosomes were measurable included three complete cells, one of which is shown in Fig. 38. The chromosome mean lengths and arm ratios are given in Table 16 and the idiogram based on these data is shown in Fig. 39c.

The chromosomes are similar to those of the two parents and the equivalent chromosomes in the three are indicated in Table 16. The lengths and arm ratios of equivalent chromosomes were compared in F tests and the only significant length differences found were between chromosome 1 of *M. hybrida* and the equivalent chromosomes of *M. suffruticosa* and the hybrid. Three chromosome classes had significant differences in arm ratio between *M. hybrida* and *M. suffruticosa*. The mean haploid complement length of three complete hybrid cells was not significantly different from the mean of the three complete *M. suffruticosa* cells.

The great similarity of the three idiograms was expected, in the light of Lesins' (1969) demonstration of the close relationship of the two species. His failure to hybridize either species with members

Figure 39

a. Idiogram of pachytene chromosomes of *M. hybrida*.

b. Idiogram of pachytene chromosomes of *M. suffruticosa*.

c. Idiogram of pachytene chromosomes of *M. hybrida* X *M. suffruticosa*.

Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. All X1250.

a
1
2
3
4
5
6
7
8
10µ
b
1
2
3
4
5
6
7
8
10µ
c
1
2
3
4
5
6
7
8
10µ

of *M. sativa s.l.* is also understandable when the great differences between the idiograms of the two groups are seen. So little have the idiograms in common that it is difficult to discern any basis for common ancestry of the two groups. This vast difference places the *M. suffruticosa - M. hybrida* group at the extreme of the genus taxonomically, and perhaps closer to the genus *Trigonella*. It is possible that these species are in fact intermediate between the two genera. Studies of the morphology of pachytene chromosomes in the genus *Trigonella* would possibly settle this problem.

VI. ANNUAL SPECIES HAVING 2n = 14 CHROMOSOME NUMBERS

1. Review of Literature

The basic chromosome number of the genus *Medicago* was established as eight by Senn (1938). However a report of species with n = 7 had been made earlier by Fryer (1930). Fryer reported n = 7 for *M. polymorpha* (synonym *M. hispida*) and *M. rigidula* and mentioned that Ghimpu (1928) had found n = 8 for the latter. Fryer also reported differences in karyotype morphology between accessions of *M. polymorpha* with the same chromosome number. Heyn (1956, 1963) gave two chromosome numbers (n = 7 and n = 8) for the species *M. polymorpha.*

Clement (1962) devoted considerable time to attempting to resolve this confusion and concluded that both of these species existed only in 2n = 14 forms. The unlikelihood of two stable base numbers existing in one sexually reproducing species, and the great variability in the plant morphology, led him to conclude that 2n = 16 forms formerly placed in these two species had been misclassified. Clement listed *M. coronata* Desr. also as having 2n = 14.

The problem raised by Clement (1962) induced Lesins and Lesins (1962) to undertake a similar reappraisal of annual species. They reported three species in which n = 7 is the unique chromosome number - *M. polymorpha, M. rigidula* and *M. praecox*, but could not confirm Clement's report of this number for *M. coronata.* In all three species confirmed as 2n = 14, two pairs of large chromosomes were noted, one pair being the satellited chromosomes. Clement (1962) had also noted the presence of a pair of large chromosomes in several 2n = 14 species, and had speculated that n = 7 was derived from n = 8.

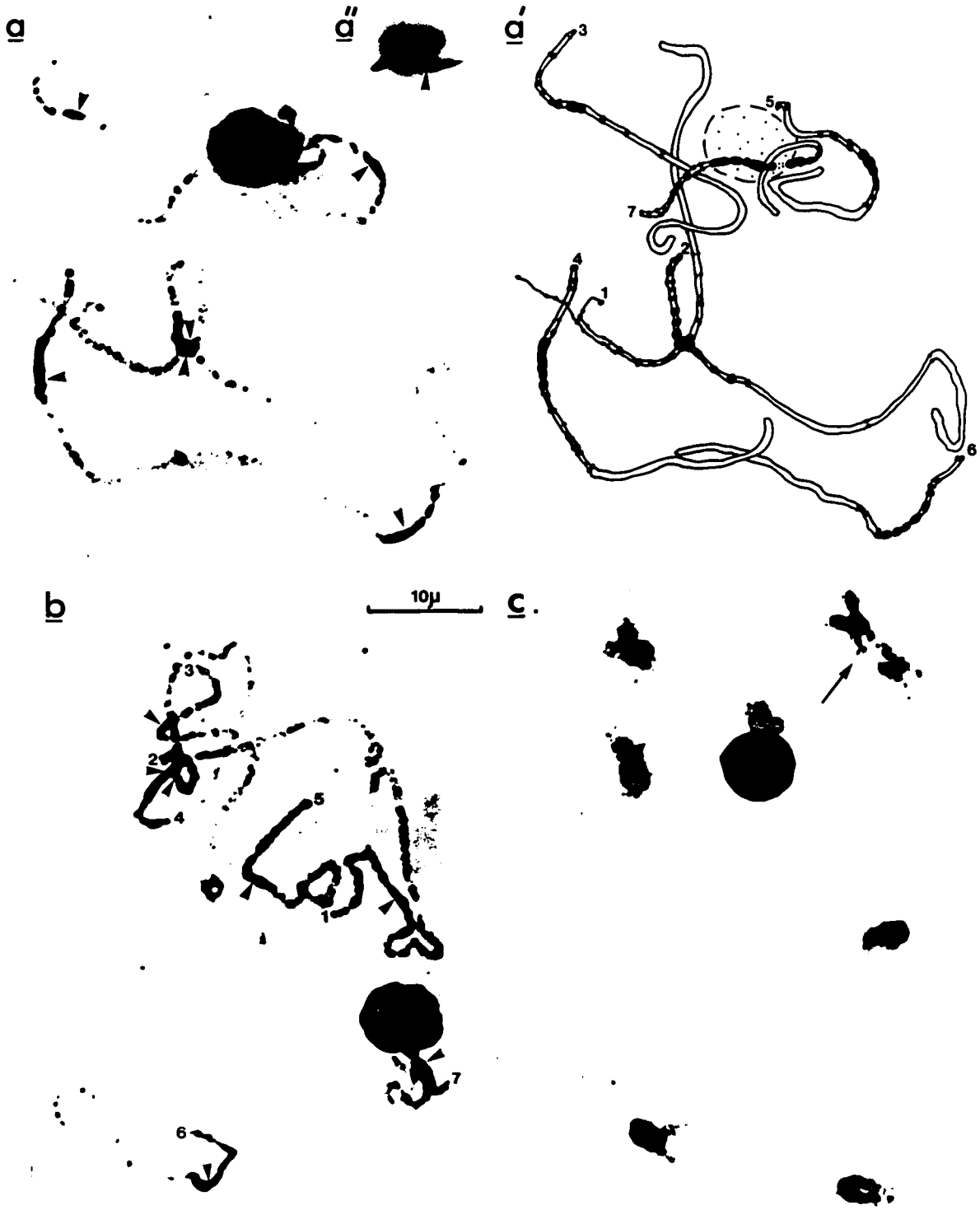
Lesins and Lesins (1963a) described *M. globosa* Presl. (more correctly called *M. constricta* Dur. - Heyn, 1963) and noted that it was the fourth species with n = 7 to be discovered. They found the satellited chromosomes to be the longest in the somatic complement. Both Heyn (1963) and Lesins and Lesins (1963a) commented on the similarity of this species to *M. rigidula*.

Simon (1965) carried out an extensive karyotype analysis of annual species of *Medicago* and confirmed that the four species *M. polymorpha*, *M. rigidula*, *M. praecox* and *M. constricta* were represented only by 2n = 14 accessions. He also concluded that *M. coronata* (L.) Bart. had 2n = 16, and that Clement's (1962) *M. coronata* with 2n = 14 was actually *M. polymorpha*. Simon and Simon (1965) gave detailed idiograms of *M. polymorpha*, *M. rigidula* and *M. praecox* based on somatic chromosome morphology. They found *M. praecox*, *M. rigidula* and the majority of the *M. polymorpha* accessions to be uniform in chromosome complement length and arm ratios, but several accessions of *M. polymorpha* had variations in the complement length and the ratio of longest to shortest chromosome. In several of the idiograms the longest chromosome was considerably longer than the next chromosome in the idiogram.

The Simons also noted that the total complement lengths of 2n = 14 and 2n = 16 species were very similar, and suggested that the former may have been derived from the latter by reciprocal translocations. They suggested that the morphological similarity of the 2n = 14 species *M. constricta* and *M. rigidula* to the 2n = 16 species *M. murex* could be a possible starting point for investigation of the genetic relationship of the two chromosome numbers.

Figure 40

Chromosomes of *M. constricta*. a and b. Pachytene cells. a! Interpretative drawing of a. Chromosomes are numbered at short arm telomeres and centromere positions are indicated by arrow heads. a'' shows the nucleolus and associated chromosome at a different focus so that the centromere of chromosome 7 is visible. c. Diakinesis cell. Arrow indicates an extremely long bivalent. X1800.


a
a''
a'
3
5
7
2
4
1
6
10µ
b
c
3
2
4
5
1
7
6

The chromosome number of *M. murex* Willd. has been given by many authors as 2n = 16 (Fryer, 1930; Heyn, 1956; Simon and Simon, 1965), and the finding of 2n = 14 chromosome numbers in addition to 2n = 16 for this species by Lesins, Lesins and Gillies (1970) was not anticipated. These authors concluded that specimens of each chromosome number existed, but that most authors had counted the same few 2n = 16 accessions or else had counted the satellites of the 2n = 14 type as chromosomes, and thus arrived at a 2n number of 16. Lesins, Lesins and Gillies found that one of Simon's (1965) accessions was 2n = 14 and not 2n = 16 as Simon had stated. Lesins et al (1970) found that plants of the 2n = 14 and of the 2n = 16 types were morphologically very similar and both conformed to the description of *M. murex*, but all attempts to cross them were unsuccessful.

2. *M. constricta* Dur.

In this species and in all the following annual species the length of staining time was approximately double that used for the perennials. Hence comparisons of chromaticity of chromosomes of the annuals and the perennials are only of limited use.

A total of seventeen pachytene stage cells of *M. constricta* were analysed from one accession, and five of these had all seven chromosomes measurable. Examples of pachytene cells are given in Fig. 40a and b. The chromosome lengths and arm ratios were calculated, and the mean values are given in Table 17. From these an idiogram was drawn, which is presented in Fig. 41.

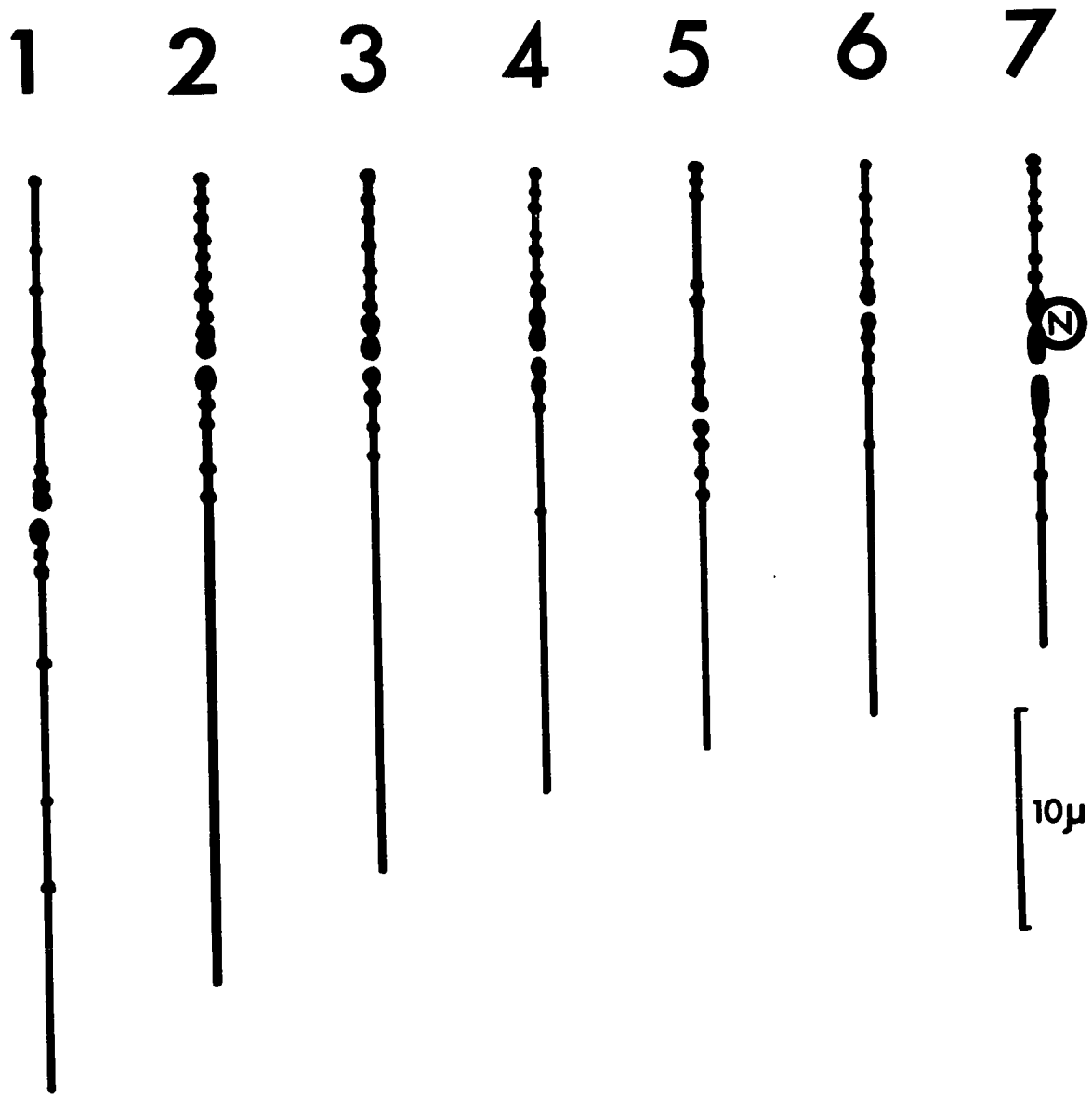
On examination of the pachytene chromosomes in Fig. 40, it is

TABLE 17. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF *M. CONSTRICTA*

Chromosome No.	Mean Length $\pm$ S_E (in μ)	Mean Arm Ratio $\pm$ S_E	Number Measured
1	41.6 $\pm$ 1.75	1.71 $\pm$ 0.112	14
2	36.8 $\pm$ 1.35	3.32 $\pm$ 0.321	10
3	31.9 $\pm$ 1.05	2.64 $\pm$ 0.152	10
4	28.4 $\pm$ 1.16	2.40 $\pm$ 0.113	9
5	26.7 $\pm$ 2.12	1.35 $\pm$ 0.090	8
6	25.2 $\pm$ 1.54	2.73 $\pm$ 0.305	9
7	22.3 $\pm$ 1.45	1.31 $\pm$ 0.110	6
Sum of Mean Lengths	212.9	-	-
Satellite (Chrom.7)	7.6 $\pm$ 0.55	-	6
Haploid Complement	212.2 $\pm$ 12.76	-	5

Figure 41

Idiogram of pachytene chromosomes of *M. constricta*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3000.


1
2
3
4
5
6
7
N
10μ

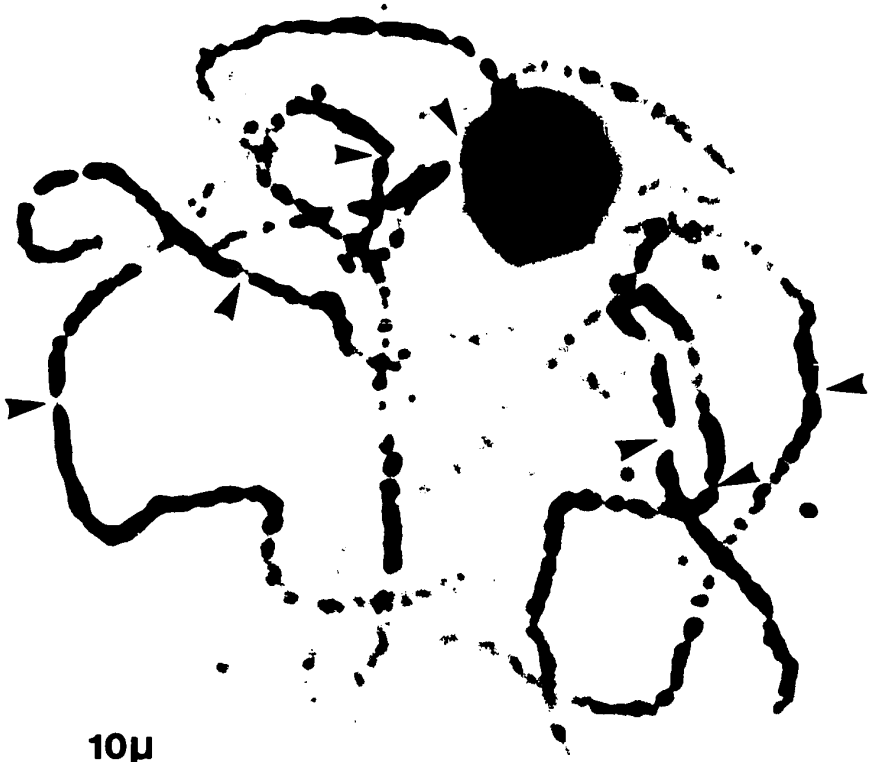
obvious that they differ from those of the perennial species previously studied. The chromatic areas are much more closely grouped around the centromere so that it is often obscured, and the few chromomeres not included in this centromeric mass are usually located close to it, mostly in the short arm of the chromosome. The long arms of chromosomes are largely achromatic for the distal three quarters or more of their length, with the exception of the nucleolar chromosome. The heavy staining of the centromeric chromatin may be a consequence of the longer staining period, which was found necessary to adequately stain the achromatic portions.

The karyotype consists of five submetacentrics and two metacentrics, one of which was the nucleolar chromosome. Chromosome 1 is unique in having the longest short arm of all the chromosomes. This short arm has a number of chromomeres distributed along it, but it is not as chromatic as the short arms of the other submetacentrics (chromosomes 2, 3, 4 and 6). The long arm of chromosome 1 also has several chromomeres in its middle portion. Chromosome 5 has an arm ratio of 1.35, but the shorter arm is fairly chromatic and the longer arm achromatic so that it has an asymmetrical chromatin distribution about the centromere. In this characteristic it differs from the symetrical distribution common in metacentrics of most perennials. Chromosome 7, the nucleolar chromosome, also has a median centromere. The N.O. is located in the short arm adjacent to the centromere so that the centromeric and N.O. chromatic areas coalesce on the short arm. The remainder of the short arm is chromatic like chromosome 5, but the longer arm is largely achromatic in its distal half.

Figure 42

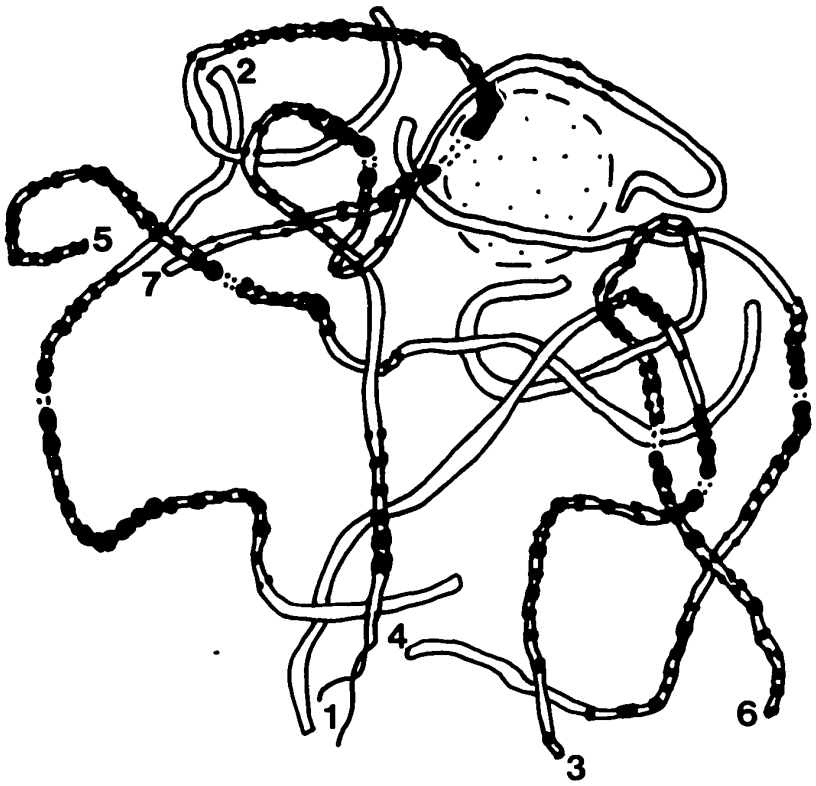
a. Pachytene cell of *M. praecox*. a! Interpretative drawing of a. Chromosomes are numbered at short arm telomeres. Centromeres are indicated by arrow heads in a and dotted gaps in a! X3500.

a



10μ

a´



The total complement length was 212.9 microns, which is considerably shorter than that found in most of the perennial species. Considering the apparent low degree of contraction evident here (as indicated by the low chromaticity of the long arms of chromosomes) this difference in chromosome length is probably a real one. This is also borne out by the gradient in chromosome lengths, it being 41.6 microns to 22.3 microns for *M. constricta*, compared with 39.6 microns to 29.8 microns for *M. sativa* No. 505.

The fact that Lesins and Lesins (1963a) had found the satellited chromosome was the longest at somatic metaphase, while in the pachytene idiogram this chromosome is the shortest, is probably due to the high proportion of this chromosome which is chromatic. All of the other chromosomes are relatively achromatic and hence subject to a higher degree of contraction. A bivalent which still has one arm much less contracted than the other is shown at diakinesis, in Fig. 40c. It is possible that this is chromosome 1 as it appears to have both arms relatively acrhomatic, although chromomeres are visible on the longer arms, and these may be the shorter arms of the pachytene stage chromosome 1.

3. *M. praecox* D.C.

Only one accession of *M. praecox* was studied. It proved relatively amenable to pachytene study so that of only thirteen cells analysed seven had all seven chromosomes measurable. Fig. 42 shows such a cell. The mean lengths and arm ratios were calculated (Table 18) and an idiogram constructed (Fig. 43).

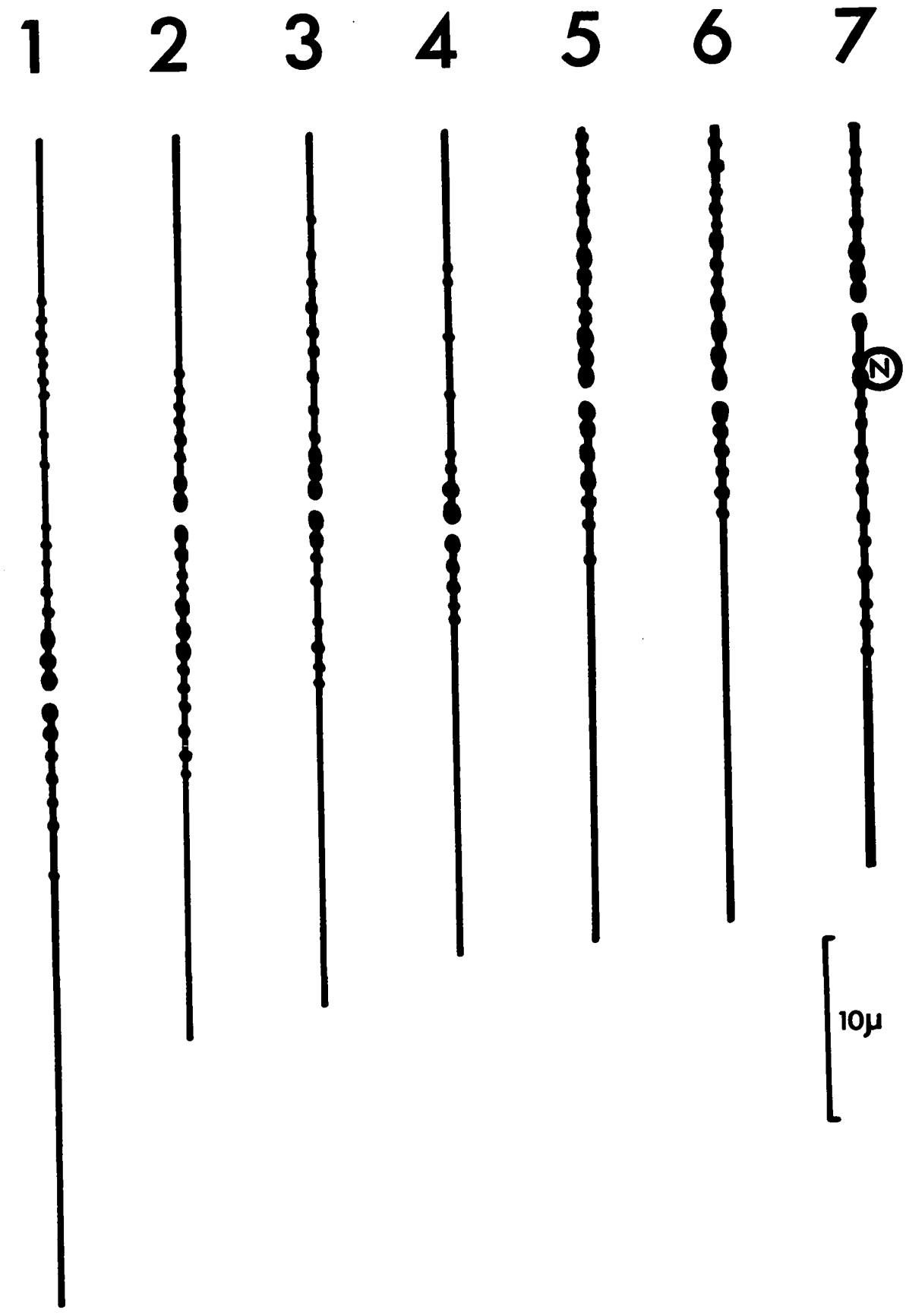
The haploid karyotype consists of four metacentric chromosomes

TABLE 18. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF *M. PRAECOX*

Chromosome No.	Mean Length $\pm$ S_E (in μ)	Mean Arm Ratio $\pm$ S_E	Number Measured
1	63.7 $\pm$ 2.00	1.10 $\pm$ 0.080	9
2	49.1 $\pm$ 0.71	1.37 $\pm$ 0.180	7
3	47.4 $\pm$ 1.76	1.35 $\pm$ 0.108	9
4	44.9 $\pm$ 1.06	1.07 $\pm$ 0.087	9
5	44.2 $\pm$ 1.82	2.08 $\pm$ 0.119	10
6	43.3 $\pm$ 0.80	1.99 $\pm$ 0.326	11
7	40.3 $\pm$ 2.61	3.09 $\pm$ 0.275	7
Sum of Mean Lengths	332.9	-	-
Satellite (Chrom.7)	27.5 $\pm$ 2.37	-	7
Haploid Complement	329.0 $\pm$ 9.10	-	7

Figure 43

Idiogram of pachytene chromosomes of *M. praecox*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3000.


1
2
3
4
5
6
7
N
10μ

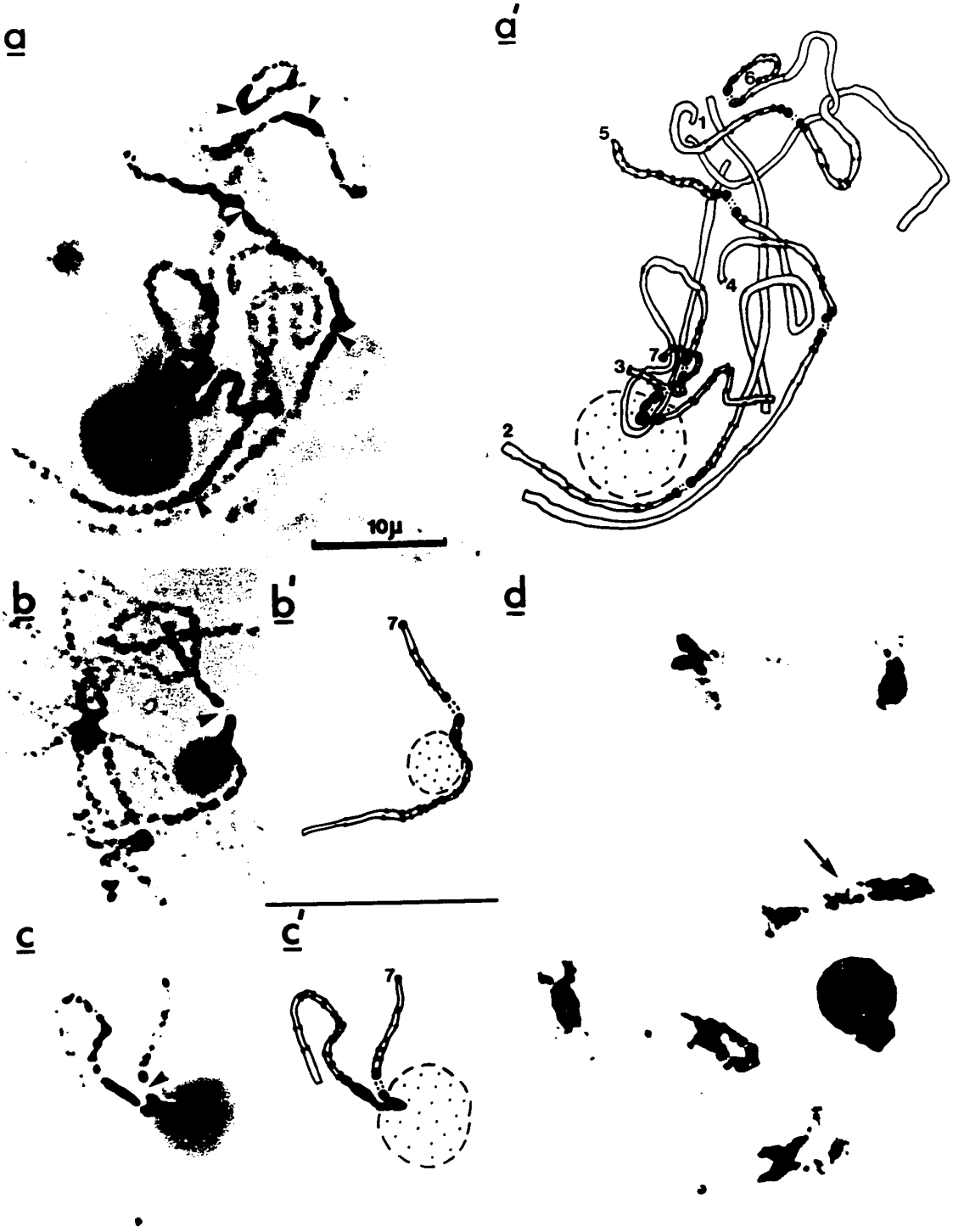
and three submetacentrics, one of which is the nucleolar chromosome. Chromosome 1 is much longer than the next chromosome and has two distinct chromatic areas, one the normal centromeric mass, and the second a group of chromomeres in the middle of the shorter arm. Chromosome 2 has a median centromere, and the proximal half of its longer arm is chromatic. Chromosomes 3 and 4 are also metacentrics but have the distal two-thirds of their long arms achromatic. Chromosome 3 has chromatic knobs scattered along the proximal two-thirds of its shorter arm, while chromosome 4 has a few large chromomeres in the same region.

Chromosomes 5 and 6 are submetacentrics with quite chromatic short arms. Chromosome 7 has a short arm having small knobs in its distal half. The N.O. is located adjacent to the centromere in the long arm. The proximal half to one third of the long arm is chromatic.

The chromosomes appear to be relatively more chromatic than those of *M. constricta*, and the complement length is 120 microns greater than in *M. constricta*. Comparison of the two idiograms reveals that structurally they have little in common. The nucleolar chromosomes have quite different arm ratios and the N.O. is on the long arm of one and on the short arm of the other. Four of the non-nucleolar chromosomes of *M. constricta* are asymmetrical submetacentrics while only two chromosomes in *M. praecox* are of this type. Chromosomes 3 and 4 of *M. praecox* have some similarities in arm ratios and chromomere patterns with chromosomes 1 and 5 of *M. constricta*, but there are no chromosomes in *M. constricta* comparable to chromosomes 1 and 2 of *M. praecox*. As the two species have been classified into different subsections of the section *Spirocarpos* this difference is to be expected. The unusual chromatic pattern and length of chromosome 1 of *M. praecox*

Figure 44

Chromosomes of *M. rigidula*. a. Pachytene cell of Acc. No. 479. b and c. Nucleolar chromosomes of Acc. Nos. 479 and 1324 respectively. a', b, and c! Interpretative drawings of a, b and c with chromosomes numbered at short arm telomeres. Centromere positions are indicated by arrow heads in a, b and c, and by dotted gaps in a', b' and c'. d. diakinesis cell. Arrow indicates an extremely long bivalent. All X2200.


a
a'
6
1
5
4
7
3
2
10μ
b
b'
7
c
c'
7
d

suggest that it may have been formed by a fusion of parts of two chromosomes in the derivation of the species from an n = 8 ancestor. This will be discussed further later.

Simon and Simon (1965) published idiograms of two accessions of *M. praecox*, one of which (N. 3195) was the accession used in the present study. A comparison of the Simons' somatic idiogram with the pachytene idiogram reveals no identifiable common features. The satellited chromosome was third longest in the Simons' idiogram and it is shortest in the pachytene idiogram. None of the somatic chromosomes had arm ratios which approach those of the two submetacentric non-satellited chromosomes at pachytene.

4. *M. rigidula* Desr.

Two accessions of this species were used, Nos. 479 and 1324, the former being the same accession as that used by Simon and Simon (1965), N.3196. Some difficulty was experienced with No. 1324 and only a few cells of it were measurable. As the chromosomes appeared to be similar to those of No. 479 the data from the two accessions were pooled. Out of a total of 60 cells analysed only seven had all seven chromosomes measurable. Fig. 44a shows a cell from No. 479 and Figs. 44b and c show the nucleolar chromosomes from the two accessions. As in *M. praecox*, chromosome 1 was much longer than the second chromosome, the length difference appearing to persist into diakinesis (Fig. 44d).

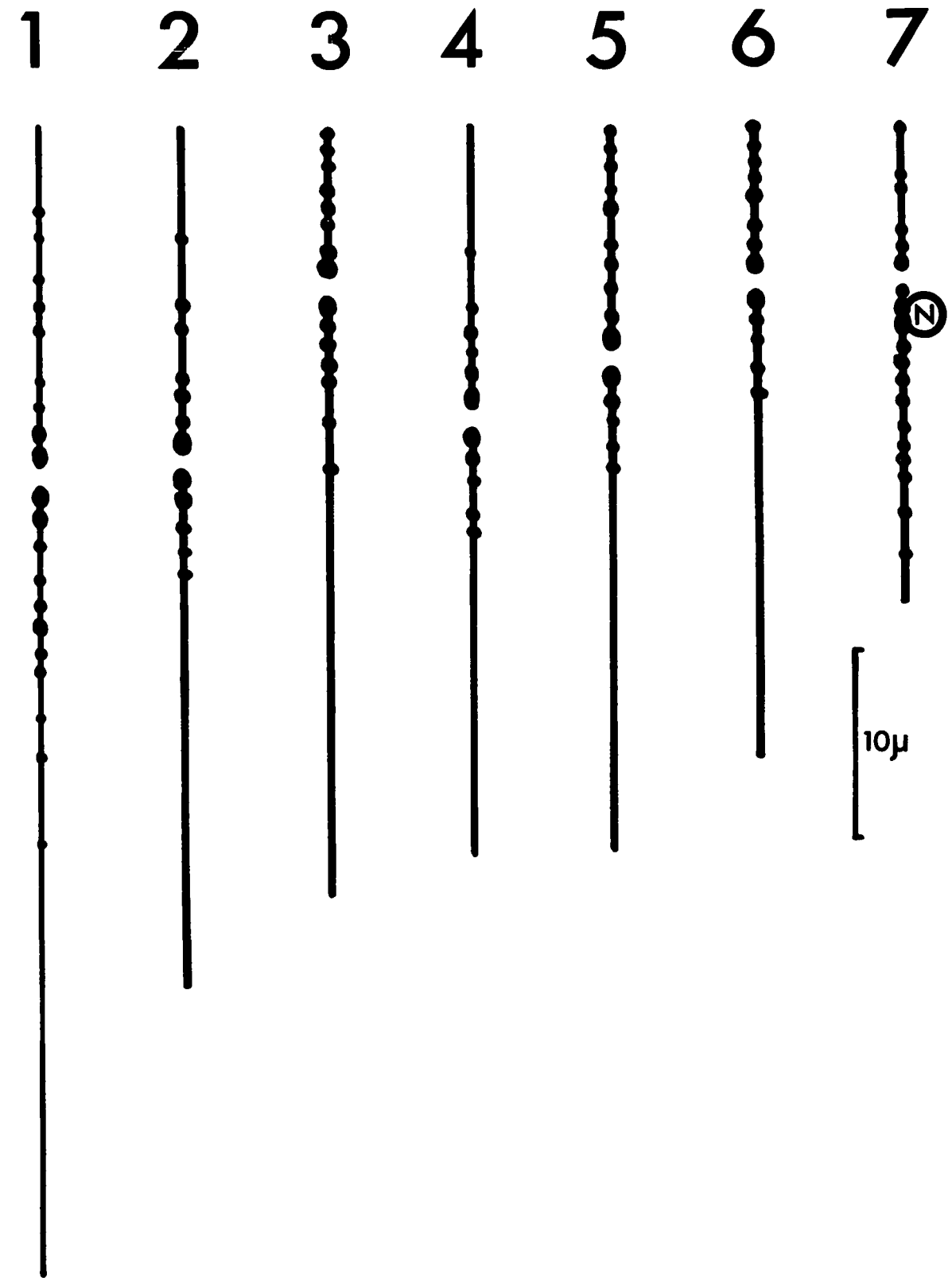
The mean lengths and arm ratios of the chromosomes are given in Table 19 and the idiogram constructed is shown in Fig. 45. The sum of the haploid chromosome lengths was 277.5 microns, which is intermediate between those of the two annual species previously discussed. As

TABLE 19. MEAN LENGTHS AND ARM RATIOS OF CHROMOSOMES OF *M. RIGIDULA*

Chromosome No.	Mean Length $\pm$ S_E (in μ)	Mean Arm Ratio $\pm$ S_E	Number Measured
1	58.9 $\pm$ 1.93	2.30 $\pm$ 0.210	22
2	44.1 $\pm$ 1.20	1.58 $\pm$ 0.067	26
3	40.3 $\pm$ 1.04	4.10 $\pm$ 0.192	25
4	38.1 $\pm$ 1.17	1.58 $\pm$ 0.069	25
5	38.0 $\pm$ 1.50	2.24 $\pm$ 0.091	17
6	33.1 $\pm$ 1.03	3.27 $\pm$ 0.186	22
7	25.0 $\pm$ 0.86	2.27 $\pm$ 0.087	26
Sum of Mean Lengths	277.5	-	-
Satellite (Chrom.7)	15.3 $\pm$ 0.61	-	26
Haploid Complement	293.2 $\pm$ 14.98	-	7

Figure 45

Idiogram of pachytene chromosomes of *M. rigidula*. Short arms are uppermost and gaps indicate centromere positions. N is the position of the nucleolus organizer. X3000.


1
2
3
4
5
6
7
N
10μ

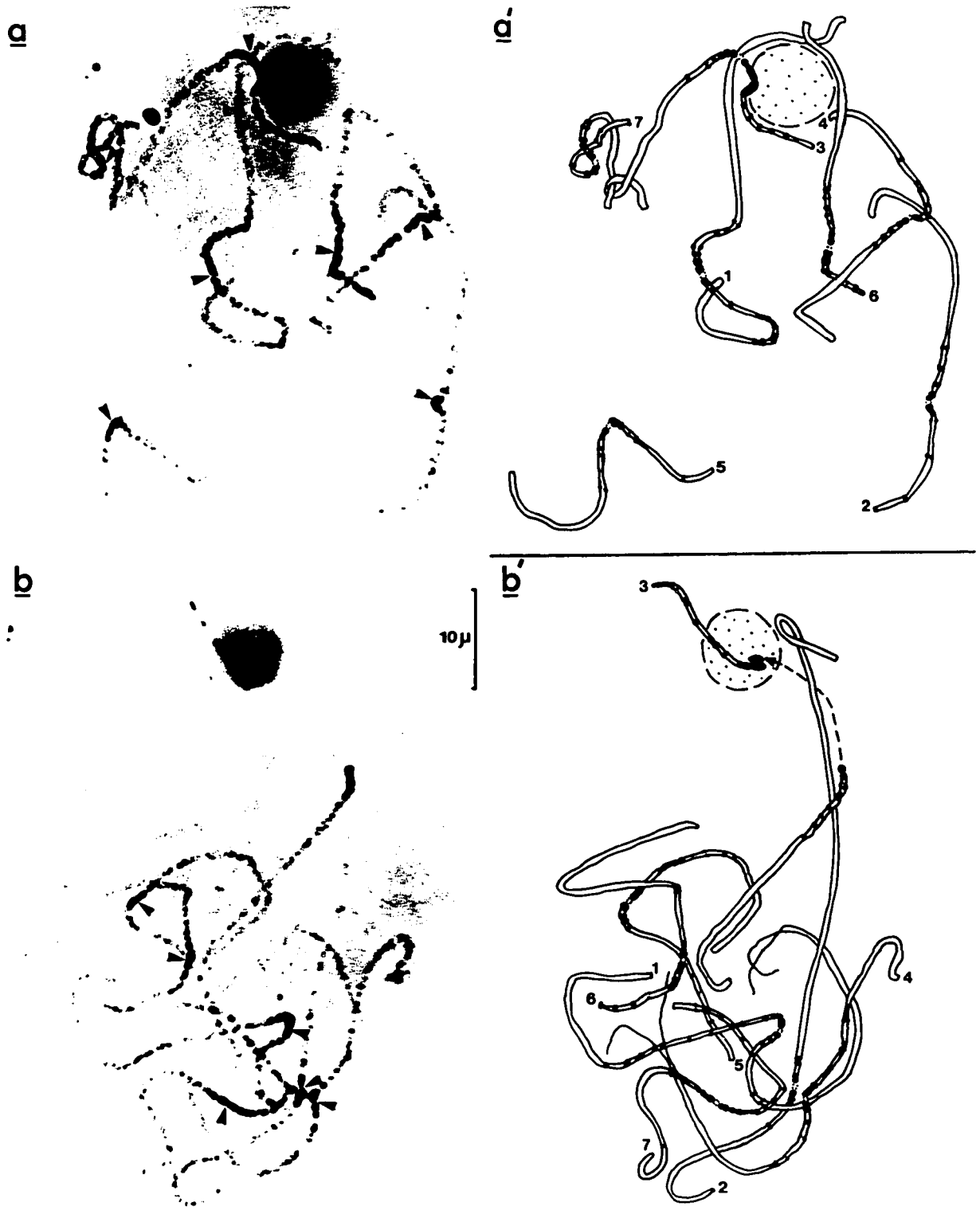
mentioned, chromosome 1 is about 15 microns longer than chromosome 2 and over twice the length of chromosome 7. Two of the chromosomes (2 and 4) have median centromeres and symmetrical chromatin distribution with achromatic distal portions in both arms. The arm ratio of chromosome 1 is 2.30, but chromatic areas are symmetrically distributed on both sides of the centromere.

Chromosomes 3, 5 and 6 have asymmetrical chromatin distribution due to their largely chromatic short arms. Chromosomes 3 and 6 have arm ratios which would classify them as subacrocentrics. All three are achromatic in the distal three-quarters of their long arms. Chromosome 7 is considerably shorter than chromosome 6 and is chromatic for a large proportion of its length. The N.O. is located in the long arm next to the centromere. The short arm has a distinct terminal knob.

Comparison of this idiogram with the *M. rigidula* somatic idiogram of Simon and Simon (1965) reveals little similarity, the positions of the nucleolar chromosomes again being different in the two idiograms. A comparison of the pachytene idiograms of *M. constricta* and *M. rigidula* shows that the *M. rigidula* idiogram has greater similarity with the *M. constricta* idiogram than has the *M. praecox* idiogram. This substantiates the classification of *M. rigidula* and *M. constricta* into the same subsection on the basis of morphological similarities (Heyn, 1963). Apart from total chromosome length differences, the two differ mainly in the arm ratio of the nucleolar chromosome and the position of the N.O., as well as in the presence of one more submetacentric in *M. constricta* than in *M. rigidula*. These differences could be caused by no more than an inversion and a translocation. The nucleolar chromosomes

Figure 46

Pachytene cells of *M. polymorpha*. a. Accession No. 427. b. Accession No. 401. a' and b' Interpretative drawings of a and b. Chromosomes are numbered at short arm telomeres. Centromeres are indicated by arrowheads in a and b and by dotted gaps in a' and b'. Chromosome 3 in b has a break at the centromere indicated by a broken line and arrow. X1600.


a
a'
7
4
3
1
6
5
2
b
10μ
b'
3
1
6
4
5
7
2

are very similar in length.

5. *M. polymorpha* L.

Four accessions of this species were used, two (Nos. 427 and N. 3176) being the same as used by Simon and Simon (1965) (their numbers N. 3199 and N. 3176). Sufficient cells of each accession were studied for an idiogram of each to be constructed. On statistical analysis of the four sets of mean chromosome lengths and arm ratios it was found that one accession, No. 401, had chromosomes considerably longer than those of the other three accessions. These three were found to be not significantly different in haploid complement length, with only two individual chromosome lengths being significantly different. Chromosome arm ratios of these three accessions were not significantly different except for the nucleolar chromosome in which there was significant difference at the 5% level but not at the 1% level.

In view of the great similarity between the chromosomes of Accession Nos. 409, 427 and N. 3176, it was decided to pool the data from these three. A representative cell of No. 427 is shown in Fig.46a, and a cell from No. 401 is shown in Fig. 46b. The pooled data from the three accessions and that from No. 401 are given in Table 20. The great differences in chromosome length between the two is obvious . In t tests all lengths except those of the nucleolar chromosomes were highly significantly different, the difference in chromosome3 being significant only at the 5% level. The chromosome arm ratios, however, were not significantly different except for the nucleolar chromosome in which the arm ratio was much lower in No. 401 than in the others, being significantly different at the 1% level. Figs. 47a and b show the difference

TABLE 20. MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF CHROMOSOMES OF *M. POLYMORPHA*

Chromosome No.	Item+	Accession No.s 409, 427 & N.3176 (pooled)	Accession No. 401	t Test - 401 vs. pooled data ‡	Mean of all 4 Accessions
1	L	55.7 ± 0.83	75.5 ± 2.61	**	58.7
	AR	1.20 ± 0.024	1.13 ± 0.048	n.s.	1.19
	PL	0.2000	0.2027	-	0.2016
	N	71	13		84
2	L	42.8 ± 0.71	59.7 ± 1.72	**	45.4
	AR	1.50 ± 0.045	1.44 ± 0.110	n.s.	1.49
	PL	0.1536	0.1601	-	0.1556
	N	67	12		79
3	L	42.3 ± 1.12	50.6 ± 3.52	*	43.3
	AR	2.34 ± 0.087	1.69 ± 0.163	**	2.26 §
	PL	0.1518	0.1357	-	0.1485
	SAT.L.	10.1 ± 0.22	16.0 ± 1.01	**	10.8 §
	N	50	7		57
4	L	38.0 ± 0.52	51.9 ± 1.28	**	39.8
	AR	1.44 ± 0.037	1.42 ± 0.107	n.s.	1.44
	PL	0.1366	0.1393	-	0.1365
	N	75	11		86
5	L	34.6 ± 0.12	46.9 ± 1.45	**	36.3
	AR	1.35 ± 0.032	1.39 ± 0.100	n.s.	1.36
	PL	0.1244	0.1258	-	0.1246
	N	89	14		103
6	L	33.9 ± 0.12	45.0 ± 0.98	**	35.3
	AR	3.18 ± 0.087	3.21 ± 0.178	n.s.	3.18
	PL	0.1217	0.1208	-	0.1211
	N	68	10		78
7	L	31.1 ± 0.11	43.1 ± 1.37	**	32.7
	AR	1.26 ± 0.023	1.11 ± 0.036	n.s.	1.24
	PL	0.1118	0.1156	-	0.1121
	N	75	11		86
Haploid Compl.	L	275.9 ± 5.20	379.6 ± 11.51	**	288.2
	N	37	5		42
Sum of Mean Lengths		278.4	372.7	-	291.4

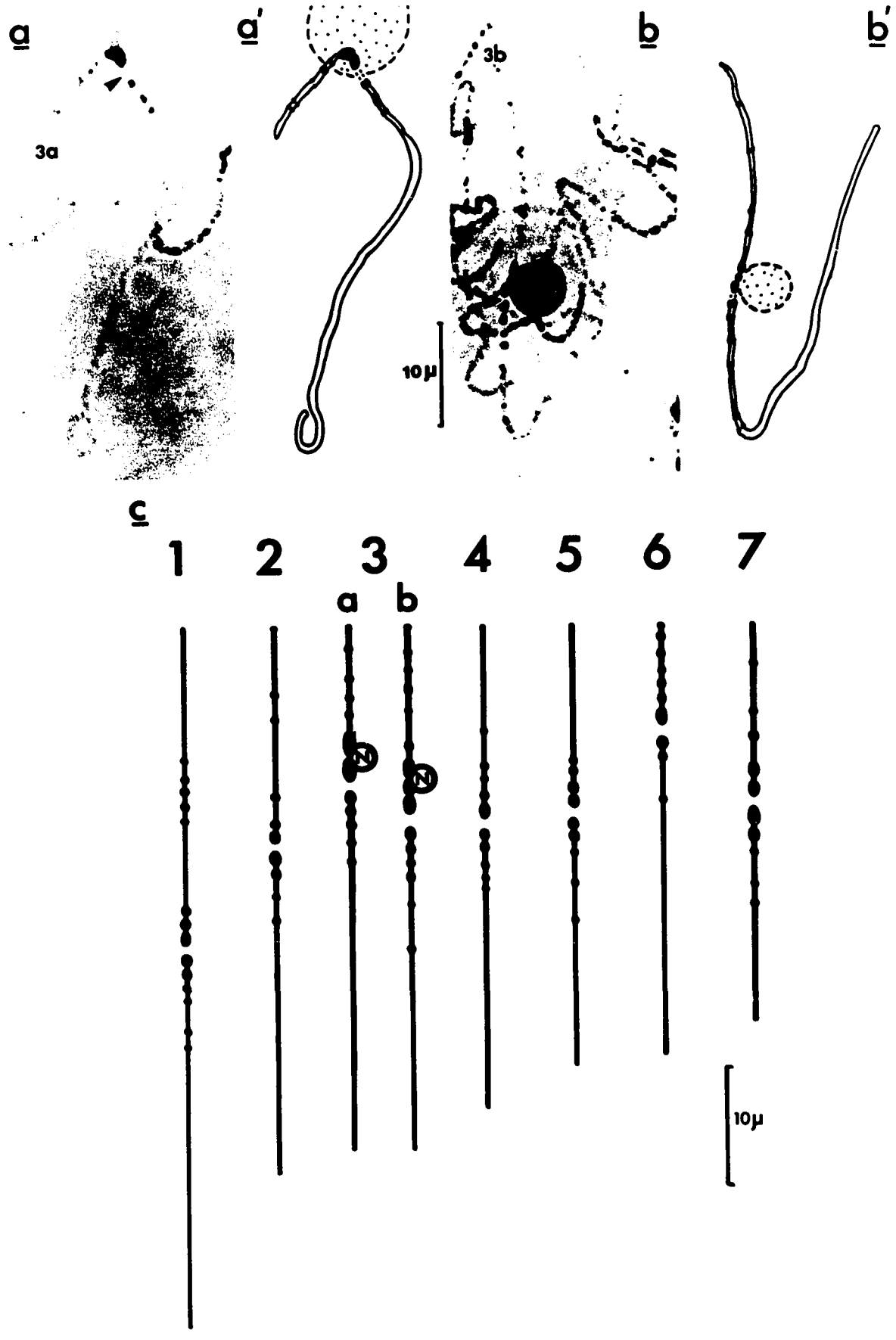
+ L - length ± S_E (in μ); AR - arm ratio ± S_E; PL - proportional length; SAT. L. - length of satellite ± S_E (in μ); N - number measured.

‡ * - significantly different at 5% level; ** - significantly different at 1% level; n.s. - not significantly different.

§ these values not used in idiograms.

Figure 47

a and b. Pachytene nucleolar chromosomes of Acc. Nos. 427 and 401 respectively. a' and b' Interpretative drawings of a and b. Chromosomes are numbered at short arm telomeres and centromeres are indicated by arrow heads in a and b and dotted gaps in a' and b'. X1750. c. Idiogram of pachytene chromosomes of *M. polymorpha*. Short arms are uppermost and gaps indicate centromere positions. N indicates the position of the nucleolus organizer. Two versions of chromosome 3 are shown. 3a is based on the arm ratio and relative satellite length of the pooled data from Acc. Nos. 409, 427 and N. 3176. 3b is based on the arm ratio and relative satellite length of Acc. No. 401. X2000.


a
a'
b
b'
3a
3b
10μ
c
1
2
3
4
5
6
7
a
b
10μ

in the arm ratios of chromosome 3 from No. 427 and No. 401 respectively.

A comparison of the proportional lengths of chromosomes in the two groups in Table 20 reveals that all are fairly similar, the one exception again being chromosome 3. It was therefore decided that differences in length of the six non-nucleolar chromosomes were probably the result of contraction differences and hence the results of all four accessions were pooled and mean values calculated. These also are given in Table 20. The pooled results for chromosome 3 are included. In constructing the idiogram in Fig. 47c. these mean lengths and arm ratios were used for the non-nucleolar chromosomes. For chromosome 3 the mean length (43.7 microns) was used, but two figures were constructed, one using the mean arm ratio and proportional satellite length of accessions No. 409, 427 and N. 3176, and the other using the mean arm ratio and proportional satellite length of accession No. 401 (chromosomes 3a and 3b respectively of Fig. 47c).

In the above results it was assumed that, with the exception of chromosome 3, the four accessions have identical karyotypes, and that chromosome length differences in the non-nucleolar chromosomes are probably the result of contraction. However, this does not adequately explain the differences between the nucleolar chromosomes. The six non-nucleolar chromosomes of No. 401 are 1.36 times the length of the six non-nucleolar chromosomes of the other three accessions. If chromosome 3 of No. 401 were also this multiple of the chromosome 3 in the other three accessions, it would be 57.5 microns long, not 50.6 microns. On comparison of the proportional lengths of the long arms and short arms of the chromosome 3 in the two groups, it was found that

the short arms had similar proportional lengths (both approximately 0.050) whereas the long arms differed markedly (0.085 in No. 401 compared with 0.105 in the other three). This leads one to conclude that the reason for the differences in proportional lengths and arm ratios of chromosome 3 in the two groups is a loss of portion of the long arm of chromosome 3 in No. 401. If the length of this chromosome is increased to 1.36 times that of chromosome 3 in the other accessions by adding to its long arm only, its arm ratio then becomes 2.06, much closer to that of the other three accessions.

This decrease in the length of the long arm of chromosome 3 of No. 401 could have been the result of an unequal translocation with one of the other chromosomes of the complement. However none of the other six chromosomes of the No. 401 idiogram is sufficiently longer than its counterpart in the other three accessions to have accounted for the whole of the lost portion of chromosome 3. The decrease could also have been a result of several smaller translocations involving several different chromosomes, each resulting in a progressive reduction in the length of chromosome 3. The alternative to translocation is a deletion of portion of the long arm of chromosome 3, an occurrence more likely to be lethal. The exact nature of the difference between No. 401 and the other accessions can be determined only by crossing them and observing the chromosome pairing configurations at pachytene and metaphase I in meiosis of the hybrids.

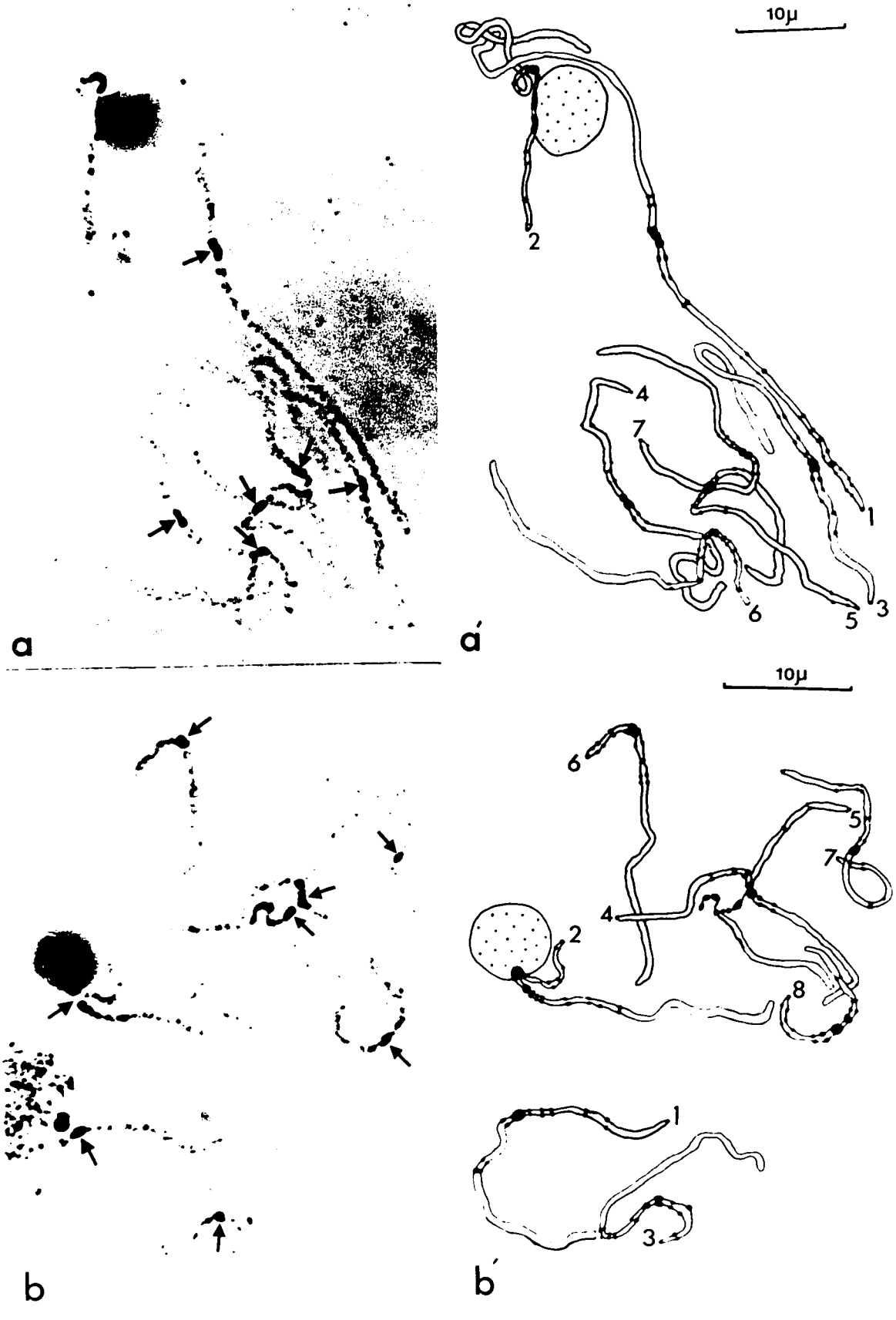
Simon and Simon (1965) had suggested that karyotype differences they had observed between accessions of *M. polymorpha* were probably a form of subspeciation. They had observed differences between two

accessions, N. 3199 (No. 427 of this study) and N. 3176, in chromosome length and ratio of longest to shortest chromosome. In the present study these two accessions were extremely similar, having complement lengths of 274.9 microns and 270.4 microns respectively, and little difference in their ratios of longest to shortest chromosome. In these two accessions Simon and Simon found the satellited chromosomes to be the longest in the idiogram, but in several other accessions that they studied the longest chromosome was a metacentric which was considerably longer than the second chromosome in the idiogram. Clement (1962) also noted two exceptionally large chromosomes in the somatic complement of *M. polymorpha*. The idiogram that the Simons presented for their accession N. 3293 is very similar to the pachytene one, having the satellited chromosome third in length.

M. polymorpha is in the subsection *Leptospirae* with *M. praecox*. On comparison of the *M. polymorpha* idiogram with the three given previously (Figs. 41, 43 and 45) it is obvious that it is much more like that of *M. praecox* than it is like that of either *M. constricta* or *M. rigidula*. Both of the latter two have short nucleolar chromosomes and four and three submetacentrics respectively. The chromosome 1 of *M. polymorpha* is very similar to chromosome 1 of *M praecox*, but quite unlike chromosome 1 of either of the other two species mentioned. *M. praecox* has one more submetacentric asymetrical chromosome than *M. polymorpha*, and also has its N.O. on the long arm of the nucleolar chromosome, whereas the N.O. is on the short arm of chromosome 3 in *M. polymorpha*. Both of these differences could be the result of structural rearrangements during the divergence of the two species from

Figure 48

Pachytene cells of *M. murex*. a. 2n = 14 Acc. No. 2143. b. 2n = 16 Acc. No. 1075. a' and b' Interpretative drawings of a and b. Chromosomes are numbered at short arm telomeres and centromeres are indicated by arrows. There is a break in one of the chromosomes of the chromosome 5 bivalent near its centromere in b. a and a' X 1800. b and b' x 2100.


10µ
2
4
7
1
6
5
3
a
á
10µ
6
5
7
4
2
8
1
3
b
b́

a common 2n = 14 ancestor which originated from a 2n = 16 species. The differences between these two species and *M. constricta* and *M. rigidula* are more pronounced and may be the result of their being derived from different 2n = 16 ancestors.

6. *M. murex* Willd.

Among the fairly comprehensive collection of annual medics of omni - Mediterranean origin, at the University of Alberta, there are 54 accessions identified as *M. murex* Willd. (according to the descriptions of Urban (1873) and Heyn (1963)). When Lesins and Lesins (personal communication) found that only four of these accessions were 2n = 16 and that the rest were 2n = 14, it was decided to investigate the pachytene karyotypes of both types to see if their relationship could be determined. The four 2n = 16 accessions included one (No. 1982) which had smooth pods. Five 2n = 14 accessions were selected, all having spiny pods.

a. 2n = 14 accessions

With the exception of Accession No. 1980, adequate numbers of cells of each accession were produced for their karyotypes to be determined. A typical cell of one of the accessions is shown in Fig. 48a. The mean chromosome lengths, arm ratios and complement lengths of the five accessions are given in Table 21. All accessions had fairly similar values, and in F tests no significant differences were found in arm ratios or in haploid complement lengths. Chromosomes 1 and 2 among the five accessions were not significantly different in length, but the other five chromosomes had significant length differences. When

TABLE 21. MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF CHROMOSOMES OF 2n = 14 ACCESSIONS OF *M. MUREX*

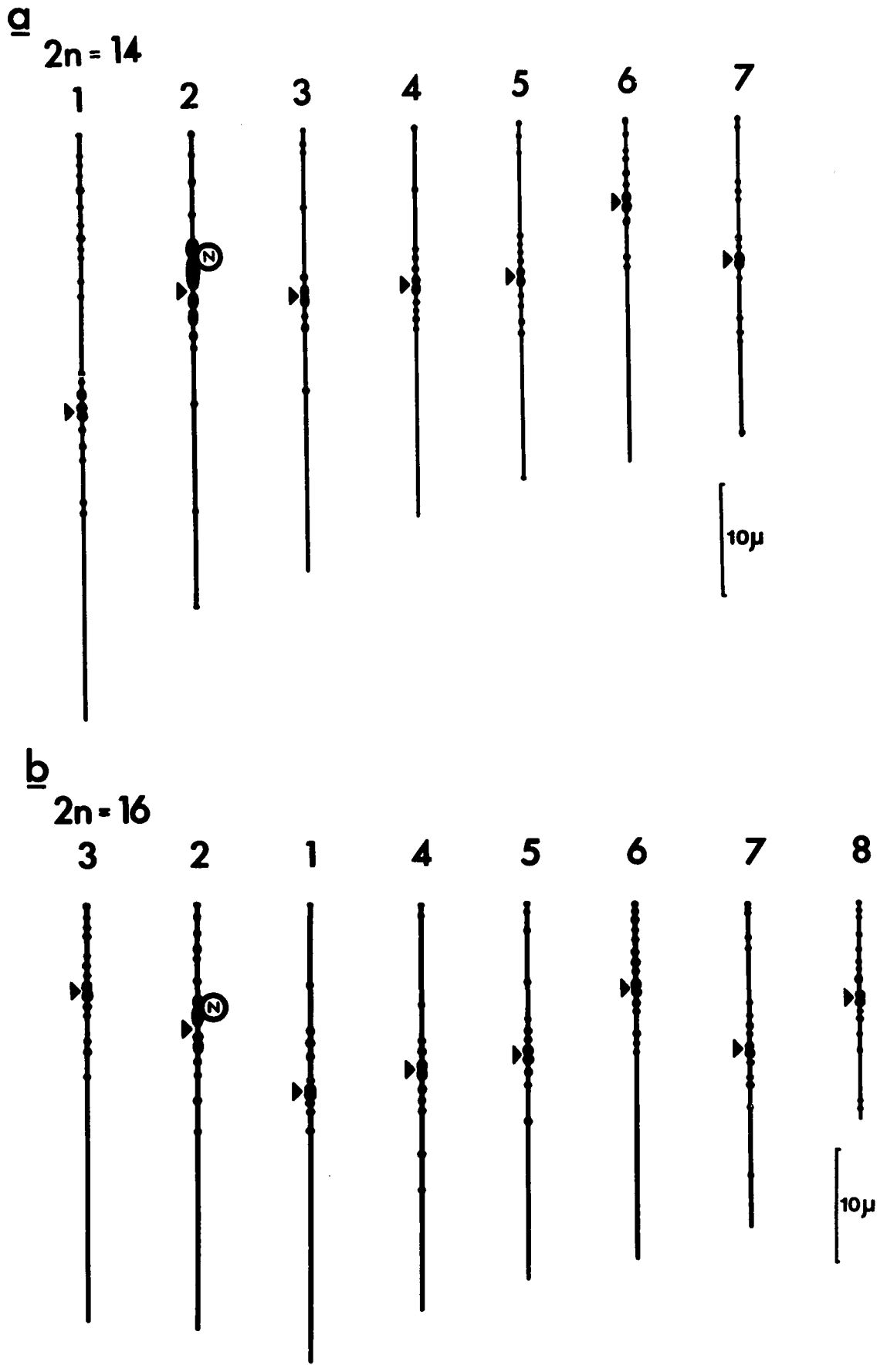
Chromosome		Mean Values of Accessions					F test‡	Mean of all 5
No.	Item+	No. 1521	No. 1980	No. 1981	No. 2065	No. 2143	Signif.	Accessions
	L	52.7	51.8	47.4	58.4	54.8	n.s.	52.9 ± 1.31
	AR	1.12	1.10	1.10	0.99	1.05	n.s.	1.08 ± 0.02
	PL	0.199	0.214	0.205	0.195	0.200	-	0.201
	N	21	4	13	10	13		61
	L	42.6	32.3	39.3	44.8	48.7	n.s.	42.9 ± 1.27
	AR	1.87	1.87	2.23	2.22	1.99	n.s.	1.98 ± 0.08
	PL	0.161	0.133	0.170	0.149	0.178	-	0.163
	SAT.L.	11.3	9.0	10.3	11.2	12.3	n.s.	11.2 ± 0.52
	N	20	2	4	6	5		37
	L	40.1	39.9	34.7	44.6	40.5	*	40.0 ± 0.59
	AR	1.63	1.75	1.72	1.36	1.83	n.s.	1.67 ± 0.06
	PL	0.152	0.165	0.150	0.149	0.148	-	0.152
	N	22	4	10	8	16		60
	L	35.4	34.8	30.3	42.0	35.3	**	35.3 ± 0.74
	AR	1.48	1.39	1.34	1.74	1.47	n.s.	1.48 ± 0.05
	PL	0.134	0.144	0.131	0.140	0.129	-	0.134
	N	22	3	12	9	17		63
	L	32.6	30.2	28.1	38.5	33.2	**	32.2 ± 0.63
	AR	1.32	1.26	1.42	1.15	1.37	n.s.	1.33 ± 0.04
	PL	0.123	0.125	0.121	0.128	0.121	-	0.122
	N	22	5	16	8	20		71
	L	32.0	27.1	26.6	36.9	31.6	**	31.1 ± 0.74
	AR	3.19	3.75	2.86	2.93	3.18	n.s.	3.12 ± 0.09
	PL	0.121	0.112	0.115	0.123	0.115	-	0.118
	N	22	4	14	9	18		67
	L	29.0	26.0	24.9	34.7	29.9	**	28.8 ± 0.63
	AR	1.16	1.27	1.15	1.25	1.21	n.s.	1.18 ± 0.02
	PL	0.110	0.107	0.108	0.116	0.109	-	0.109
	N	22	2	14	6	17		61
Haploid Compl.	L	268.8	233.5	246.0	313.9	304.3	n.s.	275.3 ± 8.1
	N	17	1	3	4	3	-	28
Sum of Mean Lengths		264.4	242.1	231.3	299.9	274.0	-	263.2

+ L - length (± S_E where applicable) in μ; AR - arm ratio (± S_E where applicable); PL - proportional length; SAT.L. - length of satellite (± S_E where applicable) in μ; N - number measured.

‡ * - significant at 5% level; ** - significant at 1% level; n.s. - not significant.

Figure 49

Idiograms of pachytene chromosomes of *M. murex*. a. 2n = 14 accessions. b. 2n = 16 accessions. The chromosomes in b are arranged so that they are below similar or related 2n = 14 chromosomes in a (see Table 23). Chromosomes are numbered according to length at their short arm telomeres. Centromeres are indicated by arrow heads. N indicates the position of the nucleolus organizer. Both X1850.


a
2n = 14
1
2
3
4
5
6
7
10μ
b
2n = 16
3
2
1
4
5
6
7
8
10μ

the proportional lengths of chromosomes in different accessions were compared (Table 21) it was found that all agreed well, except for some variations in the chromosomes of No. 1980, for which only a limited number of cells were studied. Because of these similarities in arm ratios and proportional lengths of chromosomes, it was felt that the data from all five accessions could be pooled to produce the mean values given in Table 21. These are based on a total of 82 cells, including 28 complete cells.

From these mean values an idiogram was constructed (Fig. 49a) showing the arm ratios, lengths and chromomere distribution in the chromosomes. The karyotype consists of two submetacentric chromosomes, one of which is the N.O. carrying chromosome, and five metacentric chromosomes. Chromosome 1 is ten microns longer than the second chromosome and has a median centromere. Its shorter arm is characterized by a region approximately 10 microns long (separated by a similar distance from the centromeric chromatic region) which contains a group of chromatic knobs. Chromosomes 3, 4 and 5 have similar arm ratios, with symmetrical chromatin distribution about their centromeres and achromatic distal regions on both arms. Chromosome 5 has small telomeric knobs terminating both arms.

Chromosome 7 has a median centromere and symmetrical chromatin distribution. Chromosome 6 is the only non nucleolar submetacentric, and its short arm is more chromatic than those of the metacentrics. Chromosome 2, the nucleolar chromosome, has the N.O. surrounded by large chromatic blocks on the short arm adjacent to the centromere. The short arm distal to the N.O. has a number of spaced chromomeres. The long arm

has several large centromeric chromatin knobs but the distal portion is achromatic.

On comparison of the 2n = 14 *M. murex* idiogram with those of the other 2n = 14 species it is immediately obvious that it is very similar to the idiogram of *M. polymorpha* (Fig. 47c). The total lengths of their seven chromosomes differ by almost 30 microns, *M. polymorpha* having the longer chromosomes. The arm ratios and proportional lengths of the individual chromosomes in the two species are quite similar, with the exception of the proportional length of the nucleolus chromosome, which is third in length in *M. polymorpha*. Both idiograms have similar chromosome 1's with chromatic areas in the distal half of the shorter arm. The nucleolar chromosomes, although differing in proportional length and arm ratio, have similar chromatic patterns and N.O. positions.

Although *M. murex* has been classified in the subsection *Pachyspirae* with *M. constricta* and *M. rigidula*, its idiogram has practically nothing in common with the pachytene idiograms of these two species (Figs. 41 and 45). As mentioned above, its idiogram is quite similar to that of *M. polymorpha*, which has been classified in the subsection *Leptospirae*. The similarities of the karyotypes of *M. murex* and *M. polymorpha* could be the result of a common 2n = 14 ancestry, or of parallel evolution from the same or different 2n = 16 ancestors.

b. 2n = 16 accessions

Chromosomes were measured from 63 incomplete cells and 24 complete cells from the four accessions. The chromosomes of a cell of accession No. 1075 are shown in Fig. 48b. The mean lengths and arm ratios of the chromosomes from each accession are given in Table 22.

TABLE 22. MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF CHROMOSOMES OF 2n = 16 ACCESSIONS OF *M. MUREX*

Chromosome		Mean Values of Accessions				F test‡	Mean of all 4 Accessions
No.	Item+	No. 353	No. 1075	No. 1982	No. 1983	Signif.	
1	L	49.2	36.9	36.0	43.6	**	41.0 ± 1.09
	AR	1.54	1.63	1.65	1.64	n.s.	1.63 ± 0.05
	PL	0.159	0.154	0.147	0.155	-	0.154
	N	7	14	15	29	-	65
2	L	43.7	33.8	34.1	43.7	**	38.0 ± 1.39
	AR	2.46	2.07	2.50	2.55	n.s.	2.38 ± 0.12
	PL	0.141	0.141	0.140	0.155	-	0.143
	SAT.L.	11.1	8.6	7.8	10.0	**	9.2 ± 0.54
	N	7	7	9	3	-	26
3	L	41.9	33.3	35.1	39.1	*	37.4 ± 0.85
	AR	4.09	3.50	3.98	3.85	n.s.	3.85 ± 0.12
	PL	0.135	0.139	0.144	0.139	-	0.140
	N	7	10	17	29	-	63
4	L	43.9	32.0	33.3	37.8	**	36.4 ± 0.84
	AR	1.35	1.45	1.43	1.55	**	1.49 ± 0.05
	PL	0.141	0.133	0.136	0.134	-	0.137
	N	7	12	16	36	-	71
5	L	40.6	29.8	31.7	34.3	**	33.4 ± 0.78
	AR	1.59	1.35	1.60	1.33	n.s.	1.42 ± 0.06
	PL	0.131	0.125	0.130	0.122	-	0.125
	N	7	14	15	29	-	65
6	L	36.6	29.1	28.7	33.2	**	31.7 ± 0.78
	AR	3.82	3.08	3.44	3.19	n.s.	3.30 ± 0.09
	PL	0.118	0.122	0.118	0.118	-	0.119
	N	7	12	16	30	-	65
7	L	33.7	27.2	26.7	29.7	*	28.9 ± 0.62
	AR	1.37	1.09	1.33	1.23	*	1.23 ± 0.03
	PL	0.109	0.114	0.109	0.105	-	0.109
	N	6	15	13	32	-	66
8	L	20.8	17.2	18.6	20.6	*	19.5 ± 0.44
	AR	1.21	1.35	1.37	1.27	n.s.	1.30 ± 0.03
	PL	0.067	0.072	0.076	0.073	-	0.073
	N	8	14	15	34	-	71
Haploid Compl.	L	298.3	228.4	233.8	259.7	**	251.6 ± 8.9
	N	6	7	8	3	-	24
Sum of Mean Lengths		310.4	239.3	244.2	282.0	-	266.3

+ L - length ($\pm S_E$ where applicable) in μ; AR - arm ratio ($\pm S_E$ where applicable); PL - proportional length; SAT.L. - length of satellite ($\pm S_E$ where applicable) in μ; N - number measured.

‡ * - significant at 5% level; ** - significant at 1% level; n.s. - not significant.

Considerable differences in length exist between equivalent chromosomes in the different accessions, all seven chromosomes classes having significant length differences in F tests. The arm ratios are similar in five of the seven chromosomes, there being significant differences in chromosomes 4 and 7. However, on comparing proportional lengths of chromosomes in the different accessions most were found to be very similar. Hence it was concluded that differences in chromosome length were due mainly to contraction, and so the results from the four accessions could be pooled. The mean values of lengths, arm ratios, proportional lengths, and haploid complement length are given in Table 22.

From these values an idiogram was constructed and is given in Fig. 49b. The order of chromosomes 1, 2 and 3 has been altered to facilitate comparison with similar or equivalent chromosomes in the 2n = 14 idiogram (see below).

In the majority of features the 2n = 16 idiogram is similar to the 2n = 14 idiogram. Chromosomes 1, 4 and 5 have median type centromeres and symmetrical chromomere distribution about their centromeres. Chromosome 2 contains the N.O. on its short arm adjacent to the centromere. The chromatic blocks of the N.O. and the centromere are fused in this arm while the long arm of the chromosome is achromatic in its distal three-quarters. Chromosome 6 is submetacentric with a fairly chromatic short arm, while chromosome 7 has a median centromere and a symmetrical chromomere pattern.

Chromosome 3 is a subacrocentric chromosome with a chromatic short arm, and is unlike any chromosome of similar length in the 2n = 14 idiogram. Chromosome 8 is almost ten microns shorter than chromosome 7,

has a median centromere and is characterized by many chromomeres on both arms. Its resembles no chromosome in the 2n = 14 idiogram.

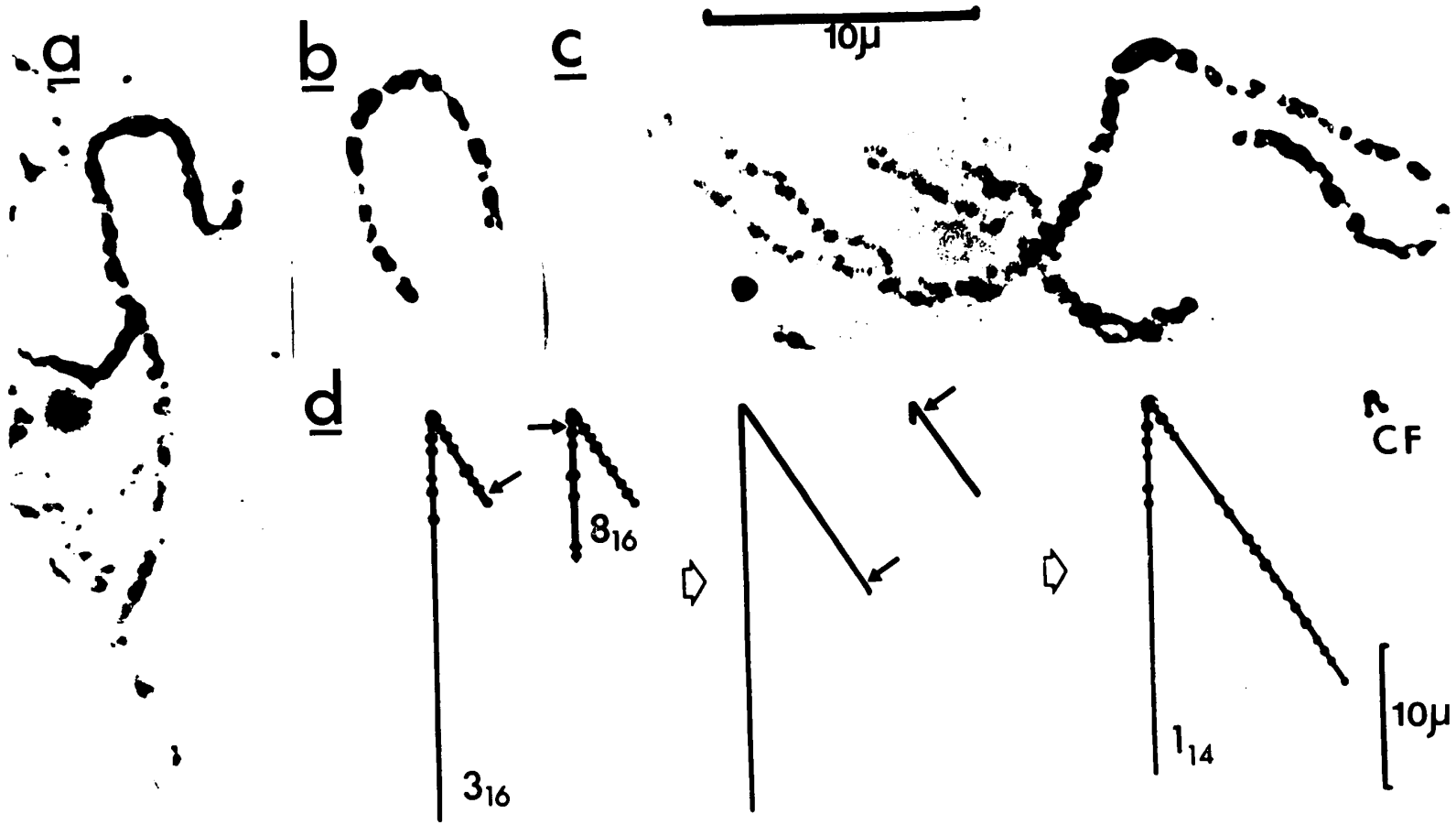
Thus six of the chromosomes in the 2n = 16 idiogram are similar to chromosomes 2 to 7 in the 2n = 14 idiogram, but neither of the remaining two, a subacrocentric and a metacentric, resembles the remaining member of the 2n = 14 idiogram. In having two asymetrical sub-metacentric type chromosomes, the 2n = 16 *M. murex* idiogram resembles the *M. praecox* idiogram. The total lengths of the two *M. murex* idiograms are almost identical. The 2n = 16 idiogram shows some similarities with the somatic idiogram given by Simon and Simon (1965). They indicated that the satellited chromosome was the longest, but their ratio of longest to shortest chromosome was similar to that obtained in this study. It is interesting to note also that Fryer (1930), who gave 2n = 16 for *M. murex*, stated that the somatic chromosomes ranged from 2.2 microns to 1.5 microns but that there was possibly one short pair of length 1 to 1.5 microns.

c. Relationship of 2n = 14 and 2n = 16 *M. murex*, with a reference to other 2n = 14 species

The obvious similarity in morphological characters of the two *M. murex* types (Lesins, Lesins and Gillies, 1970) suggests that any differences in their karyotypes are probably fairly recent evolutionary events involving a minimum of chromosomal rearrangement. This is evident also from the similarities shown in the comparison of the idiograms in Figs. 49a and b. The mean haploid complement lengths of the two karyotypes were not significantly different in a t test. Chromosomes

Figure 50

M. murex pachytene chromosomes. a. Chromosome 3_{16}. b. Chromosome 8_{16}. c. Chromosome 1_{14}. All X 4100. d. Hypothesised derivation of chromosome 1_{14} from chromosomes 3_{16} and 8_{16} by two unequal translocations, (at the small arrows). The centromeric fragment (CF) is subsequently lost. X2100.


a
b
c
10μ
d
8_{16}
3_{16}
CF
1_{14}
10μ

3_{14}*, 4_{14}, 5_{14}, 6_{14} and 7_{14} were not significantly different in length or arm ratio from chromosomes 1_{16}*, 4_{16}, 5_{16}, 6_{16} and 7_{16} respectively, in t tests (Table 23). The similarity of the nucleolar chromosomes of the two types has been noted previously, but they were significantly different in both length and arm ratio. The smaller number of nucleolar chromosomes measured (due to obstruction and breakage at the N.O.) may be partially to blame for this variation.

In examining the relationship of the remaining two 2n = 16 chromosomes, 3_{16} and 8_{16}, to chromosome 1_{14}, consideration was given to the suggestion made by Simon and Simon (1965) that reciprocal translocations might be involved in the derivation of 14 chromosome species from 16 chromosome species. It was noted that the total length of chromosome 1_{14} was almost the same as the sum of the lengths of chromosomes 3_{16} and 8_{16}. In a t test (Table 23) it was found that the difference was significant at the 5% level but not at the 1% level. Furthermore, if the length of chromosome 8_{16} was added to the short arm of chromosome 3_{16} and the arm ratio calculated using this value as the short arm, the mean value obtained was not significantly different from the arm ratio of chromosome 1_{14} (Table 23). These data lead one to conclude that chromosome 1_{14} may have been derived from chromsome 3_{16} by the addition of chromosome 8_{16} to the short arm, in an unequal reciprocal translocation.

*The subscripts 14 and 16 will be used after chromosome numbers to indicate from which complement the particular chromosomes come.

TABLE 23. COMPARISON OF MEAN LENGTHS, ARM RATIOS AND PROPORTIONAL LENGTHS OF CHROMOSOMES OF 2n=14 AND 2n=16 *M. MUREX*

2n = 14			2n = 16			t test Significant Difference§
Chromosome No.	Item+	Mean Value	Mean Value	Item+	Chromosome No.	
1	L	52.9	56.9	L	3+8‡	*
	AR	1.08	1.09	AR		n.s.
	PL	0.201	0.213	PL		-
2	L	42.9	38.0	L	2	*
	AR	1.98	2.38	AR		**
	PL	0.163	0.143	PL		-
3	L	40.0	41.0	L	1	n.s.
	AR	1.67	1.63	AR		n.s.
	PL	0.152	0.154	PL		-
4	L	35.3	36.4	L	4	n.s.
	AR	1.48	1.49	AR		n.s.
	PL	0.134	0.137	PL		-
5	L	32.3	33.4	L	5	n.s.
	AR	1.33	1.42	AR		n.s.
	PL	0.122	0.125	PL		-
6	L	31.1	31.7	L	6	n.s.
	AR	3.12	3.30	AR		n.s.
	PL	0.118	0.119	PL		-
7	L	28.8	28.9	L	7	n.s.
	AR	1.18	1.23	AR		n.s.
	PL	0.109	0.109	PL		-
Haploid Compl.	L	275.3	251.6	L	Haploid Compl.	n.s.

\+ L - length in μ; AR - arm ratio; PL - proportional length.

‡ total length of chromosome 8 added to short arm of chromosome 3.

§ * - significantly different at 5% level; ** - significantly different at 1% level; n.s. - not significantly different.

This raises the problem of the removal of the extra centromere. Examination of the chromomere pattern of the shorter arm of chromosome 1_{14} supports this hypothetical origin and gives some clue to the manner in which it may have occurred. The long arm of chromosome 1_{14} is very similar in length to the long arm of chromosome 3_{16} (27.6 microns compared with 29.7 microns), and the proximal chromatin on both sides of the centromere in the former is very similar to that of the latter. The chromomere pattern on the distal part of the shorter arm of chromosome 1_{14} resembles the chromomere pattern of chromosome 8_{16} minus some of the centromeric knobs. Thus the transfer of the greater part of chromosome 8_{16} to chromosome 3_{16} could have involved two unequal translocations, each transferring most of one arm of chromosome 8_{16}. This transfer is illustrated in Fig. 50. The remaining centromeric fragment of chromosome 8_{16}, being largely chromatic, might not form chiasma readily at meiosis (Clement and Stanford, 1961b) and the formation of univalents would result in the fragment being excluded from some gametes. As the fragment contained little genetic material, the gametes, and hence progeny, formed without it would probably be viable.

Similar rearrangements were suggested by Tobgy (1943) and Walters (1946) to explain the evolution of n = 3 and n = 4 species of *Crepis* from n = 5 species. The ultimate proof of such an origin of 2n = 14 *M. murex* from the 2n = 16 type would be the occurrence of trivalent pairing at pachytene and metaphase I in hybrids between them. Unfortunately, all attempts to cross the two types have been unsuccessful (Lesins, Lesins and Gillies, 1970). Simon (1965) had noted ovary stimulation and pod development to the fifteenth day in crosses between

M. rigidula (2n = 14) and *M. littoralis* and *M. truncatula* (2n = 16), so embryo culture may be necessary in order to obtain hybrids. The failure of crosses between 14 and 16 chromosome *M. murex* indicates that although they may have diverged only recently, the sudden cytological events involved in the hypothesized evolution of the 2n = 14 type from the 2n = 16 type have been sufficient to create a sterility barrier between the two strains, thus ensuring the maintenance of stable chromosome numbers in the two types.

The similarity of the karyotypes of *M. polymorpha* and *M. praecox* (particularly the morphology of chromosome 1) to that of 2n = 14 *M. murex* suggests that they may have originated, by a similar method, from 2n = 16 species. *M. rigidula*, also has an exceptionally long chromosome 1, but its submetacentric nature suggests that different translocations may have been involved in its derivation. The absence of an exceptionally long chromosome 1 in *M. constricta* may mean that the translocations involved in the elimination of the eighth chromosome have included more than two different chromosomes. The similarity of the idiograms of these latter two species could mean that they have a common 2n = 16 ancestor, but their great difference from the 2n = 16 *M. murex* idiogram suggests that this 2n = 16 ancestor is not closely related to *M. murex*, as was suggested by Simon and Simon (1965). The similarity of the karyotypes of *M. murex* and *M. polymorpha*, species which have been classified in different subsections of the genus, must cast some doubt on the validity of the taxonomic grouping, if pachytene karyotypes are indeed a valid means of assessing species relationships.

B. ELECTRON MICROSCOPE STUDIES

I. REVIEW OF THE LITERATURE CONCERNING THE SYNAPTONEMAL COMPLEX

1. Name and definitions

Light microscope studies have been unable to answer questions concerning the mechanism of meiotic chromosome pairing, but electron microscopy has revealed the presence of a complex structure apparently involved in chromosome synapsis. Moses (1956a, b) and Fawcett (1956) described the occurrence of these complexes in the primary spermatocytes of an invertebrate and several vertebrates. In a subsequent study of salamander spermatocytes, Moses (1958) coined the term "synaptinemal complex" to describe this structure. Recently the term "synaptonemal complex" has been used by some authors (Wettstein and Sotelo, 1967; Smith and King, 1968) in place of "synaptinemal complex". Moses (1969) discussed the etymological validity of the two terms and decided that "synaptonemal complex" is the more correct of the two and hence should be favoured. In this thesis "synaptonemal complex" will therefore be used.

Since the first reports in 1956 there have been numerous descriptions of synaptonemal complexes in meiotic prophases of many plants and animals. These have agreed on the basic form and dimensions of the complex although variations peculiar to certain species or groups have been noted.

The synaptonemal complexes appear in low magnification electron micrographs as tripartite structures consisting of three parallel

electron dense strands which appear to run along the axes of the bivalents. The central strand is narrower and less dense than the two lateral strands. A region of very low density about 500 to 800 A° wide separates the central strand from each lateral strand.

Moses (1968) has listed a number of terms (with definitions) for the various parts of the synaptonemal complex. They are as follows:

Axial Complex (AC) - the tripartite complex, whenever it appears with the characteristics that it has when associated with the meiotic chromosomes.

Synaptonemal Complex (SC) - the axial complex as it appears in association with meiotic prophase (bivalent) chromosomes.

Lateral Element (LE) - one of the two dense flanking elements of the complex that are invariably components of it; lateral elements may themselves be subdivided into finer components.

Central Element (CE) - the medial component of the tripartite complex; present in varying degrees of complexity depending on species, stage, method of preservation, etc.

Central Region (CR) - the less dense space between the lateral elements, that contains the central element.

Moses (1968) also defined "frontal view", "lateral view" and "cross sectional view" in terms of three intersecting perpendicular planes running through the complex, the first two along the longitudinal axis of the complex, and the third across these two. In a frontal view and a cross sectional view all three dense elements can be seen, while in a lateral view only the lateral elements or the central region are seen.

2. General morphology of the complex

The ten years of work following the discovery of the SC has resulted in the emergence of a consistent description. The lateral elements are continuous on their outer extremities with the fibrillar chromatin of the chromosome. In some material, such as *Acheta* and salamander (Moses, 1958), the chromatin is extremely diffuse and less dense than the LE, but in other material such as *Gallus* (Moses, 1968) the chromosome material is condensed and densely opaque so that its boundary with the LE is indistinct. For this reason the reported width of the LE has ranged from 300 to 1000 A°, the former value being the most common. The LE varies from a coarse condensed filament (Fawcett, 1966) to a flat or concave laminate structure (Moses, 1960), to a loose fibrous band. In some instances two distinct sub-components of the LE are visible - an outer and an inner segment. The inner segment is generally thinner and less dense than the outer segment (Moses, 1968). The LE is composed of microfibrils of 100A° or less in diameter, which integrate with the chromatin material (Moses, 1968). Sotelo and Wettstein (1966) noted that at magnifications of 80,000 to 800,000 the LE was seen to be composed of 15 to 20 A° fibrils which were compacted along the inner part of the LE.

The central element varies in width from 100 - 300 A° (Moens, 1968a). It is sometimes extremely lightly stained or even apparently absent (Sotelo and Trujillo-Cenoz, 1958). In the crayfish (Moses, 1956a) it is a dense rod, in the lily (Moens, 1968a) several thin parallel lines, and in the cricket (Sotelo and Trujillo-Cenoz, 1960; Moens, 1969a) it has a lattice-like appearance with longitudinal and transverse

periodic arrays. In cross sectional views the CE is usually a dense dot or line surrounded by the less dense cylindrical central space. Sotelo and Wettstein (1966) consider it to be composed of an ordered pattern of 15 to 20 A° filaments.

The central region is the less dense space between the lateral elements and ranges in total width from 600 to 1200 A° (Moens, 1968a). It contains microfibrils of about 50 A° diameter which cross from the lateral elements to the central element, apparently meeting in, or radiating from, the CE (Moses, 1968). Sotelo and Wettstein (1966) suggested that these fibrils were aggregates of the 15 to 20 A° filaments visible in the LE and CE.

Thus it can be seen that the total width of the SC ranges from 1600 to 2000 A° (Moens, 1968a), with chromatin up to several thousand A° wide on each side of the lateral elements, so that in late pachytene the total width of the bivalent chromosome is 5000 A° to 1 μ or greater.

3. Composition of the complex.

In attempting to understand the role of the SC in genetic recombination it is necessary to know to what extent the SC is composed of DNA. Nebel and Coulon (1962) used RNase, DNase and trypsin digestion of pigeon spermatocytes in combination with electron microscope examination, and concluded that the LE was mainly protein with some RNA, while the lateral diffuse material was mainly DNA. Coleman and Moses (1964) stained rooster spermatocytes with the selective nucleic acid stain indium trichloride. Indium was bound to the lateral chromatin and to the inner part of the LE. Most of the indium binding was prevented by prior DNase treatment. By use of contrast amplification of the electron

microscope images, Coleman and Moses were able to demonstrate indium positive filaments in the central region. Brinkley and Bryan (1964) report in an abstract only, the use of a silver-aldehyde modification of the Feulgen reaction to show the presence of DNA in the LE, as two parallel 100 A° fibrils about 200 A° apart and continuous with the lateral chromatin.

The presence of both RNA and protein in the SC was demonstrated by Nebel and Coulon (1962). Several other workers have reported evidence for the presence of RNA in the lateral elements (see Moses, 1968) but Coleman and Moses (1964) concluded from their indium staining that no appreciable RNA was present, and Moses (1968) considers the question unresolved. The protein present in the SC has been demonstrated by cytochemical methods (Nebel and Coulon, 1962; Moses, 1964). Sheridan and Stern (1967) found a unique histone in meiotic cells of lilies which was synthesised prior to meiosis and declined in concentration after meiosis. Subsequently, Sheridan and Barrnett (1969) used alcoholic phosphotungstic acid (PTA) to stain basic protein in the SC and found that the LE was particularly densely stained and showed a double structure. The presence of both DNA and protein in the SC has also been supported by the results which have shown that the interruption of meiotic pairing occurs if DNA synthesis or protein synthesis is inhibited at certain stages of prophase I (Hotta, Ito and Stern, 1966; Parchman and Stern, 1969).

4. Formation and function of the complex

Moses (1958) originally named the synaptinemal (sic) complex because of its association with synapsed chromosomes. Numerous studies since then have confirmed that the SC is seen under the electron microscope in sections in which chromosomes are synapsed at prophase I of meiosis. These studies have been dependant upon accurate identification of the stage of meiosis. For this purpose Franchi and Mandl (1962) used coital induction of meiosis in rat oocytes, Lu (1967) used the synchrony of meoisis in the fruiting body of the basidiomycete *Coprinus*, and Moens (1968a) used the relationship of bud length to meiotic stage in lily. All found the SC occurred in maximum frequency when synapsis was largely complete in zygotene and pachytene.

Wettstein and Sotelo (1967) used serial sections to demonstrate that the number of SC present in a cricket spermatocyte nucleus equalled the haploid number of autosomal chromosomes. Moens (1969a) also found that at pachytene the 22 autosomes of *Locusta migratoria* formed 22 SC's at the nuclear membrane where both ends of each bivalent were attached. Roth (1966) estimated the total lengths of the SC's in a mosquito oocyte and concluded that there was only one SC per bivalent. Wettstein and Sotelo (1967) traced the SC for their whole length in a cell and found they were of uniform dimension along their total length.

The occurrence in early prophase I of axial elements which resemble the lateral elements of the SC, and which appear to lie in the central axes of unpaired chromosomes, led to the suggestion that the axial elements paired to form the SC (Moses, 1958; Franchi and Mandl, 1962). The axial elements of univalents hence would become the LE's

of the bivalent SC, and the central element would be formed as a consequence of pairing. By following sequential stages of prophase I, Franchi and Mandl (1962), Lu (1967), Moens (1968a), and others, have shown that the axial elements align at precise intervals and a central element appears so that a complete SC is formed. Moens (1968a) suggested that the CE was formed after the 600 A° long filaments which radiate from each axial element interdigitate and lock the LE's at the correct pairing distance (about 1000 A°). Moens (1968b, 1969b) has studied the SC in triploid lily sporocytes and found that there was exchange of axial element partners at intervals between the homologous bivalent and univalent. Some abnormalities in the LE were attributed to segmented allotriploid difference between the chromosomes.

As well as being associated with synapsis in meiosis, the SC has been linked with crossing over and chiasma formation. Meyer (1960, 1964) has found that chiasma formation is correlated with the presence of SC. However, some workers (Menzel and Price, 1966; Gassner, 1967, 1969) have found SC in achiasmatic individuals. Hence it is concluded that the SC is a necessary but not completely sufficient prerequisite for chiasma formation. This is supported by the results of Moens (1969c) who found that desynaptic tomato plants had normal synaptonemal complexes, although the univalent frequency at diakinesis was quite high. La Cour and Wells (1970) studied prophase I in an asynaptic wheat and found that there was a complete absence of SC formation, although axial cores (elements) were prominent. These two reports demonstrate the functional difference between *asynapsis* (failure to pair) and *desynapsis* (failure to maintain pairing until metaphase I).

Moses (1969) has speculated on the possible roles of the SC in crossing over. His suggestions include its function as a pairing mechanism only, as a structural basis for crossing over, or as a means of disjunction of recombination DNA. Smith and King (1968) suggested that the major function of the SC was to ensure proper meiotic disjunction of chromosomes, whether or not crossing over had occurred. Lu (1969) has recently demonstrated that in *Coprinus* recombination occurs in pachytene after pairing is completed, and this finding has bearing on the work of Stern who has found that completion of DNA synthesis in late zygotene is connected with pairing, but this does not appear to be related to crossing over (Stern and Hotta, 1969). Ito, Hotta and Stern (1967) found some DNA synthesis during early pachytene but inhibition of it appeared to have an effect only in the fragmentation of chromosomes at anaphase II. They interpreted this as an inhibition of repair synthesis normally associated with crossing over at this stage.

Chiasma formation could, however, be prevented by inhibition of protein synthesis at the end of zygotene or in early pachytene (Parchman and Stern, 1969). This inhibition also prevented the axial elements from forming normal SC, the CE not being formed (Roth and Parchman, 1969). Smith and King (1968), after observation of the cytological effects of the crossover suppressor c(3)G in *Drosophila*, decided that the mutant was deficient in the synthesis of structural subunits necessary for SC formation. Hecht and Stern (1969) reported the isolation of a unique DNA - nonhistone protein complex from prophase I in Lilium. The inhibition of either zygotene DNA synthesis or protein synthesis prevented the appearance of the complex. These authors

suggested that the complex was related to chromosome synapsis and formation of the synaptonemal complex.

5. Anomalous complexes and the nucleolus

A confusing feature of some studies has been the reporting of SC-like structures in positions where bivalent chromosomes were not present. With few exceptions these structures, although morphologically similar to SC, have features which distinguish them from true SC. Moses (1968) has categorised these anomalous complexes into

i polycomplexes - stacks of fused complexes often found in insect meiosis

ii multiple complexes - laminate or tubular arrays of complexes found associated with the "round body" of spermatids

iii supernumeraries - short lengths of complexes which fuse with autosomal chromosome SC

iv univalent chromosome complexes - often formed between the X chromosome and the nucleolus in prophase and also in spermatids, also form between haploid autosomes and the nuclear membrane

v nucleolus associated complexes - single and multiple arrays of complexes embedded in and extending from the nucleolus.

Roth (1966) has speculated that polycomplexes in mosquity oocytes are formed by elimination and fusion of SC from bivalents as the bivalents contract in diplotene. In other organisms, some workers have concluded that polycomplexes are unrelated to SC (Moens, 1969d).

The complexes associated with the nucleolus (iv and v above) have been noted mainly in insect meiotic prophases, particularly in

Gryllus (Sotelo and Trujillo-Cenoz, 1960; Sotelo and Wettstein, 1966; Wettstein and Sotelo, 1967) where they are often in the form of multiple complexes and tubular structures unassociated with chromosomes. Single complexes between the one X chromosome and the nucleolus have also been noted in *Gryllus*. Solari (1969) has found that a normal SC is formed between short homologous terminal portions of the X and Y chromosomes in mouse spermatocytes, but that anomalous complexes having no connection with the homologous synapsed chromosomes were also formed in the associated nucleolus.

II. MATERIALS AND METHODS

Buds from plants of the double trisomic TR $39^{L}2$ were collected in the early morning, as for light microscope study. The unfixed anthers were dissected out and two from each bud (one of the largest anthers and one of the smallest) were squashed in acetocarmine and examined under the light microscope. If prophase I meiotic stages were observed the remainder of the anthers from the bud were fixed for electron microscopy.

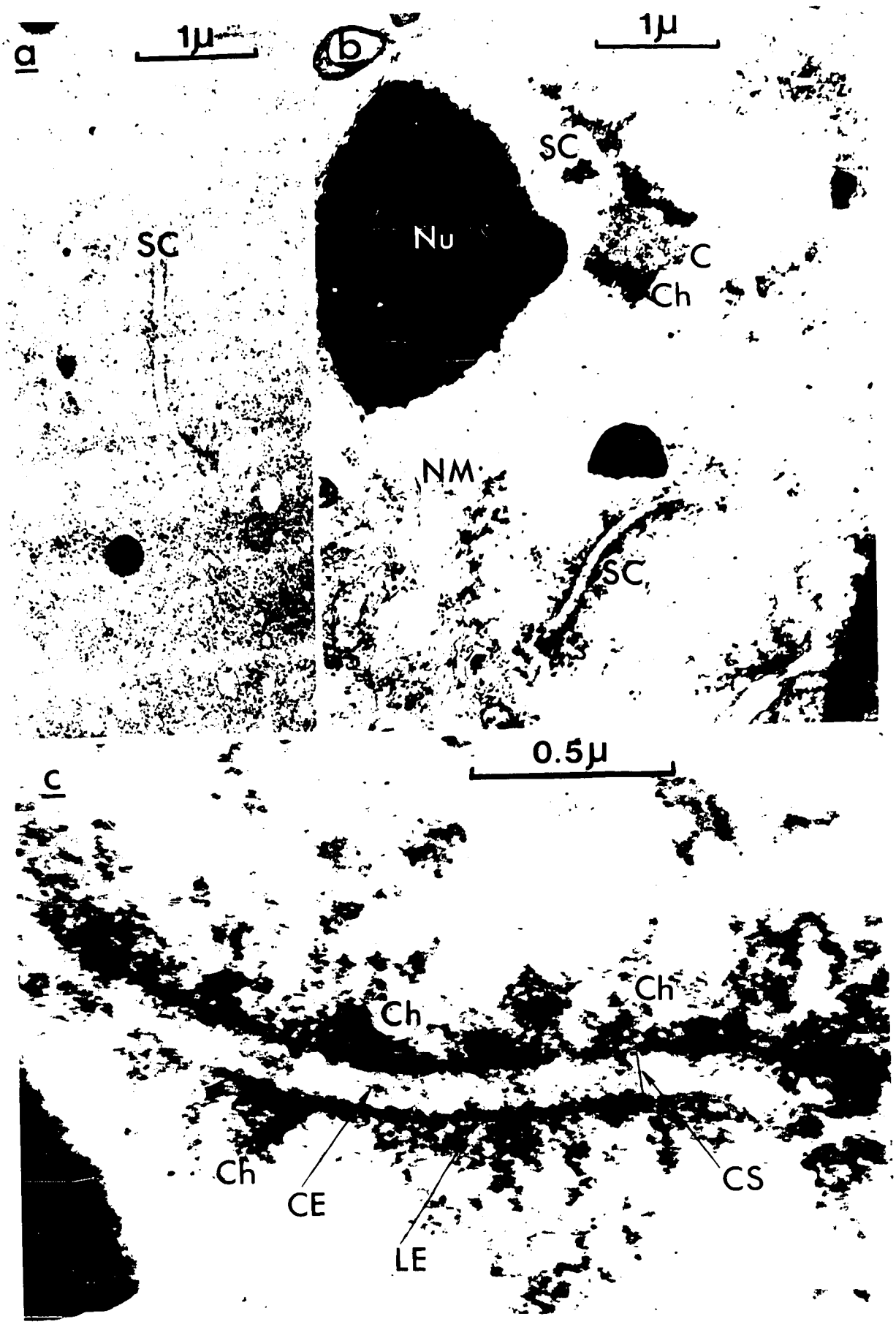
Anthers were fixed for 1 1/2 hours in a 6% glutaraldehyde solution which was made up in 10% sucrose in 0.1M Sorensen's phosphate buffer at pH 6.8. Anthers were then washed in three changes of buffered 10% sucrose for 1 hour each, and post-fixed for 1 hour in 2% OsO_4 in buffer. Dehydration was carried out in an alcohol series: 30, 50, 70, 80, 90, 100% for 30 minutes each, followed by two changes of 30 minutes each in propylene oxide. Anthers were embedded in Araldite 502 (Luft, 1961) by standing overnight in a 1:1 mixture of resin and propylene oxide, and then transferring to fresh resin in capsules, aspirating, and polymerising in a 60°C oven overnight.

The material was sectioned on a Porter Blum MTII ultramicrotome using glass knives, and thin sections (silver to gold) were picked up on collodion coated 150 mesh copper grids. Thick sections of each anther were collected on glass microslides, stained with Nile blue and examined under the light microscope to check the meiotic stage of the sporocytes. Thin sections were stained by floating the grids, section side down, on drops of the staining solution in a covered petri dish.

Staining was for 2 hours in 2% aqueous uranyl acetate, followed by 10 minutes in lead hydroxide (Millonig, 1961), with one minute washes in several changes of distilled water after each stain. Stained sections were examined with a Phillips 100B electron microscope at a voltage of 60 Kv. Photographs were made on Kodak Fine-grain Positive film and developed with Kodak D-19 developer. Prints were usually made at eight times the negative enlargement.

Figure 51

Sporocyte cells of TR39^{L}2 at late zygotene - early pachytene stage. Synaptonemal complexes (SC) can be seen in a and b, and c is higher magnification of the bottom SC in b. Chromatin (Ch) is only moderately condensed at this stage. Details of the central element (CE), lateral element (LE) and central region (CS) can be seen in c. A centromere (C) can be seen in b as well as the nucleolus (Nu) and nuclear membrane (NM). a and b. X approx. 21,000. c. X approx. 72,000.


a
1μ
SC
b
1μ
SC
Nu
C
Ch
NM
SC
0.5μ
c
Ch
Ch
Ch
CE
LE
CS

III. RESULTS AND DISCUSSION

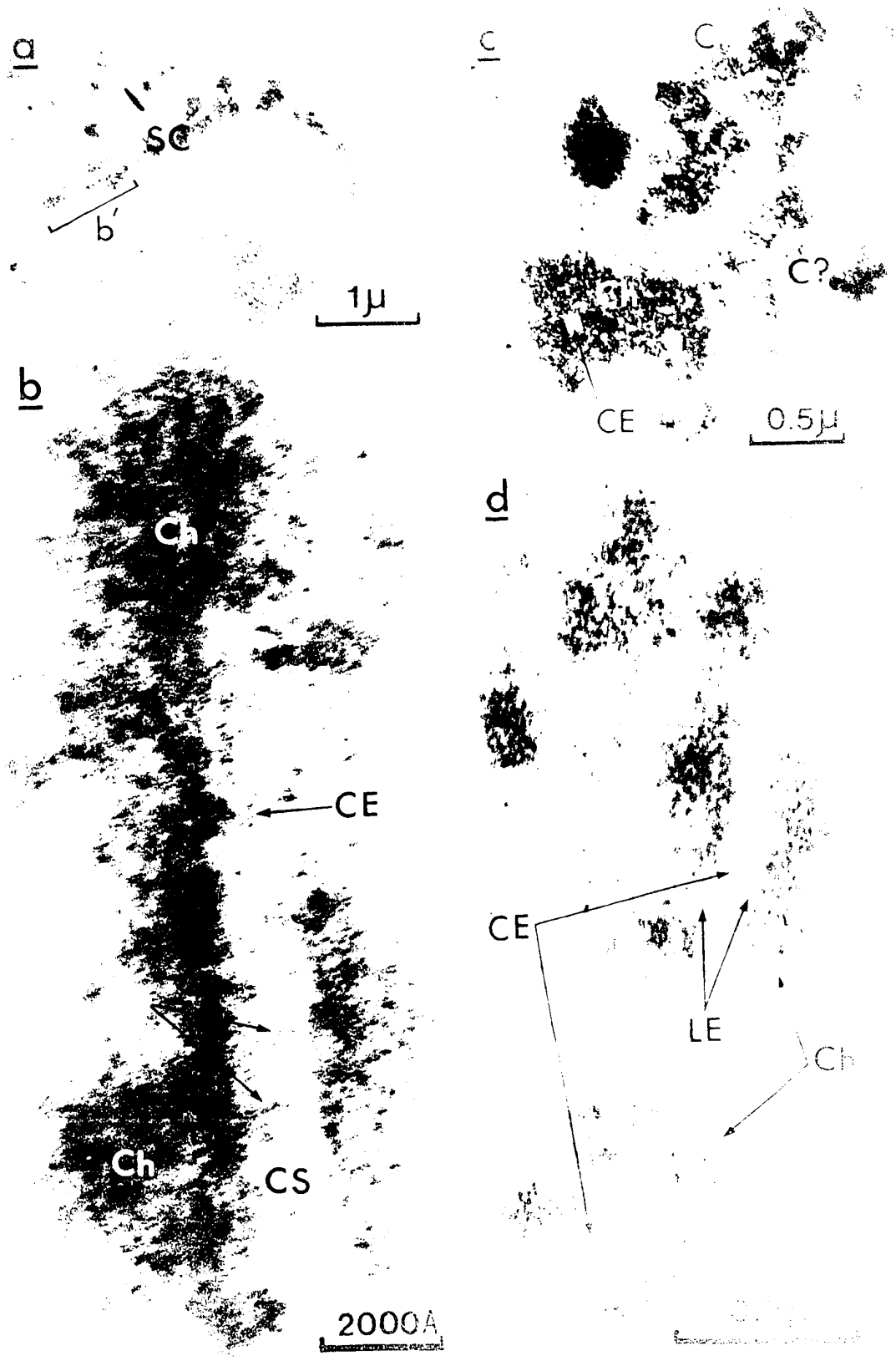
It was found that the meiosis of *Medicago* sporocytes is not completely synchronised since cells from leptotene to late pachytene were often found in the one lobe of an anther. The cells containing synaptonemal complexes which were examined could be classified on the basis of the chromatin condensation into zygotene - early pachytene (Fig. 51) and mid-late pachytene (Fig. 52). The nucleolus was usually observed as a lens shaped body appressed to the nuclear membrane.

The zygotene - early pachytene SC were characterised by the presence of relatively uncondensed chromatin adjacent to the lateral elements, so that the latter were readily distinguishable (Fig. 51). The central element was visible but not very electron dense, so that at higher magnifications it often was not clearly distinguished against the central region. The loose packing of the chromatin fibrils made it difficult to determine how far into the nucleoplasm they extended.

In slightly later stages the chromatin was much more densely packed and it was uncertain where the boundary between the LE and the chromatin was located (Fig. 52). Because of this, accurate measurements of the width of the LE were not always possible, but the mean width was approximately 275 A°, with a range of 200 to 400 A°. The chromatin appeared more granular than in earlier stages and usually extended only about 1500 to 2000 A° from the inner edge of the LE. The central region had a mean width of 1000 A° (range 750 to 1300 A°) and the CE had a mean with of 175 A° and a range of 100 to 250 A°. The range in measurements is probably due partly to the inclusion of tangential as well as true cross sections.

Figure 52

Synaptonemal complexes (SC) at mid to late pachytene. The portion b' of the synaptonemal complex in a is shown at a higher magnification in b. A cross sectional view of a bivalent is shown in c and the central element (CE) can be seen inside the central region (CS). The central elements are faintly visible in b and d, but the lateral elements (LE) cannot be clearly distinguished from the highly condensed chromatin (Ch). Possible transverse filaments (TF) can be discerned in b. One or possibly two centromeres (C, C?) are visible in c with SC running through them. a. X approx. 17,000. b. X approx. 100,000. c. X approx. 31,000. d. X approx. 60,000.


a
SC
b'
1μ
c
C
C?
CE
0.5μ
b
Ch
CE
Ch
CS
2000A
d
CE
LE
Ch

In some SC it was possible to see what appeared to be transverse filaments crossing the central region from the LE to the CE (Fig.52b), and the CE and LE were sometimes evident as longitudinal filaments (Fig. 51c). In Fig. 51c the CE appears to consist of two filaments of about 25 - 40 A° in diameter separated by about 100 A°. In Fig. 52c a cross sectional view of the pachytene SC is shown and it can be seen that the CE is a dot in the centre of a hollow cylinder (central region) surrounded by condensed chromatin.

The synaptonemal complexes here described are very similar to those of lily described by Moens (1968a). The dimensions of the central space are the same although lateral and central elements appear to be thinner in *Medicago*. The electron density of the *Medicago* SC is not as great as that of lily, but this may be partly a result of differences in technique. However the prophase chromosomes of lily are considerably more chromatic in light microscope preparations than those of *Medicago*. As in lily the *Medicago* SC lacks the more complex arrangement of the CE and LE found in many insects (cf. Moses, 1968).

The chromaticity of *Medicago* non-nucleolar chromosomes is much greater adjacent to the centromere than in distal regions of the chromosomes. In several cells fine grained, less densely stained regions were noted on SC, surrounded by highly condensed chromatin, and on comparison with previous reports (Brinkley and Stubblefield, 1966; Moens, 1968b) these were identified as centromeres (Figs. 51b and 53). The fine granular structure of the centromere distinguished it from the rest of the chromatin of the chromosome. The LE and the CE of the SC transversed the centromere apparently without any change in their

Figure 53

Serial sections through an early pachytene sporocyte cell showing an approximately 3 micron long bivalent with a centromere (C). The synaptonemal complex (SC) passes along the full length of this bivalent, and clumps of condensed chromatin (Ch) can be seen, especially on each side of the centromere. All X approx. 21,000.


a
1μ
C
b
SC
C
Ch
c
C
SC

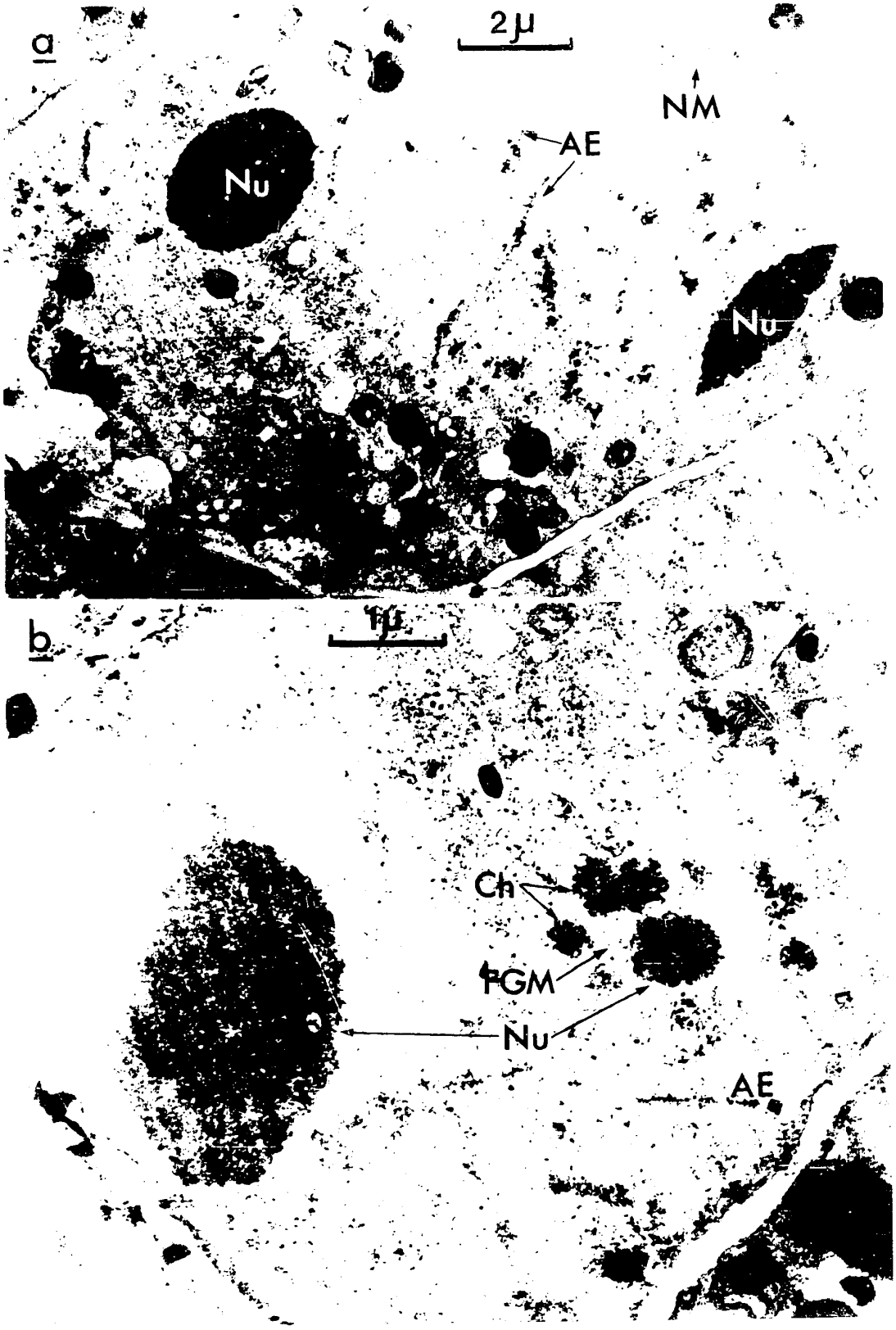
structure. The occurrence of several regions of condensed chromatin (chromomeres) along the bivalent can be seen in Fig. 53.

Brinkley and Stubblefield (1966) described the centromeres of mammalian mitotic metaphase and anaphase chromosomes as consisting of a dense 200 to 300 A° diameter core made up of axial filaments, with a surrounding less dense 200 to 600 A° wide zone of microfibrils, the whole having a lampbrush-like structure. They considered that the centromere had much in common with the axial elements of the SC. The centromeres found here are similar to the mitotic ones, with the lateral elements of the SC comprising a centromeric core about which the fine granular material (fine fibrils) is organised.

As the buds used were from a plant which was trisomic for two chromosomes it was expected that some variation in synaptonemal complexes corresponding to the trivalents of the trisomic chromosomes would be observed. However no true serial sections were prepared and hence no structures positively identifiable as trivalents were found. As TR $39^{L}2$ is trisomic for the nucleolar chromosome it was also expected that it might be possible to recognize a univalent of the nucleolar chromosome if it occurred, by its attachment to a secondary nucleolus. Light microscope studies had, however, suggested that this event was relatively rare. Two sporocyte cells were found in electron microscope studies in which there were two nucleoli present at the stage when SC were also observed (Fig. 54). Unfortunately none of the sections of either cell had both nucleoli associated with chromosomes. In Fig. 54a, due to the plane of sectioning, the circular nucleolus is in a lobe of the nucleus which appears unattached to the rest of the nucleus. The smaller

Figure 54

Two sporocyte cells each having two nuceloli (Nu). Axial elements (AE) are visible in both cells and the nuclear membrane (NM) can be seen in a. In b the smaller nucleolus has chromatin (Ch) and fine grained material (FGM) associated with it. a. X approx. 9,600. b. X approx. 21,000.


a
2μ
NM
AE
Nu
Nu
b
1μ
Ch
PGM
Nu
AE

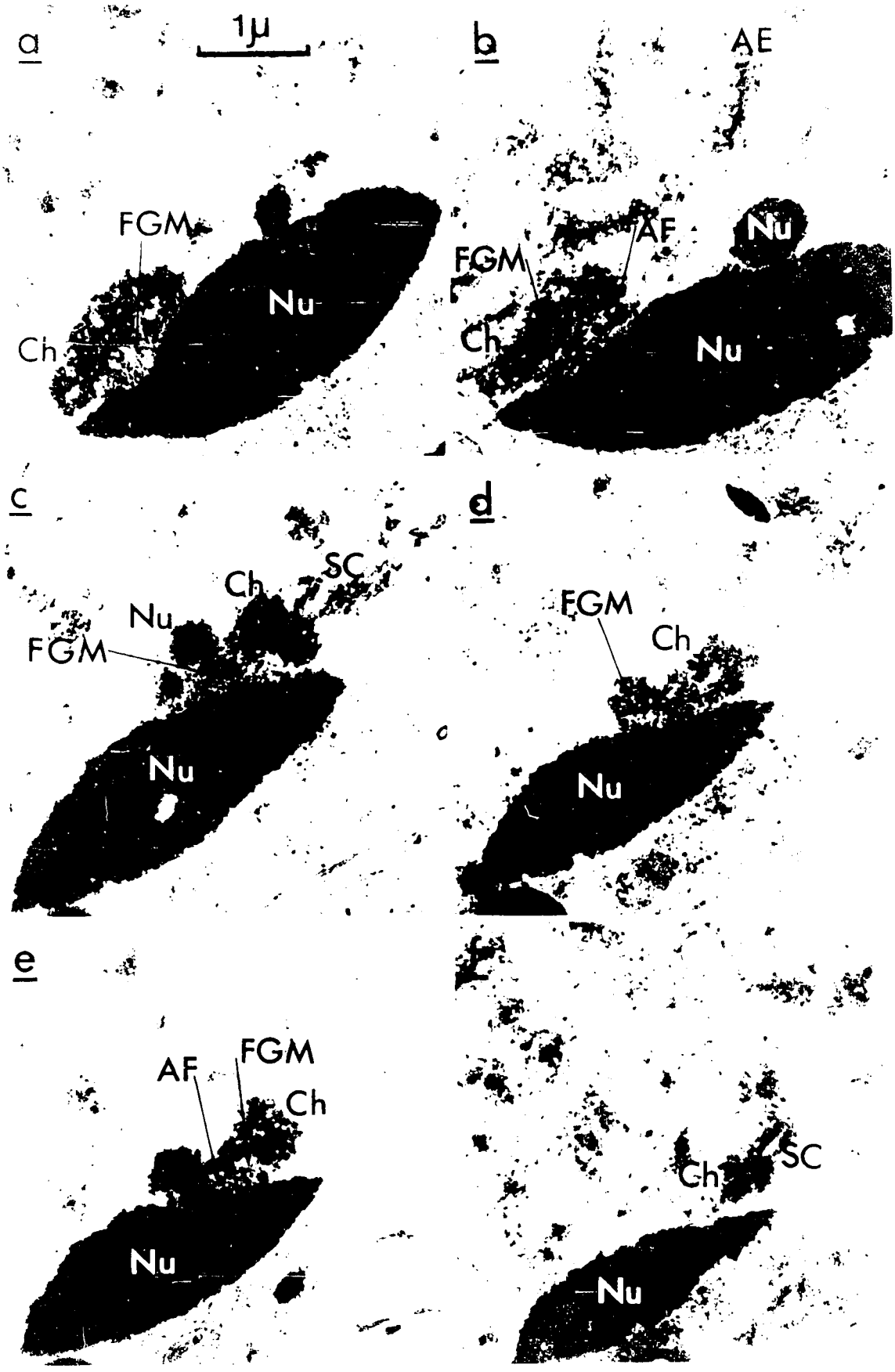
nucleolus in Fig. 54b has the fine granular structure which was found to be characteristic of chromosome-nucleolus junctions. The lens shaped nucleolus in Fig. 54a was also found in other sections to have a similar association with the chromatin material.

The nucleolus was usually lens shaped, occasionally circular, and appressed to the inside of the nuclear membrane. The association of the chromatin with the nucleolus was quite characteristic, and as mentioned above, involved a fine grained component which appeared extremely similar to the centromere (Figs. 55 and 56). The junction usually consisted of a band of fine grained material, less dense than the nucleolus and having a mean width of about 2500 A°, but ranging from 1500 to 3500 A° wide. It was flanked on one side by the nucleolus and on the other by a combination of dense chromatin material and lobes of nucleolar material. The granular band ran for a distance of from 5000 A° to 1.5 microns along the inner edge of the nucleolus.

The material on the non-nucleolar edge of the granular band was in some cases obviously part of the nucleolus. These nucleolar lobes were usually circular (Fig. 55a, b, c and Fig 56a) and in some cases (Fig. 55a, b) could be seen connected to the nucleolus proper. The dense chromatin was composed of larger diameter grains or fibrils than the nucleolus, and in some instances appeared to have some axial filaments running through it. The SC could not be positively identified in either the dense chromatin or the less dense granular band, although in some sections (Figs. 55b, e) there were indistinct structures suggestive of the SC in the granular band, and the chromatin in Fig. 56c appears to have some axial structures and lighter areas.

Figure 55

Series of sections through the junction of a nucleolus (Nu) with the nucleolar bivalent and synaptonemal complex (SC). Lobes of nucleolus (in b and c) and condensed chromatin (Ch) can be seen separated from the main nucleolus by a band of fine grained material (FGM), which in b and e appears to have an axial filament (AF) running through it. All X approx. 19,000.


a
1μ
FGM
Nu
Ch
b
AE
AF
Nu
FGM
Ch
Nu
c
Ch
SC
Nu
FGM
Nu
d
FGM
Ch
Nu
e
AF
FGM
Ch
Nu
Ch
SC
Nu

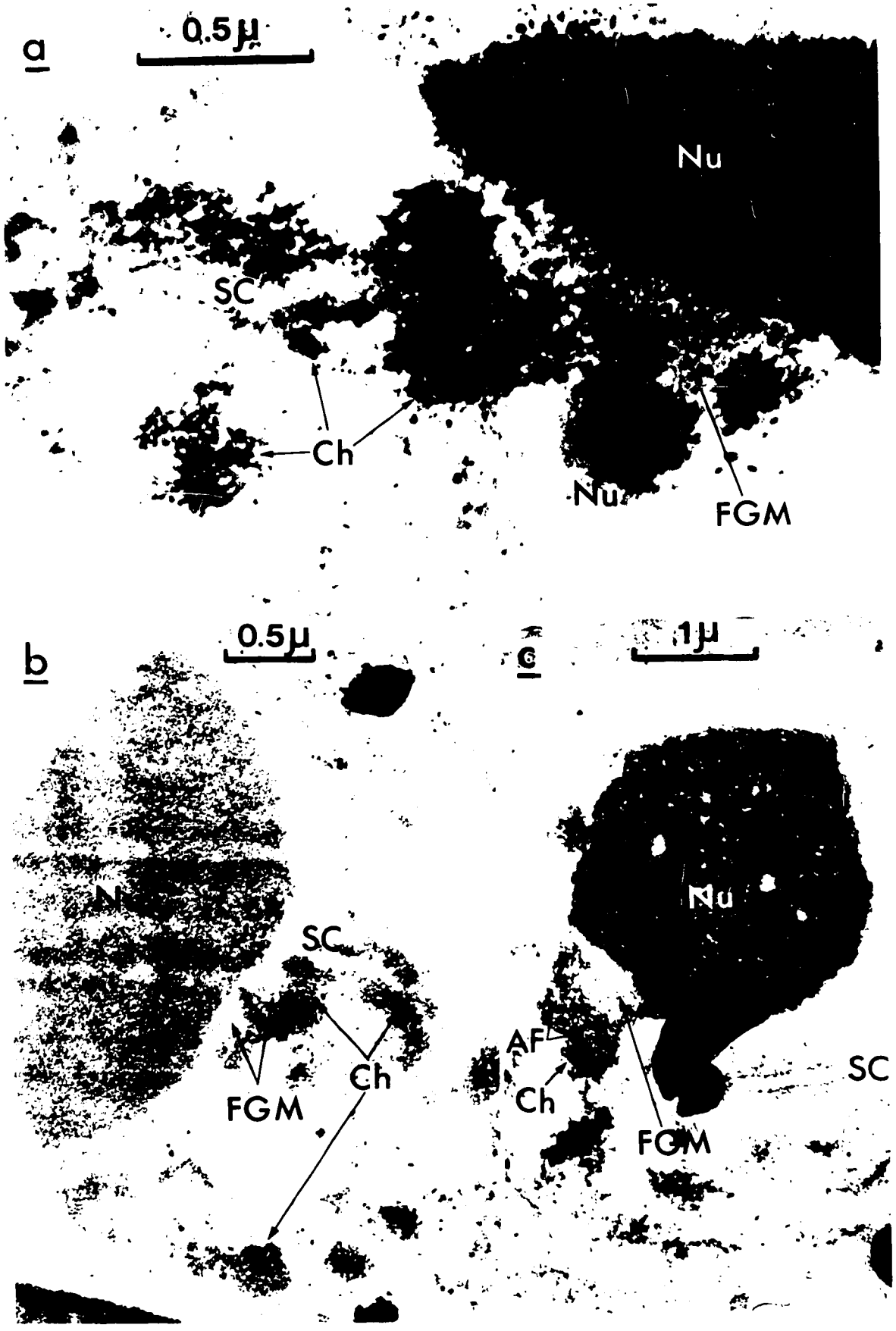
Although the presence of the SC in the fine granular band was not demonstrated, it is of interest to note that the width of this band at its narrowest corresponds approximately to the width of the SC (without associated chromatin). The dense chromatic material associated with the junction in Figs. 55a, e and Figs. 56b, c corresponds to the chromatic knobs found associated with the N.O. in light microscope studies. The fine grained material may correspond to the achromatic gap sometimes seen at the N.O. in light micrographs, between the two chromatic knobs.

The fine grained nature of the chromosome-nucleolus junction at the presumed N.O. is similar to that described by Hsu, Brinkley and Arrighi (1967) in mammalian mitotic metaphase chromosomes at their secondary constrictions. Hsu et al found that the N.O. region was less dense because it consisted of smaller diameter filaments than the rest of the chromosome (50-70 A° compared with 150 - 200 A°), and these filaments were more loosely packed than the rest of the chromatin. The N.O. filaments were not as lampbrush-like as the low density zone of the mammalian mitotic chromosome centromere. In the present case the reverse appears true as the centromeres in Figs. 51 and 53 are composed of slightly larger diameter granular material than the N.O.

Wettstein and Sotelo (1967) described SC-like structures which lay between chromatin and the nucleolus in *Gryllus* spermatocytes. Jaworska and Lima-de-Faria (1969) attributed multiple SC found associated with the nuclear DNA body in *Acheta* to some form of pairing between amplified DNA present in this body. The association found in *Medicago* between the chromatin and the nucleolus at prophase is in

Figure 56

Nucleolus - chromosome junctions. a is a higher magnification of Fig. 55c (inverted). Synaptonemal complexes (SC) can be seen ending in condensed chromatin (Ch) adjacent to the fine grained material (FGM) and nucleolus (Nu) in a and b. In c axial filaments (AF) are apparent in the nucleolus associated chromatin. a. X approx. 60,000. b. X approx. 29,000. c. X approx. 21,000.


a
0.5μ
Nu
SC
Ch
Nu
FGM
b
0.5μ
SC
FGM
Ch
c
1μ
Nu
AF
Ch
SC
FGM

some respects similar to the chromatin-nucleolus association in *Gryllus*, except that no central axial component is obvious. It is possible that the chromosomes are so decondensed in the N.O. region because of their active synthetic state that any SC formation is too dispersed to be visible. Possibly the protein components necessary for SC formation (Hecht and Stern, 1969) are not present in the N.O. region and hence a normal SC cannot form, and the chromosomes are held together by their attachment to the one nucleolus. The fine grained material may also represent immature nucleolar material formed between the chromatin of the N.O. region and the mature nucleolus. In this case one would expect the occurrence of the SC, if present, to be in the nucleolus associated chromatin.

As the plant studied is trisomic for the N.O. chromosome and all three N.O. chromosomes were found associated with the nucleolus in light microscope studies, the possibility must be considered that the presence of all three chromosomes may affect synapsis of the N.O. region. The exact nature of the N.O. region in paired *Medicago* chromosomes must be investigated by serial sectioning of the nucleolus of normal disomic plants, before it will be possible to decide if the N.O. structure described here is a common feature or is peculiar to the trisomic condition of the nucleolar chromosomes.

SUMMARY

A. LIGHT MICROSCOPE STUDIES

Squashes of pachytene stage cells of annual and perennial species of *Medicago* were prepared by Snow's (1963) method. The chromosomes of each species or hybrid were measured and idiograms were prepared from the data.

In the group of diploid perennials which readily cross with *M. sativa*, viz. *M. falcata*, *M. glomerata*, *M. coerulea* and *M. glandulosa*, the pachytene karyotypes were found to be extremely similar in chromosome arm ratios and proportional lengths to that of diploid *M. sativa*. Absolute length differences between species appeared to be caused by differences in chromosome contraction. Hybrids of *M. sativa* and *M. falcata* with *M. glomerata* were found to have normal pachytene pairing, demonstrating the close relationship of the three species. The five species mentioned, because of their close relationship, were considered a coenospecies, and a common idiogram designated *M. sativa sensu lato (s.l.)* was constructed, based on the arm ratios and proportional lengths of their chromosomes.

Tetraploid *M. glutinosa* was found to have chromosomes which were similar in morphology to those of *M. sativa*, and the presence of quadrivalents suggested that *M. glutinosa* might be an autotetraploid member of the coenospecies. *M. prostrata* had a karyotype which differed little from *M. sativa s.l.*, hence it may also be a member of the coenospecies.

Three groups of perennial species not usually considered to be related to *M. sativa* were analysed. *M. daghestanica*, *M. pironae* and

their sterile hybrid had similar idiograms and they are probably closely related. The hybrid sterility was caused by other than gross chromosomal differences. The idiograms differed from that of *M. sativa s.l.* only in proportional lengths of chromosomes, hence the two groups may share a common ancestor.

M. rhodopaea and *M. rhodopaea* X *M. rupestris* had almost identical idiograms, leading to the conclusion that *M. rhodopaea* and *M. rupestris* are very closely related. The idiogram of *M. rhodopaea* was very similar to the *M. sativa s.l.* idiogram, and in a triploid hybrid between diploid *M. sativa* and autotetraploid *M. rhodopaea* trivalent pairing was observed, confirming that some homology existed between the chromosomes of the two species. Thus the *M. sativa* group and the *M. rhodopaea* group may have diverged only fairly recently.

M. hybrida, M. suffruticosa and their hybrid were found to have almost identical karyotypes, confirming the close relationship of the two species. The idiograms were quite different from those of all perennials previously examined, the chromosomes being longer, more chromatic and having different arm ratios. It is possible that these species are related to the genus *Trigonella*.

Five annual species having 2n = 14 chromosome numbers were analysed and idiograms prepared. The *M. constricta* idiogram was unique among the 2n = 14 idiograms in having an even gradient of reduction in chromosome length. All the other 2n = 14 idiograms had their longest chromosome at least 10 microns longer than the second chromosome in the idiogram. *M. murex* was represented by both 2n = 14 and 2n = 16 accessions, and the differences between the idiograms of the two sug-

gested that the 14 chromosome type had evolved from the 16 chromosome type by two unequal reciprocal translocations which had transferred most of the shortest metacentric chromosome to a submetacentric chromosome, which became the extremely long metacentric of the 14 chromosome type. Similar extremely long chromosomes in *M. rigidula*, *M. praecox* and *M. polymorpha* suggested that they may have arisen in a similar manner. The *M. polymorpha* idiogram was very similar to the 2n = 14 *M. murex* idiogram, a fact not expected as the two species are usually classified into different subsections of the genus. The differences between the 2n = 14 and 2n = 16 *M. murex*, together with their reported inability to cross, suggest that they should be reclassified as two species.

Analysis of pachytene chromosomes of diploid, tetraploid and hexaploid *M. sativa s.l.* showed that there was a reduction in chromosome length with the increase in ploidy level, the reduction from diploid to tetraploid being more pronounced than from tetraploid to hexaploid. It was decided that this length change was probably caused by contraction of achromatic portions of chromosomes. In submetacentric chromosomes this resulted in a simultaneous reduction in arm ratio.

Pachytene karyotypes of five dihaploids derived from tetraploid alfalfa had normal pachytene pairing and were similar to the *M. sativa s.l.* karyotype. This lent weight to the argument that tetraploid alfalfa is essentially an autotetraploid. A hybrid of one of the dihaploids with diploid *M. falcata* had a normal pachytene karyotype, demonstrating the close relationship of tetraploid alfalfa and diploid *M. falcata*.

The *M. sativa s.l.* idiogram was used to identify by cytological

means the chromosomes present in triplicate in eight trisomics and one double trisomic of diploid alfalfa. All trisomics were tentatively identified, but require confirmation by further pachytene studies and the use of linkage data.

In pachytene cells of *M. rhodopaea* and two of the *M. rhodopaea* X *M. rupestris* hybrids, darkly stained bodies were observed in the nucleoplasm. These were shown by pyronine/methyl green staining to contain RNA, and in their behaviour they resembled nucleoli.

B. ELECTRON MICROSCOPE STUDIES

The structure of the paired chromosomes at early meiotic prophase was investigated in sporocytes of double trisomic TR $39^{L}2$, using the electron microscope. Synaptonemal complexes were described which were similar to earlier descriptions for other species of plants. The central element of the synaptonemal complex was often difficult to distinguish. The centromeres of paired chromosomes were found to consist of fine grained material through which the synaptonemal complexes passed. In its fine structure the centromere resembled mitotic chromosome centromeres.

The association of the nucleolar chromosomes with the nucleolus was investigated and it was found that a band of fine grained material, similar to the centromere material, was found at the junction of the chromosome and the nucleolus. The synaptonemal complex was not identified in either the chromatin or the fine grained material at the presumed nucleolus organizer. It was uncertain whether or not this was a result of the trisomic condition of the nucleolar chromosome.

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