SOMATIC AND SECONDARY PAIRING IN *ORNITHOGALUM*

EEVA THERMAN

State Horticultural Institute, Piikkiö, Finland

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I. INTRODUCTION

CONSIDERING the great amount of cytological work done on Liliaceous plants, the genus *Ornithogalum* has remained comparatively neglected. The chromosome number has been determined in several species, but the genus as a whole has not been the subject of cytological analysis. From the papers of Delauney (1927), Heitz (1926), Geitler (1929), and Nakajima (1936) it is seen that the chromosome numbers vary considerably in the different species. So far the following diploid numbers, which seem to fit into an aneuploid series, have been encountered: 10, 12, 14, 16, 18, 24, 27, 30, 32, 50, 54.

The present study deals with the cytology of two *Ornithogalum* species, *O. graminifolium* Thunb. ($2n = 10$) and *O. caudatum* Ait. ($2n = 54$), both native to the Cape. The distinguishing feature in their chromosome behaviour is a strong tendency to somatic and secondary pairing. This feature, which has been overlooked by the earlier investigators of the genus, might prove a valuable tool in a comparative study of cytogenetic relations in the genus *Ornithogalum*. That such a study would yield interesting results seems evident, not only on account of the fragmentary knowledge of the chromosome numbers, but also from the taxonomy and the geographical distribution of the genus (*cf.* Baker, 1873; Feinbrun, 1941). Of the 73 species recognised by Baker, 39 belong to the Cape, a few species are native to tropical Africa, one grows in Chile and Peru, and the rest are spread around the Mediterranean.

2. MATERIAL AND METHODS

Both mitosis and meiosis were studied in *Ornithogalum graminifolium* (Botanical Garden of Coimbra) and *O. caudatum* (Botanical Garden of Pisa). The plants were received as bulbs and cultivated in a hot-house at a suitable temperature. The somatic chromosomes of *O. graminifolium* were examined in acetic-alcohol-Feulgen squash preparations made from root tips (*cf.* Darlington and La Cour, 1947). Slides made according to this method were not very favourable for the demonstration of the somatic pairing of the chromosomes. Unfortunately the plant in question had died before this cytological feature had been discovered. Additional material could therefore not be obtained. Meiosis in the PMC was studied in smear preparations, a part of which had been fixed in acetic-alcohol and stained with Feulgen. Another part was fixed in La Cour's 2 BD and stained with crystal violet.

The root tip mitoses of *O. caudatum* were studied both in squashes made as above and in sectioned material fixed in 2 BD and stained with Feulgen. The PMC were made into preparations by the same methods as in *O. graminifolium*. The magnification of the camera lucida drawings is $\times 2000$, that of the microphotographs being about the same.

3. OBSERVATIONS

(i) *Ornithogalum graminifolium*

Mitosis.—This species has five pairs of somatic chromosomes, three pairs of which are long and two pairs short. The homologous chromosomes show a great tendency to somatic pairing. This is seen in prophase where the chromosomes, as a rule, lie in pairs. This phenomenon is, however, more easily examined in metaphase (fig. 1a), different metaphase plates showing variation in this respect. The same tendency gives a characteristic appearance also to the mitotic anaphases. Anaphases and telophases often exhibit chromatid bridges between the separating chromosome groups (fig. 1b). In interphase nuclei some ten chromocentres and two nucleoli are visible.

Meiosis.—In pachytene the chromocentres appear as more condensed parts in the thread-like chromosomes. They seem to vary slightly in number between eight and ten, in any case exceeding the bivalents in number. The big chromocentre (fig. 1c, at 4 o'clock) is constant in appearance, and it could be distinguished in all the nuclei examined. The chromocentres do not show any tendency to run together, appearing in fact, even more condensed than other chromosome regions.

Though the pachytene stages in this plant cannot be analysed as elaborately as has been done by Coleman (1940, 1941) in *Veltheimia* and *Rhoeo*, it is obvious that the chromocentric regions, as in these plants, are tightly coiled parts of the chromosomes. Consequently they are incapable of real pairing. This is especially clear in the big chromocentre mentioned above, which in most cells reveals a distinctly bipartite structure.

In diakinesis the chromosomes in most cells form three large and two small bivalents. Not uncommonly, however, one or more chromosome pairs remain as univalents. The univalents then show the same general repulsion as the bivalents. An example of such a diakinesis stage is seen in fig. 1d. One of the short chromosome pairs has remained unpaired, and the two univalents lie on opposite sides of the cell.

The first meiotic metaphase exhibits several features which deviate from the normal. Fig. 1e illustrates the most regular type of metaphase plate found in *O. graminifolium*. In this case all the chromosomes have paired to form five bivalents, which are fairly regularly orientated in a metaphase plate. Disturbances in bivalent orientation are, however, common, one or more of the bivalents in many cells lying outside the plate. In the cell in fig. 1f one of the large bivalents is

unorientated, while the other four form a regular plate. In fig. 1*g* again we see a side view of a first division metaphase in which con-

FIG. 1.—*Ornithogalum graminifolium*.

- (a) Metaphase from a root tip cell with ten chromosomes ; the chromosomes show somatic attraction.
- (b) Telophase from a root tip cell with chromatid bridges.
- (c) Pachytene showing a nucleolus and nine chromocentres.
- (d) Diakinesis with four bivalents and two small univalents.
- (e) Metaphase I with five orientated bivalents.
- (f) Metaphase I with five bivalents, one lying outside the plate.
- (g) Metaphase I with five unorientated bivalents.
- (h) Metaphase I with two bivalents and three pairs of univalents, one of which lies somatically paired on the plate, one at the one pole, while one pair is separated.

gression has failed and the five bivalents are scattered around the place where the metaphase plate ought to be situated.

Chiasma frequency in *O. graminifolium* is low. In ten cells only 45 chiasmata in all could be recorded, which gives a chiasma frequency of 0.9 per potential bivalent. The low chiasma frequency is reflected by the frequency of univalents. Contrary to observations made on other organisms (*cf.* Darlington, 1937) no greater tendency for the longer chromosomes to be effectively paired could be established. In ten cells which contained univalents it was observed that seven pairs of the smaller chromosomes had remained as univalents, and nine pairs of the larger chromosomes.

The behaviour of the univalent chromosomes is the most interesting feature in the meiosis of *O. graminifolium*. The tendency to somatic pairing seen in the root tip mitoses also governs the behaviour of univalents. The repulsion they have shown in diakinesis (fig. 1*d*) is replaced by an attraction in the first meiotic metaphase. As a rule the homologous univalents come together and pair somatically—naturally without chiasma formation—on the metaphase plate or sometimes outside it (plate I, fig. 2). Obviously this somatic pairing is often very brief, the chromosomes separating almost immediately.

Fig. 1*h* illustrates several modes of chromosome behaviour. Two pairs of chromosomes have formed bivalents and lie on the plate. One pair of long chromosomes has paired somatically and is co-orientated on the plate. One univalent pair lies close together at one pole, while the third pair of univalents has obviously paired and separated, and now shows a position correlation (*cf.* Ribbands, 1937). In the cell shown in fig. 2*a* one bivalent is outside the plate, and one pair of the longer chromosomes lies somatically paired on the plate. Fig. 2*b* represents a further metaphase in which the chromosomes are defectively orientated. One small chromosomes pair, which obviously has been paired somatically, has separated in advance. A further chromosome pair, which is somatically paired, is interesting in that it is auto-orientated on the plate. In the cell in fig. 2*c*, again, the members of the two univalent pairs lie at opposite poles showing position correlation (the cell axis is slightly twisted in smearing). An example of rarer chromosomes behaviour is furnished by the cell in fig. 2*d*. The two homologous univalents are situated on the same side of the plate; it is not certain whether or not they have been somatically paired. In fig. 2*e* a situation is represented in which the three univalent pairs, which obviously have previously been paired somatically, are separated while the two bivalents still lie on the plate. An interesting feature makes its appearance in this cell. Usually only the somatically paired univalents seem to be capable of auto-orientation; in this cell the upper bivalent is obviously auto-orientated. The cell represented in fig. 2*f* provides an example of great variation in the chromosome behaviour of a single cell. The large bivalent at the left contains two chiasmata. The large chromosome pair at the right has formed only one chiasma and is separating precociously. The two short chromosome pairs have paired somatically;

one of the pairs is co-orientated, while the other shows auto-orientation. The fifth chromosome pair lies separated at one of the poles. In fig. 3a an extreme case is illustrated. In this cell all the chromosomes

FIG. 2.—First meiotic metaphases in PMC of *Ornithogalum graminifolium*.

- (a) Three bivalents are orientated, one lies off the plate, while the two univalents are somatically paired.
- (b) Three bivalents, one pair of separated chromosomes; and two somatically paired auto-orientated univalents.
- (c) Three bivalents and two pairs of separated univalents.
- (d) Four bivalents and one pair of univalents.
- (e) Two bivalents: the left auto-orientated, the right co-orientated; three pairs of precociously separated chromosomes, which obviously have been somatically paired.
- (f) Two bivalents and three pairs of univalents, of which two lie on the plate and one off the plate; the right univalent pair auto-orientated.

have remained as univalents. In the first metaphase they show more or less strong attempts at somatic pairing, lying scattered around the equator.

To obtain an idea of the frequency of the various modes of chromo-

some behaviour the conditions in 71 cells at first meiotic metaphase have been analysed as follows :—

32 cells : all the chromosomes had conjugated and the five bivalents were orientated fairly regularly.

15 cells : only bivalents present but they showed disturbances in their orientation.

20 cells : one pair of chromosomes had remained as univalents. These were situated as follows :—

(i) 9 cases—the members lay correlated on opposite sides outside the metaphase plate.

(ii) 4 cases—the univalents were still paired somatically and were situated on the plate.

(iii) 7 cases—again the two univalents lay more or less side by side outside the plate.

2 cells : two pairs of univalents. In one of them one pair of the univalents was on the same side of the plate, while the members of the other pair lay on opposite sides. In the other cell the members of both the pairs occupied correlated positions at opposite poles.

One cell : three pairs of univalents. In this case the members of all the pairs were situated correlated on opposite sides. And finally, one cell (fig. 3a) with all the chromosomes unpaired was scored.

As seen from the figures and the above analysis the differences in pairing and orientation lead to desynchronisation in anaphase separation of the different chromosome pairs. The first to separate are the somatically paired univalents which have been co-orientated on the metaphase plate. The next to separate seem to be the halves of the bivalents, but even these do not segregate simultaneously. The auto-orientated chromosome pairs necessarily encounter great difficulties in their separation. No rule can be given in regard to somatically paired univalents which have never reached the metaphase plate ; their defective orientation also renders their separation variable. In fig. 3b an incipient anaphase is seen. Two pairs of chromosomes, which probably have been somatically united, have already separated. The third pair of univalents is auto-orientated and consequently shows no attempt to separate. The halves of the two bivalents are only just about to separate. It must be pointed out that the univalents, once separated, never return to the metaphase plate to divide in the first division, as is the case in many other organisms (*cf.* Ribbands, 1937).

In view of the great deviations from normal meiosis characteristic of *O. graminifolium* it is remarkable that the results of the first division are not more abnormal. This fact must in the first place be ascribed to the property of somatic attraction which allows the fairly regular somatic pairing of the univalents and their subsequent segregation. Though the chromosome pairs differ in the timing of their separation, in the end the chromosomes segregate, ensuring the formation of anaphase groups with five chromosomes each. In fig. 3c a regular anaphase separation is illustrated. Naturally, the unorientated chromosomes are bound to give rise to uneven chromosome numbers. That this is not, however, of very common occurrence is seen from

the normal course of the second division. A regular second division metaphase with five chromosomes in each plate is seen in fig. 3*d*. The tetrads also appear normal, no micronuclei being visible.

FIG. 3.—Meiotic stages in PMC of *Ornithogalum graminifolium*.

- (a) Metaphase I showing five pairs of somatically paired univalents.
- (b) Anaphase I with two bivalents, two pairs of precociously separated univalents, and one auto-orientated pair of univalents.
- (c) Regular anaphase I.
- (d) Metaphase II with five chromosomes in each plate.

(ii) *Ornithogalum caudatum*

Mitosis.—This species possesses 54 somatic chromosomes in its root tip cells. As appears in fig. 4*a* the smaller chromosomes, especially, often lie in pairs in metaphase, thus showing, though to a lesser degree, the property of somatic pairing which is so well developed in *O. graminifolium*. The metaphase plates vary, however, in regard to the degree of somatic pairing. In the interphase nuclei in the root tips two nucleoli and about 20 Feulgen-stained chromocentres are visible (fig. 4*b*).

Meiosis.—The pachytene nuclei reveal the paired chromosome threads, in which some ten more or less condensed regions are visible (fig. 4*c*). These chromocentres are distinct in all the pachytene

stages examined, and their number is fairly constant. They do not appear to be sticky, and accordingly they do not show any tendency to run together. As meiosis proceeds the chromocentres remain

FIG. 4.—*Ornithogalum caudatum*.

- (a) Metaphase plate from a root-tip cell with 54 chromosomes ; the smallest chromosomes lie in pairs.
- (b) Interphase from a root-tip cell showing two nucleoli and some twenty chromocentres.
- (c) Pachytene with eleven chromocentres.
- (d) Diakinesis with seven large and twenty small bivalents.
- (e) Side view of metaphase I ; the smaller bivalents show secondary association.
- (f) Metaphase I ; the smaller bivalents are secondarily associated.
- (g) Metaphase I ; the smaller bivalents are secondarily associated.

condensed, while the rest of the chromosomes gradually catch up with them.

In diakinesis (fig. 4d) 27 bivalents—7 larger and 20 smaller—are visible. They are spaced around the nuclear cavity and do not exhibit any secondary pairing.

The first metaphase, however, is characterised by a strong secondary association of the smaller bivalents (figs. 4*e*, *f*, *g*). The seven large bivalents seem to be spaced at random, whereas the twenty small ones, as a rule, lie in pairs. This is apparent in side views as well as in polar views. The different plates show slight variations in this respect, but usually the phenomenon is very regular.

The first anaphase also proceeds normally. In a few cells inversion bridges are visible. Fig. 5*a* illustrates a first division anaphase where the centromere of one chromosome has misdivided; the two longer arms are going to one pole and the two shorter ones to the other (*cf.* Darlington, 1939).

FIG. 5.—Meiotic stages in *Ornithogalum caudatum*.

- (a) Anaphase I; the centromere of one chromosome has misdivided.
- (b) Metaphase II; the smaller chromosomes show secondary association.

In the second division metaphases, secondary pairing is as pronounced as in the first. Fig. 5*b* represents a second division metaphase in which the smaller of the 27 chromosomes lie side by side. As a rule, the second division is regular and the tetrads also appear normal.

4. DISCUSSION

(i) *Chromosome attraction and repulsion in the light of the precocity theory*

It is a generally acknowledged fact that the mutual relations of the chromosomes in mitosis and meiosis are governed by two main forces. On the one hand, two homologous genes—and consequently the chromosomes containing them—attract each other specifically. On the other hand, the chromosomes show an unspecific body repulsion

(cf. Darlington, 1937). How these forces act and in what way they are interrelated with other phenomena involved in mitosis will, be discussed in the following.

In normal mitosis the intrachromosomal mechanism, *i.e.* the cycle undergone by the chromosomes, acts strictly in step with the extra-chromosomal mechanism, which is represented by the spindle. According to Darlington (1937), the meiotic phenomena can best be brought into relation with mitosis by assuming that the two mechanisms are out of step. In meiosis the external changes are precocious as compared with the internal changes. This precocity theory has been developed further by Oksala (1943, 1944). In his study on the cytology of the dragonfly genus *Aeschna*, Oksala (1944) has shown that precocity is a phenomenon which develops gradually during the spermatogonial divisions. It finally reaches a point when the extra-chromosomal mechanism acts twice while the chromosomes divide only once, which is the very process essential in meiosis. According to Oksala the precocity implies the occurrence of the polarised stage in the cell at a time when the chromosomes are still single. It has been especially emphasised by him that the question is whether the chromosomes are *functionally* single or double, irrespective of the actual number of chromonemata present in them. The effectively single chromosomes react to polarisation by pairing, while in the double chromosomes the attraction is satisfied by the presence of an identical partner chromatid. In the words of Oksala (1944) p. 26) :

“Dann würde das in frühesten Phasen der Meiose bemerkbare, eigenartige, von allen mitotischen Vorgängen krass abweichende Verhalten der Chromosomen, also gerade die Konjugation, nicht von der frühreifen Prophase, sondern von der eben in dieser Phase entstehenden frühreifen Polarität herrühren. Es würde sich mithin um eine entweder unmittelbar nach der Telophase oder in der frühen Interphase entstehende Metaphasesituation handeln, auf die die einfachen Chromosomen durch Konjugation reagieren und so die Doppelgestalt und zugleich also die Fähigkeit der polaren Orientierung erlangen.”

It may be mentioned in this connection that Klingstedt (1939), on the basis of certain purely morphological similarities, came to the conclusion that leptotene and metaphase are related stages.

As a rule, the attraction of homologous chromosomes seems to be saturated in the double chromosomes in mitotic prophase and in the paired chromosomes in meiosis. We have, however, instances in which a so-called residual attraction remains, which gives rise to somatic and secondary pairing of the chromosomes. In regard to the forces concerned we may cite Darlington (1934, p. 107) :

“It is then possible to suppose that a common force of attraction exists between identical genes and that this attraction is almost or entirely saturated by the approximation (or association) of genes in pairs. Secondary attraction, A_2 , is then a residual attraction and its variation is merely the variation of degree of saturation by pairing. In meiosis in the male *Drosophila* the saturation reaches its

lowest level. Forces of repulsion due to surface charge (R_1) and localised charge at spindle attachment (R_2) and spindle poles (R_3) can likewise all be considered most conveniently as a unit in regard to physical causation."

As was mentioned above, one of the results of residual attraction is the somatic pairing of the chromosomes. This involves a tendency of the homologous chromosomes to be situated closer together than non-homologous chromosomes during mitotic stages. This phenomenon is to a slight degree revealed by many organisms. To prove it, in many cases a statistical analysis of the frequency of the various chromosome arrangements in metaphase would be needed (*cf.* Upcott, 1936). Somatic pairing is very pronounced in polyploid plant cells which have arisen due to endomitosis, a condition realised, for instance, in many *Chenopodiaceae* plants. In the metaphase following the endomitotic chromosome doubling, the homologous chromosomes lie regularly in two's.

Somatic pairing is, however, most strongly developed in various *Diptera*. In these the homologous chromosomes, whether two or more, are regularly situated side by side. Moffett (1936), in *Culex pipiens*, has found an attraction of chromosomes at all stages of mitosis, being, however, at a minimum in metaphase. If we apply to somatic pairing the views developed above, we come to the following conclusions. It might be thought that the precocity of the external changes in the cell, which plays such a decisive role in regard to the chromosome conjugation in meiosis, also gives rise to somatic pairing in the *Diptera*. We need only assume that in the mitoses of the *Diptera* a slight precocity prevails which results in the fact that the chromosomes during the various mitotic phases are relatively single as compared with other organisms. This precocity is not strong enough to give rise to actual pairing but the functionally single chromosomes attract each other and pair somatically. This is in agreement with the fact that somatic crossing-over has occasionally been reported in *Drosophila* (*cf.* Darlington, 1937).

This ability of the chromosomes to pair somatically has provided a starting-point for the evolution of the various types of meiosis found in the *Diptera* which so greatly deviate from the normal (*cf.* White, 1949). In the most primitive forms chiasmata are present in both sexes; the sex chromosomes, however, do not form chiasmata in the male. The behaviour of the sex chromosomes resembles the distance conjugation observed in the *Neuroptera* (Klingstedt, 1933). According to White, *loc. cit.*, two main evolutionary lines may be derived from this primitive type. In one of them chiasmata are present in the male, but the sex chromosomes have disappeared as separate units. The other line again is characterised by the fact that the sex chromosome mechanism is retained, the chromosomes in the male being, however, devoid of chiasmata. From this a side line has developed, in which the whole chromosome cycle is quite anomalous. The highest *Diptera*, which may also be derived from the line in which chiasma formation

fails in the male, again have a pairing segment in their sex chromosomes. A further case which possibly has a bearing on the present problem is the touch-and-go pairing of the sex chromosomes in certain Hemiptera (*cf.* Wilson, 1905 ; Darlington, 1937).

Another phenomenon which is accounted for by the residual attraction is the secondary association of the chromosomes. It is often seen, especially in plants with small chromosomes, that the bivalents are not spaced at random on the metaphase plate, but lie in twos or in groups containing more than two chromosomes. As a rule, the bivalents involved are of the same shape and size. The first to pay conscious attention to this phenomenon has been Darlington (1928) in his study on *Prunus*. Lawrence (1931) in his comprehensive survey on this subject, has presented a great number of examples in which secondary association has been described or is to be inferred. According to Lawrence the following features are characteristic of secondary association. It is not present in diakinesis, but in the first metaphase the—obviously related—bivalents arrange themselves in pairs or groups of more than two. They lie near each other, but do not touch. The same tendency is exhibited in the second meiotic division. That this attraction of certain bivalents indicates their homology seems to be an established fact (*cf.* Meurman, 1933 ; Darlington, 1937). Indeed, this phenomenon has been used to throw light on evolution and relationships in various plant groups, secondary association being regarded as a token of ancient homology of the chromosomes involved.

If we try to establish a common ground for the interpretation of the phenomena in which chromosome attraction plays a role, we come to the following conclusions. As recapitulated above, the meiotic phenomena may best be explained as a result of the precocity of the external changes in the cell. In accordance with this, somatic pairing might be regarded as due to a slight degree of precocity. This, in turn, leads to the chromosomes being either effectively single, or in a midway state between functional singleness and doubleness (*cf.* Huskins, 1948), which again gives rise to an attempt of the homologous chromosomes to pair. It seems probable that secondary association would be explicable in a like manner. A somewhat similar view has been presented by Svärdson (1941) in regard to somatic pairing in fishes belonging to the genera *Salmo* and *Coregonus*. In these animals the somatic attraction of the chromosomes is most pronounced during anaphase and telophase, and Svärdson ascribes this fact to the singleness of the chromosomes at these stages. It must finally be stressed that the views developed above represent only the basic line of thought. The interpretation of the various individual cases will certainly require additional hypotheses.

To sum up : There seems to be two main conditions determining the degree of chromosome attraction. First, we have the relative singleness or doubleness of the chromosomes. As a rule, the highest

degree of relative doubleness is reached in the metaphase stage. This obviously causes somatic pairing in many organisms to be at a minimum in metaphase (for instance, *Culex*, *Salmo*, *Coregonus*). A factor acting in the opposite direction is that the repulsion forces between the chromosomes, which are so strong both in mitotic prophase and in diakinesis, are to a considerable extent diminished when the metaphase stage is reached. It seems that the latter factor is in many cases decisive, for instance, the secondary pairing characteristic of many plants is apparent only in metaphase. In general it can be said that the degree of somatic and secondary association is the resultant of these two tendencies.

(ii) Somatic and secondary pairing in *Ornithogalum*

The chromosome behaviour in the two *Ornithogalum* species shows that the same causes bring about the same results at the cytological level in the most diverse organisms. The phenomena which the capacity for somatic pairing has made possible in *Ornithogalum*, resemble those found, for instance, in the Diptera.

The features characterising *Ornithogalum caudatum* are common to many other plants. The lists given by Lawrence (1931) and Darlington (1937) show that a great number of plants with small chromosomes exhibit secondary pairing in meiosis. In *O. caudatum* the seven large bivalents do not show any attraction, while the twenty smaller ones are associated in pairs. This may be interpreted in either of two ways. The seven large bivalents may actually be unrelated, while doublings have occurred among the smaller chromosomes, leading to the formation of the chromosome complement of *O. caudatum*. Or some of the larger chromosomes are related, but their size prevents any secondary association. A comparative study of the genus would throw light on this point.

The somatic attraction shown by the chromosomes in *O. graminifolium* is even stronger than in *O. caudatum*. Consequently the chromosome behaviour also deviates more from the normal. On the hypothesis developed above it would be supposed that the somatic attraction in this case too, depends on a slight degree of precocity in the external mechanism of the cell. The occurrence of chromatid bridges in the mitotic anaphases also fits in well with this line of thought. If the chromosomes are not completely divided, *i.e.* functionally double, when the anaphase separation begins, the formation of chromatid bridges is only to be expected (*cf.* Darlington and La Cour, 1940).

It has been pointed out by Oksala (1943) that the variable behaviour of univalents at meiosis in different organisms—especially their division in the first or second division respectively—must depend on the degree of precocity characterising the meiosis in each individual case. If the external changes in the cell are only slightly advanced,

the univalents have time to divide during the first division. If, on the other hand, precocity is strong, their division is postponed to the second division.

The precocity of the external changes which is present in the mitotic divisions of *O. graminifolium* is naturally even more pronounced in its meiosis. A number of chromosomes which obviously have been paired in pachytene, later fall apart owing to lack of chiasmata. In diakinesis the chromosomes which have remained as univalents repel each other as do the bivalents. In prometaphase the repulsion lapses and the chromosomes come together on the metaphase plate. Now if we suppose that the precocity is strong, the univalents are not yet functionally double and the chromosomes attract each other, pairing somatically. The fact that the univalents never divide during the first division is in accordance with this interpretation.

The various chromosomes in a single cell often represent different stages of the developmental cycle, and the anaphase separation does not take place simultaneously in the different chromosome pairs. Considering the deviations from normal meiosis found in *O. graminifolium*, its ultimate results are remarkably regular. As in the Diptera, somatic attraction has enabled the development of a new type of meiosis which, in its effects, however, resembles normal meiosis.

It must be noticed that in the present case there is no greater tendency to remain as univalents shown by either the larger or the smaller chromosomes. This again ensures that the genetic variation which is brought about by crossing-over is maintained in regard to all the chromosome types.

A case which in certain of its features resembles *Ornithogalum* has been described by Ribbands (1937) in a *Lilium* hybrid. In this plant too, a variable number of chromosomes which have paired in pachytene fall apart later owing to a failure of chiasma formation. In diakinesis they repel each other only slightly, retaining a clear "position correlation." In metaphase even this slight repulsion is overcome and the homologous chromosomes pair somatically, and subsequently separate. In consequence the homologous chromosomes occupy a correlated position on the spindle during the whole first division. At late metaphase a number of the univalents return to the plate and divide.

Somatic and secondary pairing have generally been interpreted as expressions of the homology of the chromosomes concerned. The homology may be more or less pronounced, even ancient relationships of the chromosomes coming to light in this way. Another explanation has been advanced by Thomas and Revell (1946) in regard to secondary pairing of bivalents observed in *Cicer arietinum*. In this plant secondary association between the eight bivalents does not show any specificity. In an autotetraploid derivative, however, the association seems to be preferential between homologous bivalents. Thomas and Revell assume that secondary association in this case

has originated from a fusion of heterochromatic chromosome regions in pachytene, which continues through the intervening stages into metaphase.

This explanation does not seem to hold in the case of *Ornithogalum*. It is true that both the species now examined possess well-developed chromocentres. These do not, however, show any tendency to fuse either in mitosis or in meiosis. On the contrary, the heterochromatic bodies seem to be more condensed than the other chromosome regions. It should be pointed out that the heterochromatic bodies, in the pachytene of *O. caudatum*, for instance, are remarkably constant in number, no variation which could be interpreted as due to fusion being observable. In *Ornithogalum* the attraction of the chromosomes, both somatic and secondary, is quite obviously between homologous chromosomes. The units which show these kinds of association are similar in size and shape. A further fact corroborating the older view concerning secondary association is that in *O. caudatum* the chromosomes do not show any association in diakinesis, this feature becoming apparent only at metaphase. Since little attention has been paid to possible fusion of heterochromatic chromosome regions it is uncertain which interpretation applies to secondary association in other organisms in which it has been reported to occur.

5. SUMMARY

1. The chromosome behaviour of two *Ornithogalum* species, *O. graminifolium* ($2n = 10$) and *O. caudatum* ($2n = 54$), has been studied both in mitosis and meiosis. The characteristic feature of both these species is a strong tendency to somatic and secondary attraction, which is obviously an expression of the homology of the chromosomes concerned.

2. In *O. caudatum* a variable number of the smaller chromosomes lie in pairs in the mitotic metaphase plates. In the first division metaphase the twenty smaller bivalents are situated in twos, while the seven larger bivalents are spaced at random. A similar association is revealed by the chromosomes in second division metaphase plates. The course of meiosis is fairly regular.

3. In *O. graminifolium* the somatic attraction of the chromosomes, as seen especially in the mitotic metaphases, is even more pronounced than in *O. caudatum*. In meiosis the same tendency is reflected by the behaviour of the univalent chromosomes. In many cells a number of chromosomes which have been paired in pachytene fall apart owing to absence of chiasmata. They repel each other in diakinesis, but come together and pair somatically on the first division metaphase plate. Univalents never divide during the first division.

4. The somatically paired univalents in *O. graminifolium* are, as a rule, co-orientated on the metaphase plate. In a few cells, however, they show auto-orientation. In the former case a normal segregation takes place. Meiosis in this plant, though deviating from the normal,

is remarkably regular in its ultimate results. It thus bears a resemblance to certain exceptional modes of meiosis found in the Diptera, whose chromosome behaviour too is determined by the property of somatic pairing.

5. Chromosome conjugation in meiosis is brought about by the attraction of homologous chromosomes which are single. It has been supposed that for somatic and secondary attraction of the chromosomes a so-called residual attraction is responsible. These two attractions may be brought into relation with each other as follows. In accordance with the precocity theory of meiosis residual attraction may be thought to be due to the fact that the external changes in the cell are more or less precocious relative to those internal to the chromosomes. This, in turn, leads to the chromosomes being more or less *functionally single* at stages when in other organisms they are double. This state of affairs again accounts for the occurrence of residual attraction between the chromosomes. The degree of chromosome association is determined by two factors: the degree of doubleness of the chromosomes, which is at a maximum in metaphase, and the diminishing body repulsion of the chromosomes when prophase or diakinesis is succeeded by metaphase.

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Plate

Microphotographs of PMC in *Ornithogalum graminifolium* and *O. caudatum*.

- FIG. 1.—Metaphase I in *O. graminifolium*. FIG. 2.—Metaphase I in *O. graminifolium*
Cf. text fig. 2 f. with a somatically paired univalent pair
on the plate.

- FIG. 3.—Metaphase I in *O. caudatum*; the small bivalents show secondary association.
Cf. text fig. 4 g.

- FIG. 4.—Side view of metaphase I in *O. caudatum*; secondary association of the smaller
bivalents. Cf. text fig. 4 e.

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