HOMOLOGOUS AND SIMILAR CHROMOSOMES IN DIPLOID AND TRIPLOID HYACINTHS

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INTRODUCTION

The results of researches into the genetic consequences of the triploid or tetraploid state of one or more of the sets of homologous chromosomes, together with the genetic consequences of segmental interchange between paired homologous chromosomes, have shown that, with certain exceptions, a chromosome can be regarded, in its essentials, as a group of genes. When we find that there are two chromosomes of each size and shape, and that at the metaphase of the first maturation division the chromosomes are connected in pairs, forming independent bivalents, the two members of each pair passing to opposite poles, without interference with the members of any other pair; then we may infer that, apart from species hybrids and similar exceptions, the ordinary diploid segregation of genes is taking place. If the plant has in its chromosome group four chromosomes of each size and shape, and at the first maturation metaphase each of these sets of four chromosomes forms a quadrivalent, we may conclude that tetraploid segregation of genes will be found. On the other hand, if there are four chromosomes of each shape and size, which, however, form two bivalents that do not attract each other; then the plant may not be tetraploid, nor show tetraploid inheritance, but may have descended, either through
tetraploidy or by some as yet unknown process, from an ancestor with the same chromosome sizes, but with only half the number. It would then probably show duplicate genes. In this case it can be called a double diploid. It is, however, possible that such a chromosome grouping might have originated in some other way, without the presence of duplicate genes.

Several species of flowering plants, both dicotyledons and monocotyledons, have been examined by the writer with regard to this point. *Dahlia imperialis*, a wild species sometimes cultivated, was found to possess 16 pairs of chromosomes; while the ordinary garden *Dahlia* has 32 pairs (as reported by Ishikawa 1911). But the chromosomes of the latter could not be satisfactorily classified as to their sizes, and quadrivalents were not apparent. *Tradescantia virginiana* showed at the first maturation metaphase, in addition to many bivalents, occasional rings and strings of four chromosomes each, while a related genus is known to have half the number of chromosomes, but the sizes were not sufficiently distinct to use as a means of distinguishing homologous or similar chromosomes. In the oriental hyacinth of cultivation, however, as has long been known, there are four small chromosomes, four medium, and eight large. The problem was, therefore, to determine whether the four chromosomes of each size class were identical in length, breadth and position of constriction; and whether, if apparently identical, and associating in pairs at the first metaphase, as found by Miss Hyde (1909), there was any obvious mutual attraction between the two pairs themselves. Also, whether the eight large chromosomes were divisible into two classes of four each, as Miss Hyde’s observations seemed to indicate. Since triploid hyacinths were also known, the matter gained in interest, for the behavior of the triploid Daturas and Cannas at the first maturation division, only enlightened us as to the behavior of chromosomes united end to end in the bivalents or trivalents; whereas the constituent chromosomes of the large bivalents of the hyacinth were united at other points, and often had free ends. If trivalents occurred, those formed by the long chromosomes should be instructive with regard to the mutual connections. In addition, it was necessary to ascertain whether the size relations of short, medium and long chromosomes found in the haploid group in the pollen grain, where measurement is comparatively easy, agreed with those determined by de Mol (1921) from the diploid group of chromosomes in the root tip. When these points were cleared up, the work might form a contribution to the evidence as to whether such a quadruple chromosome grouping had arisen through ancestral tetraploidy, through species crosses, through inherited fractures
of chromosomes at the constrictions, through permanent combinations of chromosomes, or by some as yet unsuspected process.

HISTORICAL RÉSUMÉ

Osa wa (1920) figured and briefly alluded to the attachment of some of the univalent chromosomes to bivalents in the first metaphase of a triploid Morus. In 1921, the writer described and figured pollen mother cells of a triploid Canna ("Gladiator"), in which all the 27 chromosomes were normally in trivalents at the first metaphase (Belling 1921). Another triploid Canna ("Firebird") has since been found in the same condition. In some specially clear figures observed in late autumn, the three constituent chromosomes of each trivalent were connected by fine threads during the early anaphase; so that it could be seen that two went to one pole and one to the other, and that this was a random process, with regard to the numbers passing each way. In the next year it was shown (Belling and Blakeslee 1922) that the presence of trivalents in diakinesis and first metaphase was normal in triploid Daturas. It was also demonstrated that the assortment of the 12 extra chromosomes into two groups accorded with the laws of chance, with a slight excess, however, of the more unequal divisions. *Hemerocallis fulva* (growing by the roadside at Cold Spring Harbor) appears to be a somewhat irregular triploid, in which, however, the 11 trivalents can occasionally be made out. No one apparently has observed trivalents in triploid Oenotheras. The triploid Solanums of Winkler (1916), and the triploid mosses obtained by Schweitzer (1923), seem not to have been examined with regard to this point.

In 1921 De Mol described four triploid hyacinths, but confined his observations almost wholly to the chromosomes of the root tips. The writer then began a study of the chromosomes in the pollen grains, and the bivalents and trivalents in the pollen mother cells of hyacinths, making use of the iron-acetocarmine method (Belling 1921 b, 1923). One advantage of this method is the rapidity with which fixation occurs, though hardening of the chromosomes and cytoplasm takes some days. A criterion of good fixation for plant chromosomes is perhaps of use. The writer is inclined to consider that if the metaphase of the second division in the pollen mother cell, or the metaphase of the first division in the pollen grain, shows the longitudinal splits and the constrictions distinctly, in all the chromosomes whose positions admit of these being seen, then the fixation can not be far from complete. Applying the usual fixatives after removing the pollen mother cells from the anther, gives excellent results as to fixation, as was shown by Taylor (1922).
In Datura the two chromosomes of each bivalent are joined at the ends, and the same is true of the trivalents (Bell and Blakeslee 1922). No clear case of any other junction has yet been seen at diakinesis or first metaphase. In Canna the bivalents and trivalents resemble those of Datura in this respect. In Hyacinthus, however, the large chromosomes in the bivalents and trivalents are connected at other points; and the bivalents often resemble the single or double rings described in certain animal maturation divisions.

That there were eight bivalents in *H. orientalis*, at the first division of the pollen mother cell, was observed 15 years ago by Miss Hyde (1909). The same observer noticed that the pollen mother cells in the bulbs underwent the maturation divisions at the end of October and during the early days of November. Three years after, root tips of the variety *albulus* were found to have 8 long V chromosomes, 4 medium, and 4 short chromosomes (Müller 1912). More recently, fracture (or possibly constriction) of a chromosome was seen occasionally in the somatic divisions of the same variety (Carruthers 1921). The examination of the root tips of a number of clonal varieties of *H. orientalis* (De Mol 1921) showed 19 diploids, 4 triploids, 5 plants with fewer chromosomes, and 5 plants with more chromosomes than triploids. *Hyacinthus romanus* Desf. had only 4 pairs of chromosomes in the root tips (De Mol 1921). Three years later it was demonstrated that the eight additional long, medium and short chromosomes were distributed at random in the pollen grains of a triploid hyacinth (Bell 1924).

For three autumns the writer has observed 3 clonal varieties of Dutch hyacinth: (1) A diploid clone with pale bulbs and sulfur-yellow flowers, which can be easily identified. It is listed as “Yellow Hammer.” (2) A triploid clone, catalogued as “Lady Derby,” with purple outer bulb scales; the flowers suffused with pink, and turning red when they fade. (3) A triploid clone, listed as “King of the Blues,” flowering later; with dark purple bulb scales and deep violet-blue flowers. There has appeared no cause to consider any of these three strains as different from the strains of the same names and characters examined by De Mol (1921).

**The Diploid Hyacinth**

*Chromosomes*

This plant showed 4 long, 2 medium, and 2 short chromosomes in the pollen grain (figure 1), and also at each pole at the anaphase of the second division of the pollen mother cell (figure 2). De Mol (1921) found 8 long,
4 medium, and 4 short chromosomes in the cells of the root tips. At the metaphase of the first division in the pollen grains, only those chromosomes which happen to be horizontal can be accurately measured. This was naturally more rarely the case with the long V's than with the shorter chromosomes. In this haploid group of 8 chromosomes, two pairs of different chromosomes can be readily made out (figures 1 and 2), and the presence of four different pairs seems indicated. Of the 4 large V-shaped chromosomes, each with a clear constriction at the center of the V, 2 were sometimes seen with a second constriction (visible in three of the chromo-

![Figure 1](image1.png)

**Figure 1.**—Haploid group of chromosomes in the metaphase, in the pollen grain of the diploid hyacinth, "Yellow Hammer." The constriction where the spindle fiber is attached is evident in all the chromosomes. All but one of the long chromosomes are more or less foreshortened. (Camera drawing, made with Zeiss water-immersion apochromatic objective 70; as were all the subsequent figures.)

![Figure 2](image2.png)

**Figure 2.**—Four haploid groups at the late anaphase of the second division in the pollen mother cell of the diploid hyacinth. In three of the groups two of the long chromosomes show a second constriction.

some groups in figure 2); but it could not be determined whether this second constriction was invariably present in these two. The 2 medium J chromosomes had a clear constriction at the angle of the J, one-quarter of its length from one end. No difference in length was perceptible between the two similar J's, nor was any difference of shape noticed. These medium J's were about half the length of the long V's. The members of the pair of short similar chromosomes were not distinguishable from one another in length, nor were differences of shape seen. Each had a clear constriction one-third of its length from one end, and was about half as long as a medium chromosome.

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To test whether there might be any small difference in length between the similar chromosomes of the haploid pairs in the pollen grain, about 20 metaphase chromosomes of each kind were drawn with the camera at a magnification of 710, and measured with dividers under a binocular magnifying 3.5. The chromosomes were selected from eight pollen grains of a triploid, and were chosen for measurement because of their horizontality. Twenty of the short chromosomes varied from 5.0 to 7.0, and averaged 5.9. They seemed to belong to one size group. Twenty-one of the medium chromosomes varied from 9.5 to 12.5, and averaged 10.5. All were in one class. But 17 of the long chromosomes varied from 18.0 to 30.5, averaging 23.1. The variation in length of the large V’s was conspicuous, though care was taken only to measure those that appeared horizontal. In another pollen grain where the two groups of chromosomes at the anaphase were not far from horizontality, sufficient pressure was applied to squeeze the groups out flat, and the chromosomes were then drawn at a magnification of over a thousand, and measured under the binocular as before. The unit of measurement was twice as large as that used above. Each chromosome was matched with its fellow from which it had split, and the differences were slight, affording thus a proof of horizontality. The four small chromosomes averaged 7.2 (ranging from 6.5 to 8.2), the segments being 4.9 and 2.4. The 6 medium chromosomes averaged 13.3 (ranging from 13.0 to 13.5), the segments being 10.0 and 3.3. The 8 long chromosomes averaged 26.6 (ranging from 24.2 to 32.2), the segments being indistinguishable in length. (All these pollen grains were from the clone, “Lady Derby.”) Thus in the measurements at the metaphase, the lengths of small, medium and large were as 1.0 : 1.8 : 3.9; while in the measurements at the anaphase the ratio was 1.0 : 1.8 : 3.7. These are not far from 1 : 2 : 4. (De Mol’s measurements of root-tip chromosomes gave different figures, the long chromosomes appearing relatively much shorter.) On the whole then we may consider that each of the two pairs found in the haploid group consists of two members indistinguishable in shape and size. This is also indicated, but not yet proved, for the two pairs which probably make up the four long chromosomes of the haploid. Hence, the size formula for the diploid group may be put as 8L + 4M + 4S, or possibly 4L + 4L’ + 4M + 4S.

Bivalents

At the first division in the pollen mother cells, as has already been described (Hyde 1909), there are found 8 bivalents: 4 large, 2 medium and 2 small (figures 3 and 4). These bivalents were in the metaphase,
good examples of the late prophase not having been found in the buds studied. The bivalents were especially examined in 51 pollen mother cells selected as showing the 8 bivalents without overlap. Only those bivalents were considered which presented clear configurations with little or no foreshortening. The long bivalents included 14 cases of the loop with two free ends, 10 X's, 5 cases of the loop with four free ends, and 2 figures of eight. The medium bivalents comprised 7 truncated A's, 5 X's or crosses, and 2 loops with free ends. The 16 short bivalents were crosses, mostly with two shorter transverse arms (figures 3 and 4). The metaphase chromo-

![Figure 3](image3.png)

**FIGURE 3.**—First metaphase in the pollen mother cell of the diploid hyacinth, showing the 8 bivalents. In three of the large bivalents the constituent chromosomes are connected at two points, and in the other large bivalent, at three points. The constituents of the medium and small bivalents seem connected at one point only.

![Figure 4](image4.png)

**FIGURE 4.**—First metaphase in the pollen mother cell of the diploid hyacinth, the cytoplasm with chromosomes having been squeezed from the cell. The chromosomes of two of the large bivalents are connected in two places, while the other two show only one connection.

somes were closely corrugated. At the anaphase (figure 5), each large bivalent separated, as is usual, into two split double chromosomes; the two V's of each double V being connected, after the well-known fashion, at the constriction, where the spindle fiber was attached. Each medium bivalent also separated at the anaphase in the ordinary way into two double J's, the halves being connected at their points of constriction; and the same happened to the small bivalents. These anaphase chromosomes still showed the corrugations, but became smoother before the second division in the pollen mother cell. The methods of connection, of separation, and of splitting of the two chromosomes of each bivalent have not yet been completely followed.

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Similar bivalents

Fifty-one pollen mother cells in the first metaphase, selected as showing the 8 bivalents without overlap, were examined, as already mentioned. The number of times the two small and the two medium bivalents, respectively, were near enough together to be regarded as constituting a pair, was noted, as well as the number of cases in which other chromosomes were in equal proximity to any one of these. Considering first the small bivalents: there was, for the left-hand one of the two, a total of 114 potential partners, including the other small bivalent when necessary. For the right-hand small bivalents, there were 110 possible partners. The average then is 112 apparent pairings for either one of the two small bivalents. On mere chance proximities, the other small bivalent should only be a partner in one-seventh of these potential pairs, because it is one out of 7 other bivalents. Hence there should have been found, on the average, 16 pairs of small bivalents. But actually there were 37 cells in which the two small bivalents were close enough together to be reckoned as a pair. The difference is 21.0 ± 2.5, which is well marked. For the medium bivalents the average number of such potential pairings was 141, giving 20.1 for the calculated number of chance proximities of two medium bivalents. Actually, 26 such pairs were found. The difference is 5.9 ± 2.8, which is not significant, taken alone.

Further, the first 16 metaphase plates were drawn with the camera, at a magnification of 520, and the distances were measured under a binocular: (1) between the two small bivalents; (2) between the two medium bivalents; and (3) between each of the two small and each of the two
medium bivalents. The average distance between two small bivalents was 4.0; that between two medium bivalents was 8.3; while the average distance between a small and a medium bivalent was also 8.3. This mutual proximity of the small bivalents might have been due, wholly or in part, to the well-known fact that the smallest chromosomes are usually nearest the center of the equatorial plate, and the largest on the periphery. To ascertain this, the distances of the bivalents from the center of the cell, as seen in sectional view, were measured for the 16 pollen mother cells in question. The average distance of the 32 small bivalents from the center was 4.2, while the average distance of the

![Figure 6](image)

**Fig. 6**—Late prophase in the pollen mother cell of the triploid hyacinth, "King of the Blues." Three of the large trivalents and one of the medium trivalents overlap somewhat.

![Figure 7](image)

**Fig. 7**—Late prophase in the pollen mother cell of the triploid hyacinth, cytoplasm and chromosomes having been squeezed from the cell. The three chromosomes can be traced in most of the trivalents.

medium bivalents from the center was 8.5. Hence the small bivalents were on the average only half as far from the center of the cell as were the medium bivalents. If we imagine two circles, one with a radius twice that of the other, it is obvious that two bivalents spaced at random on the outer circle should average twice as far from one to the other as would two bivalents spaced by chance on the inner circle. The distance between two medium bivalents has already been found to average about twice the distance between two small bivalents, and the mean distances between small and medium bivalents are also in agreement. Hence, the attraction between similar small bivalents, if it exists, must be slight as compared with the drift towards the center.

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THE TRIPLOID HYACINTHS

The descriptions and figures refer to the clone, "King of the Blues," which presented more examples of favorable stages in the late prophase. Observations with regard to other stages made on the clone, "Lady Derby," gave similar results. These two hyacinths showed 6 short, 6 medium and 12 long chromosomes in the root tips (DE MOL 1921). One count made on each at the anaphase of the second division in the pollen mother cell seemed to confirm these numbers.

Trivalents

In the late prophase of the first maturation division there were 8 trivalents: 4 large, 2 medium, and 2 small (figures 6 and 7). No sexivalents were seen. Each long chromosome was usually connected with one or both of its two partners, in the comparatively few examples which showed this clearly, near one or both ends, and sometimes at intervening points. The medium chromosomes were mutually connected at fewer points, and the short chromosomes showed only 1 or 2 such junctions each. The chromosomes, especially the long chromosomes, were already more or less tortuous and somewhat corrugated. In figure 8, a and b, there are shown four trivalents of long chromosomes, from two pollen mother cells in the late prophase. In the two trivalents shown in 8a, two of the chromosomes are connected near both ends, and the third chromosome is connected with one of these junctions. The chromosomes cross and apparently fuse at several other points. These points seem to be equally distant from the connected ends of the chromosomes in question. In 8b, the chromosomes of the trivalent on the left are connected nearly end to end. In the trivalent on the right, two of the chromosomes are connected near one end.
of each, and the other chromosome is free except for one obvious lateral junction. In figure 8, one of the apparently original chromosomes of each trivalent has, for the sake of clearness, been made solid black. Nothing, however, is meant to be implied by this as to what chromosomes or parts of original chromosomes separate at the anaphase.

At the metaphase, the chromosomes of the trivalents are closely corrugated, so that the points of junction can not be clearly seen. At the anaphase of the first division, the double V's, and the medium and small double J's are still corrugated, as in the diploid hyacinth. The examination which has been made of the distribution of chromosomes in the pollen grains of the clone "Lady Derby," showed that the 8 extra chromosomes of the triploid were distributed at random to the pollen grains; and that all, or nearly all, of the pollen grains with extra chromosomes went through the first nuclear division, at least as far as the metaphase (BELLING 1924).

**DISCUSSION**

That *H. orientalis* has four equal or nearly equal long chromosomes (probably forming two pairs), one pair of similar medium, about half as long, and one pair of similar short chromosomes, about half as long as the medium, in the haploid group, might perhaps be taken as indicating near or remote descent from a species with half the number of chromosomes (namely, two long, one medium, and one short) in the haploid group. But the allied species, *Galtonia (Hyacinthus) candicans*, also has four equal or nearly equal long chromosomes, one pair of similar medium, about half as long, and one pair of similar short chromosomes, about half as long as the medium, in the haploid group, according to the figures of NEWTON (1924). These similar chromosomes do not show mutual attraction in the somatic anaphases or telophases of Galtonia, though the homologous chromosomes do. Unlike *H. orientalis*, however, the constrictions and spindle-fiber attachments in Galtonia are close to the ends of the chromosomes (NEWTON 1924). It is hence possible that the origin of the pairs of similar chromosomes in *Hyacinthus orientalis* may date back to before the separation of the closely allied genera, *Hyacinthus* and Galtonia.

With regard to the variable connections observed between the constituent chromosomes of the bivalents and trivalents, it has been assumed: (1) that they are accidental twistings of chromosomes, or openings out between chromatids; or (2) that they mark points where segmental interchange has occurred or might have occurred. There does not seem to be sufficient evidence as to which of these two is the preferable working hypothesis.
SUMMARY

1. The haploid group of the diploid hyacinth probably consists of four pairs (and certainly contains two pairs) of similar chromosomes, the members of each pair being indistinguishable in size, shape and position of constrictions. The ratio of the lengths of short, medium and long chromosomes in the pollen grain averaged in metaphase and anaphase, 1 : 1.8 : 3.8, which is not far from 1 : 2 : 4. The distances of the constrictions from the ends were one-third, one-fourth and one-half of the lengths of the chromosomes, respectively.

2. There was no perceptible attraction between the members of the pairs of similar bivalents at the first maturation division. The well-marked mutual proximity of the two small bivalents was due, mainly or wholly, to their drift to the center of the chromosome plate.

3. A sample of the long bivalents showed, at the metaphase, 19 rings or loops with two or four free ends, 10 X’s, and 2 figures of eight. The medium bivalents had 12 truncated A’s or crosses, and 2 rings; while the small bivalents gave 16 crosses only.

4. In the triploid hyacinths there were 8 trivalents: 2 pairs of large, 1 pair of medium, and 1 pair of small. At the late prophase, in the few cases which could be followed, two of the chromosomes of a large trivalent were sometimes seen to be connected near both ends, the third being joined near its end to one of these connections. Or, all three were joined end to end; or one might be connected laterally. Intermediate connections probably occurred also.

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