PRUNUS LAUROCERASUS L., A SPECIES SHOWING HIGH POLYPLOIDY.

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(With One Plate and Two Text-figures.)

In the earlier investigations of polyploid plants, the determination of the chromosome number and the deduction of the numerical relationships in the related species were the main objectives attempted. Little attention was paid to the state of association of the chromosomes in the reduction divisions and to the occurrence of irregularities. Although the knowledge of the chromosome number in different species is in itself of importance, it is probable that in many cases a closer study of the other features might often have yielded information on the nature of the polyploidy in the plant studied. The more recent investigations have indeed shown that many polyploids contain more than two homologous chromosome complements. The affinity between the corresponding chromosomes of these different complements may therefore lead to the formation of chromosome groups of a higher order, so that in the first division metaphase trivalents, quadrivalents, etc., are to be found. In the genus *Prunus* the triploid and tetraploid cherries constantly show such groups (Darlington, 1928). The polyploids of higher order in this genus, reported by Kobel (1927), had not been studied in detail in this respect. The highest chromosome number was found in *Prunus laurocerasus*, which plant is the subject of the present study.

The observations are limited to the reduction divisions of the pollen mother cells, the earlier prophase stages in a plant with more than a hundred small chromosomes naturally not being suitable for detailed observation. The material was collected from a plant in the Royal Botanic Gardens, Kew, London, on 9 February, 1928. The anthers at this time contained pollen mother cells in all stages from early prophase up to young pollen tetrads. The anthers were fixed separately in a modified Flemming solution (Darlington, 1928), embedded in paraffin and cut at 10μ. The preparations were stained by Newton's gentian violet method.

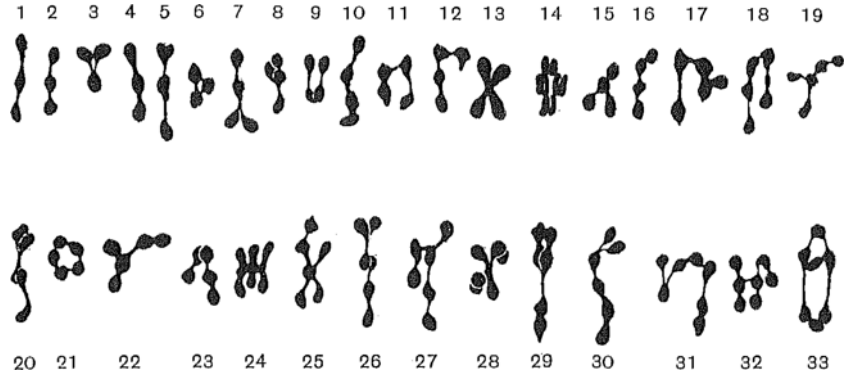
Cytological Observations.

Under low magnification, the equatorial plates seem very regular and in side views they appear as black ribbons across the cell. The separation of the chromosomes also proceeds at a regular pace and the entire process of meiosis looks normal. This appearance is, however, only due to the very high number of chromosomes; small disturbances, such as univalents outside the plates, lagging chromosomes, etc., being scarcely noticeable in relation to the whole mass. With a higher magnification it is at once obvious that the reduction division is far from normal. In side views of the first division metaphase in the pollen mother cells a few univalents are almost invariably seen scattered outside the plates. Such a view at this stage is shown in Pl. IX, fig. 1. All the univalents present, eight in number, are here figured, but only a small number of the chromosomes in the plate itself are drawn. A few very small fragments can also be seen lying in the neighbourhood of the plate or scattered in the plasma.

Although the plates mostly seem to consist of ordinary bivalents, many cases can be clearly seen where the chromosomes are associated in groups. In his detailed study of triploid and tetraploid cherries, Darlington (1928) has shown that pairing results mainly in the formation of trivalents and quadrivalents respectively. In *Prunus laurocerasus* quite similar groups of united chromosomes are to be found, but in addition there are others with still higher numbers of connected chromosomes. In Text-fig. 1 extracted chromosome groups from first division side views are shown: 1–6 trivalents, 7–16 quadrivalents, 17–23 quinquevalents, 24–30 sexivalents, and 31–33 septivalents. Judging from the appearance of the polar views it is quite evident that conjugated groups of still higher numbers of chromosomes are present, but in the side views owing to the smallness of the chromosomes it is not easy to be sure whether there is an actual connection between pairs lying close together or whether they are only in close juxtaposition. Thus in the case of an octavalent group with the chromosomes arranged as it were at the corners of a cube, the four upper chromosomes can easily cover the lower four in such a manner that the connection, if present, could not be seen. Thus in the last group figured (33), only owing to their closeness to one another can it be concluded that the middle chromosomes in the upper and lower row are associated with the five which are visibly connected.

In trivalents (Text-fig. 1, 1-6) the most common form of association

is that of a line or a V, but triangles are sometimes also found. The linear arrangement is also common in quadrivalents (Text-fig. 1, 7-16); other forms are Y's, squares and crosses similar to those described in tetraploid cherries by Darlington. The higher the number of associated chromosomes in a group, the more complicated and variable are the forms (Text-fig. 1, 17-33). In closed rings consisting of an odd number of chromosomes (6 and 21) it must be assumed that one of the chromosomes lies transversely, and is connected at the same end to the two adjacent ones. It has been especially emphasised by Belling (1927) that the two ends of a chromosome show different attractions. This being so, only the above assumption will lead to a closed ring in a string of three, five, or any odd number of homologous chromosomes, unless



Text-fig. 1. Trivalents (1–6), quadrivalents (7–16), quinquevalents (17–23), sexivalents (24–30), and septivalents (31–33) extracted from different metaphases in *Prunus laurocerasus* (×3100).

we take into consideration the possibility of segmental interchange. The smallness of the chromosomes and their spherical shape usually prevents the determination of the arrangement of the chromosomes in the rings in relation to their axes. In some cases, as in the pentaploid ring (21), it is fairly certain that the lowest chromosome lies transversely, since one can see the equational split in this direction. In wheat, where the chromosomes are much bigger, Huskins (1928) figures a trivalent ring in which there can be no dispute about the transverse position of one chromosome and its attachment by the chromatids at one end to the adjacent homologous chromosomes. Here the equational split is also marked, and apparently this chromosome will divide into two halves in the first division. Whether or not the correspondingly connected chromosomes in *P. laurocerasus* will split and separate at the first

division cannot be said, but it is fairly certain that some at least of the univalents will undergo this process.

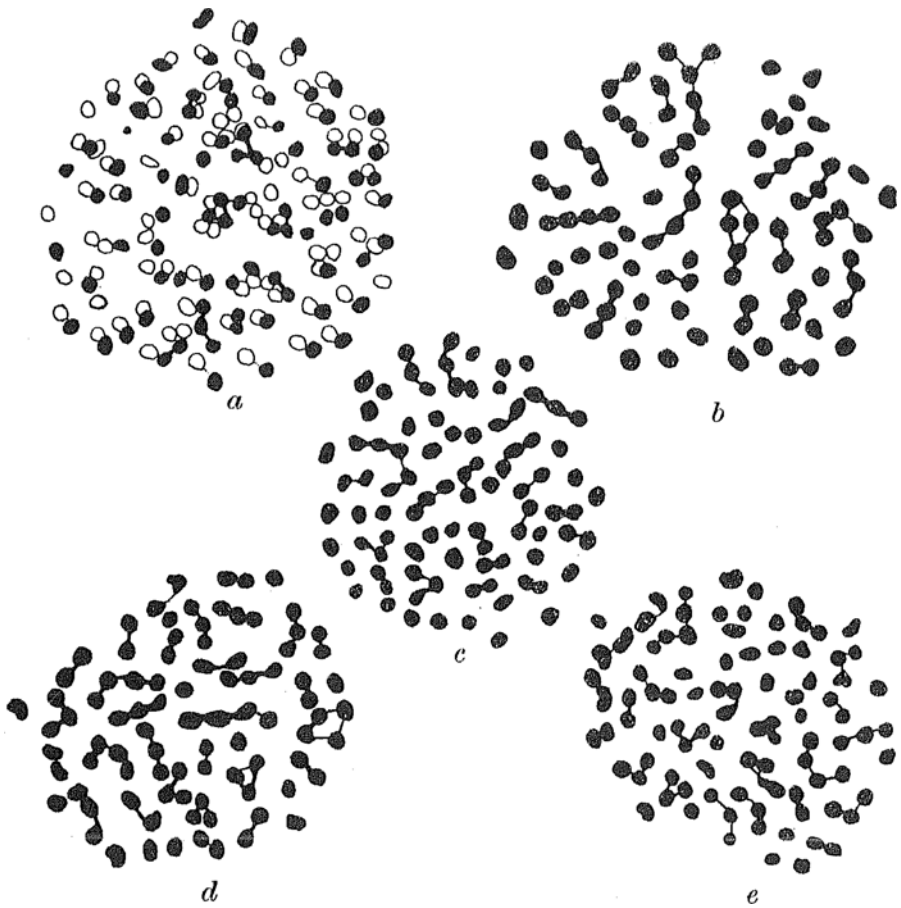
In quinquevalent, sexivalent and septivalent groups, often three and sometimes four chromosomes (25) are connected to one chromosome. This indicates that the chromosome threads must have split prior to synizesis, that is, they consist at this early stage of two chromatids each. Only by the assumption that the four ends of one chromosome have each become attached to a different homologous chromosome can one explain the above pairing. The same assumption is also necessary to explain the formation of the odd-numbered rings.

It is evident that the presence of numerous homologous chromosomes capable of conjugating to form such irregular first-division groups as are here described, together with the simultaneous occurrence of univalents, leads to a numerically irregular segregation. The scattered univalents often show marked splits in the metaphase, and in later anaphase (Pl. IX, figs. 2, 3) some at least of the lagging and divided bodies must be regarded as the result of the equational division of these univalents. Pl. IX, fig. 2 shows two lagging bipartite bodies of which the bigger might perhaps be a retarded bivalent, but the smaller one is more probably a divided univalent. The majority of the chromosomes on the other hand seem to pass to the poles in surprisingly good order. In the late anaphase or early telophase shown in Pl. IX, fig. 3 several lagging and elongated bodies, mostly divided, can be seen. Some of them are apparently split univalents, but the small pair at the right should, owing to its smallness, be more probably regarded as a divided fragment. Other very small round fragments are also present.

In the second-division anaphase and telophase, some chromosomes are often left out of the main mass and lie between the poles (cf. Pl. IX, fig. 4). Such chromosomes certainly will not be included in the daughter nuclei, but whether they form their own nuclei or are absorbed in the plasma is not quite clear. In any case they do not disturb the regular formation of tetrads.

It is obvious that in a plant of this type a haploid number of chromosomes in the strict sense does not exist. The first division metaphase plates consist of a thick layer of chromosomes in various planes, and only an arbitrary line can be drawn between the chromosomes presumed to be bound for the two poles respectively. It is no wonder, therefore, that Kobel (1927) in the metaphase plates of the first division in this plant has counted numbers varying from 70 to 80 chromosomes; but there does not seem to be any foundation for his conclusion that the

haploid number should be 72. It is possible that the somatic number could be obtained from a study of the root tips, but it can be taken as almost certain that every seedling will differ in this respect. In Text-fig. 2 *a* an early anaphase polar view is shown, in which it was possible to count with great accuracy all the chromosomes in the cell. In the higher focus there were 83 chromosomes and one very small body,



Text-fig. 2. *a*. Polar view of first-division metaphase with 83 chromosomes and one fragment at the upper focus, and 88 chromosomes at the lower focus (× 3100).

b. Polar views of first-division metaphase. Only chromosomes at upper focus are drawn; 91 in number. The association of chromosomes in groups is clearly visible (× 3100).

c and *d*. Polar views of second-division metaphase, with 89 chromosomes. Groups of two to five associated chromosomes can be seen (× 3100).

e. Polar view of second-division metaphase with 91 chromosomes (× 3100).

apparently a diminutive chromosome or fragment at 10 o'clock; in the lower focus 88 chromosomes were counted. Many of them are evidently connected as ordinary bivalents, but others show clear connections between several chromosomes. Groups ranging from trivalents up to at least octavalents are present, and a number of chromosomes lie alone as univalents. It would, however, be over-bold to state with certainty that the total chromosome number of this individual plant is 171. Although the fixation is very good and the possibility of chromosomes having been overlooked is almost excluded, it might be thought that certain of the units regarded as univalents could be closely connected bivalents. In Text-fig. 2 *b* another first-division metaphase plate is shown. Here no attempt has been made to count and figure all the chromosomes in the cell. Only those which were seen at the upper focus or in its vicinity, and were supposed to be segregating to the upper pole, are figured. The number of chromosomes is 91, but as is evident from the data given, this is not to be thought of as indicating any "haploid chromosome number," any more than 83 or 88 chromosomes in the previous case. The figure merely illustrates the grouping and connections between the chromosomes as seen in polar views of this stage. The highest groups here show connections between five associated chromosomes, and as the same number of similarly arranged and linked chromosomes could in some cases be found in the lower focus, this strongly indicates that groups as high as decimivalent can be formed. This pairing between chromosomes going to the same pole persists through interphase, so that in the second-division metaphase plates the association of chromosomes in groups is still easily seen, a fact pointed out by Darlington in the tetraploid cherries. The counting of the chromosome number in the second-division metaphase plates is easier than in the first division. This follows from the almost spherical shape of the chromosomes at this stage, and the fact that the split is not at first so marked as to cause difficulty. In some anthers containing pollen mother cells at the second-division metaphase the fixation was remarkably good, and in several plates it was possible, in spite of the high number, to count the chromosomes with great accuracy. In the twenty best plates the number varied from 84 to 92; 88, 90 and 91 were counted once, 85, 86, 87 and 92 twice, 84 three times, and in six plates the number was 89. In Text-fig. 2 *c–e* three such plates are figured, two with 89 chromosomes and one with 91. The association of chromosomes in groups similar to those seen in the first division is clearly seen. The mean of the above twenty counts is 87·7. This would almost suggest that the plant could be 22-ploid, but

considering the irregularities observed there is no reason to suppose that the somatic chromosome number must be an even multiple of 8, the basic number in this genus. It can hardly be supposed that gametes with 88 chromosomes would be the only viable ones, since the tetrads formed after unequal segregations appear quite normal. Moreover, the method of division makes it plain that only an exceptionally rare chance would give 88-chromosome gametes consisting of eleven complete sets. Most probably, therefore, the somatic number of the "species" *P. laurocerasus* varies to a remarkable degree in different individuals, as has been suggested above.

DISCUSSION.

The most interesting feature of the chromosome behaviour in *Prunus laurocerasus* is perhaps the association of the chromosomes in groups at the reduction division. In this respect, however, it does no more than follow the habit of many other autopolyploid plants, and especially that of the tetraploid species of the genus, described by Darlington (1928). The chromosome complement of the polyploid *Prunus* species seems to consist of a number of sets in which the corresponding chromosomes are so similar that they are all capable of conjugating with one another. The irregularities observed at the reduction divisions are thus caused by the presence of more than two homologous units. The position in the tetraploid cherries is neatly explained by Darlington, when he says (*loc. cit.*, p. 245): "Irregularity in segregation, including irregular division and abnormal pairing in the tetraploids, springs no more from an unlikeness between the chromosomes than from a too-great likeness." He also predicts that "Species with high multiple chromosome numbers might be expected to show some of the symptoms of autopolyploidy," and in fact *P. laurocerasus* reveals such symptoms in abundance.

The association of more than two chromosomes in groups of the type described in *P. laurocerasus* is considered by some authors as secondary pairing. This term indicates that the pairing here would not be of the same kind as in the case of normal formation of bivalents resulting from true synapsis. This opinion was held by Darlington, and he regarded the quadrivalent groups in tetraploid cherries as due not to a development of a prophase relationship but to a secondary association. If we assume that the chromosomes found connected in groups at the reduction division are homologous—the only plausible explanation for such behaviour —it is in my opinion evident that this pairing is the result of a real synaptic conjugation between them. No other forces are needed to explain this association of more than two chromosomes in groups of

higher number, especially as it has been shown in tetraploid material where the synapsis can be studied in detail that four homologous chromosomes can be found conjugated with each other at this stage (Darlington, 1929). Strong evidence for this opinion is also to be found in the polyploid *Prunus* species in the cases where odd-numbered rings or peculiar groups with three or four chromosomes connected to one chromosome are found. In the first case the fact that the two ends of the chromosome show different attractions will allow these rings to be formed only if one of the chromosomes is connected at one of its ends with two adjacent chromosomes; in the latter case the connections clearly indicate that the conjugation is a real one between the chromatids. Mr Darlington tells me that he is now of this opinion also, and considers that this "secondary pairing" only appears strongest at the first metaphase as the result of mechanical conditions; that, in fact, actual structural conditions are always responsible for "secondary pairing," but that when the chromosomes are distributed throughout the nucleus at diakinesis these connections may be too tenuous to be visible. Naturally where the chromosomes are as small as they are in *Prunus* the interpretation of these various associations must to some extent depend on inference.

The other interesting result of the study of this highly polyploid species is that although the association of numerous homologous chromosomes leads necessarily to a certain degree of irregularity in the reduction divisions, these as a whole are not greatly influenced, and tetrad formation proceeds with remarkable regularity. It is, therefore, evident that although a few lagging chromosomes would form extra cells after the telophase of the second division in a diploid, in this plant where the basic chromosome complement is so many times duplicated they are from this point of view entirely negligible. Gametes are probably viable independent of their actual chromosome number, for a single chromosome will scarcely affect the balance of the eight fundamental types. Kobel's (1927) study of the chromosome numbers in *Prunus laurocerasus* varieties also indicates that the variation in the somatic number in different individuals may be wide. This can be taken as a good illustration of the fact that the chromosome number of itself is not necessarily of importance for the viability of a zygote, and in many species with a high degree of polyploidy the chromosome number may be found to fluctuate to a considerable degree in different individuals. Many examples of aneuploidy pointing in the same direction are already known and need not be quoted here.

It may be surmised that in the course of evolution the originally identical homologous chromosomes in autopolyploid plants may undergo differentiation in different directions, so that finally they are no longer capable of free conjugation with one another. A number of new and more constant forms with fixed chromosome numbers may thus arise. These plants, although related, need not then necessarily have multiples of the basic chromosome number. Aneuploid series are known in a number of plant families, as for instance in *Carex* (Heilborn, 1924), *Viola* (Clausen, 1926) and others, and could be explained as having arisen in this way through plants showing some of the phenomena of autopolyploidy. In families on the other hand where the serial arrangement of the chromosome numbers is clear and the chromosome numbers constant, a strongly allopolyploid condition seems to be indicated, and hybridisation with alien species has probably provided one of the means of evolution.

SUMMARY.

The somatic chromosome number of *Prunus laurocerasus* is considered to be variable and to consist of numerous homologous basic complements. Counts made in the first and second metaphases indicate that the individual investigated is nearly 22-ploid, $2n = 170–180$, the basic number in the family being 8.

No haploid chromosome number in the strict sense exists in this species, because segregation is irregular and gametes with various numbers of chromosomes are found.

The irregularity observed at meiosis is due to the fact that the homologous chromosomes of the original basic complements are capable of conjugating with one another. Trivalent, quadrivalent, quinquevalent, sexivalent, and septivalent groups can be seen in side views of the first-division metaphase. Groups of still higher numbers of associated chromosomes are almost certainly formed, judging from the grouping of the chromosomes in polar views. A few univalents are also almost invariably present, owing to the occasional formation of odd-numbered groups.

The connection of the chromosomes in groups of more than two units can also be clearly seen in the first division polar views, and these connections persist through interkinesis between chromosomes going to the same pole, so that in second-division metaphase plates these groups are still to be found.

Lagging pairs and univalents are common in the late anaphase side views. In some cases the univalents apparently split, the halves separat-

ing at the first division. Small fragments are also to be seen both in metaphase and anaphase; they may split like the univalents at the first division.

The lagging chromosomes do not interfere with tetrad formation and it is assumed that gametes with varying numbers of chromosomes are viable.

An original autopolyploid condition may be thought to be one of the essential stages in the formation of aneuploid series.

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EXPLANATION OF PLATE IX.

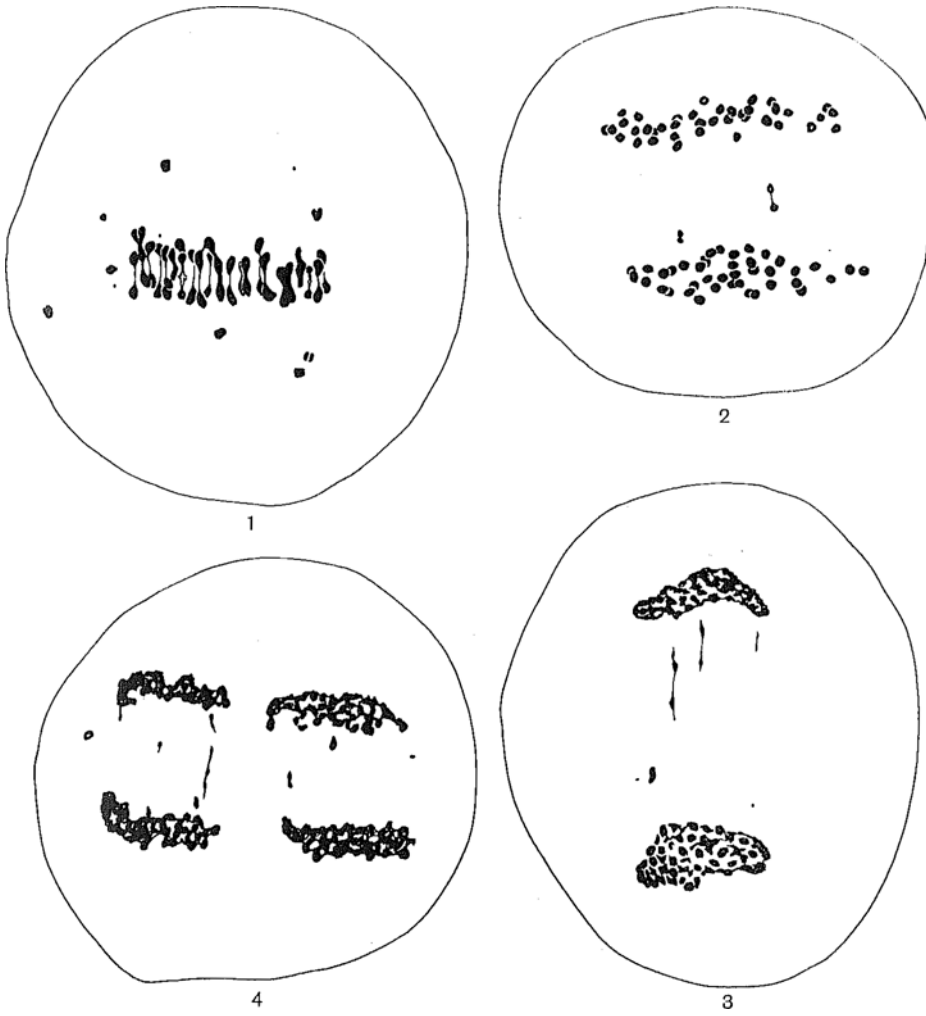
All drawings were made with a Zeiss objective 1·5 mm., N.A. 1·3 and ocular × 25 at bench level, with the aid of an Abbé camera lucida, and reduced in reproduction to the magnification given.

Fig. 1. Side view of first-division metaphase. Univalents and fragments scattered in the plasma. In the plate bivalents, quadrivalents and one quinquevalent group can be seen. Only a part of the chromosomes in the plate are drawn (× 2800).

Fig. 2. First-division anaphase with lagging chromosomes (× 2800).

Fig. 3. Late anaphase of first division with lagging and divided univalents and fragments (× 2800).

Fig. 4. Side view of second-division anaphase with lagging chromosomes and fragments between the plates (× 2800).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