A TRANSMISSIBLE DICENTRIC CHROMOSOME

E. R. SEARS [1] AND A. CÂMARA [2]

*U. S. Department of Agriculture, Columbia, Missouri, and Estação
Agronómica Nacional, Sacavem, Portugal*

DICENTRIC chromosomes have been observed in many types of material, particularly following X-ray treatment, but they are usually eliminated within a few cell generations. An apparent exception to this rule is maize endosperm, where dicentrics are still found many divisions following their inception; but here a breakage-fusion-bridge cycle is set up (MCCLINTOCK 1943, 1944), so that the dicentric is broken at each cell division, with sister ends uniting to form new dicentric chromosomes. Secondary centromeres have been described in rye by KATTERMANN (1939), PRAKKEN and MÜNTZING (1942), and ÖSTERGREN and PRAKKEN (1946); and in maize by RHOADES and VILKOMERSON (1942). These are not true centromeres, however, but are active at a particular stage of meiosis only. PIZA (1941) thought that the chromosomes of *Tityus bahiensis* were dicentric, but RHOADES and KERR (1949) have shown that this organism has either diffuse centromeres or many localized centromeres. The same is true of *Luzula purpurea* (CASTRO, CÂMARA and MALHEIROS 1949), and of certain coccids (HUGHES-SCHRADER and RIS 1941).

A transmissible dicentric chromosome in common wheat, *Triticum aestivum* L. *emend.* Fiori et Paoletti, was mentioned by SEARS (1946) and described in some detail by SEARS and CÂMARA (1950). Further observations on the behavior of this chromosome will be presented here, along with brief accounts of the findings reported in our previous paper, which was in Spanish. The chromosome has a " primary " centromere which is submedian and apparently normal, and a subterminal " secondary " centromere which is weaker than the primary but is active in both meiosis and mitosis. Figure 1 shows the relative lengths of the components of the chromosome, which are estimated at 30 : 10 : 1 for the long arm, the intercentromeric region, and the short arm, respectively. This chromosome was derived from an isochromosome for the short arm of chromosome VII. It was first observed in a plant which carried both the isochromosome and the dicentric. The parent of this plant had a single isochromosome and no other chromosome VII or derivative. Possible methods of origin of the dicentric will be discussed later.

THE UNPAIRED DICENTRIC AT MEIOSIS

At meiosis the two centromeres of the unpaired dicentric chromosome al-
most always oppose each other, attenuating the intercentromeric region and
orienting this region perpendicular to the metaphase plate. Thus the behavior
of the dicentric is typical of that of a bivalent, except that it ordinarily lies
off the plate nearer the pole toward which the primary centromere is directed
(figs. 2 and 3). Rarely, as in figure 4, the dicentric chromosome is found lying
on the plate. During anaphase, the intercentromeric region usually becomes
further attenuated (fig. 5), through poleward movement of the primary cen-
tromere. The secondary centromere makes little, if any, progress toward its
pole. Usually the entire chromosome is included within the one telophase
group. Occasionally, however, breakage occurs in the intercentromeric region,
and monocentric chromosomes are formed.

In about one percent of first meiotic divisions, the dicentric chromosome
behaves as a univalent, coming late onto the plate and dividing. When this
occurs, a bridge involving the intercentromeric region is usually formed be-
tween the two daughter chromatids (figs. 6 and 7). In the one microsporocyte
(fig. 9) where no bridge was formed following division of the dicentric at AI,
it was clear that the two centromeres of each chromatid were directed toward
the same pole.

When it divides at the first division, the dicentric chromosome is subject
to misdivision, like any ordinary univalent. The two misdivisions observed,
one of which is shown in figure 8, were identical in character. The primary
centromere misdivided, breaking one chromatid at that point. The long arm
of this chromatid remained attached to the primary centromere of the other
chromatid, while the intercentromeric region and minute arm formed a di-
centric fragment.

When the dicentric has failed to divide at the first division, it divides at the
second division. This division is usually normal, as in figure 10. Bridges have
been observed only occasionally. Presumably at this stage the two centromeres
of each chromatid are usually oriented toward the same pole, with no twists
or half-twists of the chromatids about each other in the intercentromeric
region. Where a half-twist occurs, the two centromeres of each chromatid
proceed toward opposite poles, forming a two-strand bridge. Such bridges
may eventually resolve themselves without breakage.

The much higher frequency of bridges following division of the dicentric at
the first division may be attributed to the more tightly coiled condition of the
chromosome at that stage, such that there is usually at least a half-twist in
the region between the two centromeres.

PAIRING BEHAVIOR OF THE DICENTRIC

Several plants obtained had a pair of dicentric chromosomes. In 93 percent
of 200 microsporocytes classified in these plants, the two dicentrics were
paired with each other. Where they were unpaired (figs. 2 and 3), each
showed the typical behavior already described—that is, with both centromeres
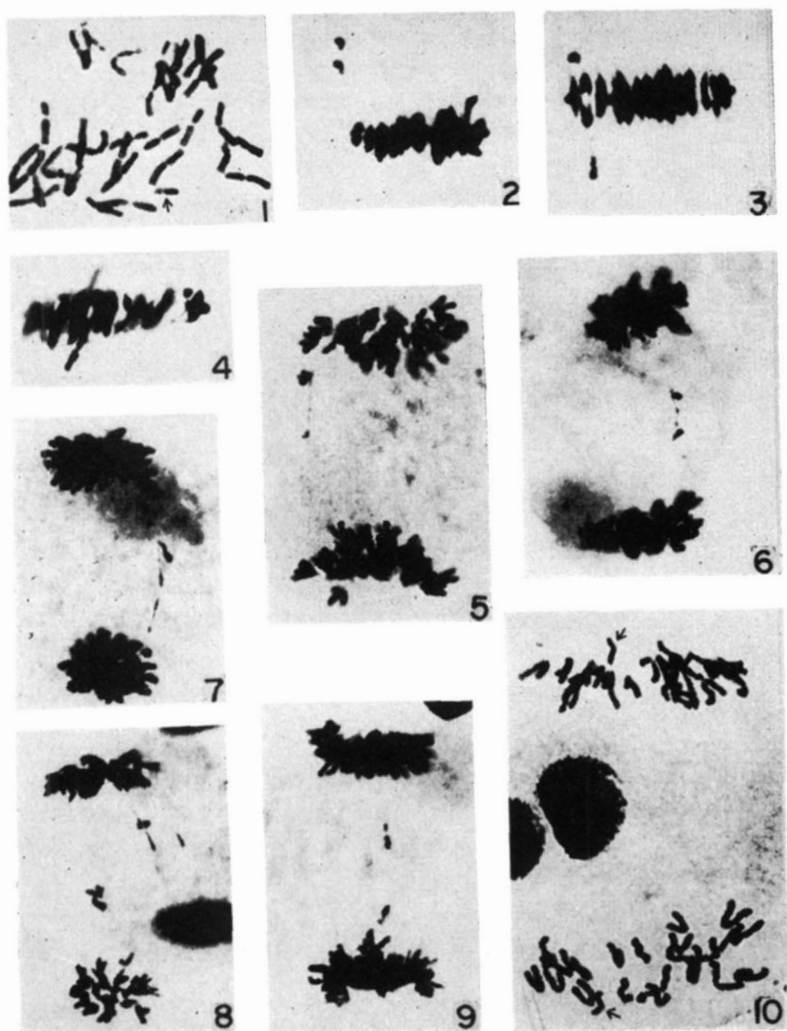
FIGURES 1–10.—The dicentric chromosome in mitosis and meiosis. From aceto-carmine smears. ×790. FIGURE 1.—Part of a mitotic metaphase plate from a root-tip smear. The dicentric chromosome is indicated by arrow. FIGURES 2, 3.—Characteristic behavior of unpaired dicentrics at MI. FIGURE 4.—Unpaired dicentric (second from right) on plate at MI. FIGURE 5.—Typical behavior of unpaired dicentric at AI, passing undivided toward upper pole. Intercentromeric region attenuated. FIGURES 6–9.—Un-paired dicentrics dividing at first division. Bridges are present in figures 6 and 7. In figure 8 the dicentric has misdivided at the primary centromere. A portion of one chromatid, consisting of the intercentromeric region and minute arm, lies at the right. This fragment is presumably dicentric. The long arm of the broken chromatid remains attached to the primary centromere of the other chromatid. In figure 9, the secondary centromere of the upper daughter chromatid is leading the way toward the pole. FIGURE 10.—Typical AII, with a daughter dicentric at each pole.

active. Where they were paired, only one centromere of each chromosome was active, as a rule. The typical bivalent had a chiasma between the long arms of the two dicentrics, with the short arms (intercentromeric region plus minute arm) lying free. The chiasma was either interstitial (fig. 11) or terminal (figs. 12 and 13). These bivalents could only be distinguished from other bivalents of the complement by their smaller size and more extreme arm ratio.

In some microsporocytes, chiasmata occurred on both sides of the primary centromere, so that ring bivalents were formed (fig. 14). Occasional bivalents were found with a chiasma only in the intercentromeric region or perhaps in the minute arm. It could not be determined whether or not the secondary centromere, as well as the primary, was active in these bivalents.

In another type of bivalent (fig. 15) both centromeres of each chromosome were active, but the two centromeres of each chromosome opposed each other rather than their homologues in the other chromosome. This resulted in metaphase behavior like that of two univalent dicentrics, but with the long arms of the two chromosomes joined. Presumably the two long arms had been paired in prophase throughout part of their length, while the centromere regions were involved in intrachromosomal pairing. Since both primary centromeres were oriented toward the same pole, the bivalent was displaced toward that pole.

Besides the symmetrical bivalents, there were a very few of asymmetrical type. In these bivalents (fig. 16) the primary centromere of one chromosome was the active one, while in the other chromosome it was the secondary which was active. Since the primary centromere of one chromosome was opposed by the secondary of the other, the bivalent lay off the plate toward the pole toward which the primary centromere was directed.

Following pairing of two dicentrics, bridges were seen at first telophase in 13 percent of 108 microsporocytes. These bridges, two of which are shown in figures 17 and 18, always involved the intercentromeric region. It could not be ascertained whether any of these bridges were due to activity of the secondary centromere.

The dicentric chromosome was also obtained in plants which had a normal chromosome VII. Here pairing occurred in only 59.5 percent of 168 microsporocytes, and interstitial chiasmata were not found. The long arm of the dicentric usually paired with the short arm of chromosome VII, as in figure 19. In these bivalents the primary centromere was active, the secondary inactive. One bivalent was found in which the secondary centromere of the dicentric was active, with the primary centromere presumably inactive, and the long arm of the dicentric lying free. Bridges were rarely seen at first telophase, only one definite bridge having been found in about 50 cells in which pairing had occurred. This bridge apparently involved the intercentromeric region of the dicentric chromosome.

As mentioned earlier, the dicentric chromosome frequently gives rise to derivative chromosomes which have lost the region containing the secondary centromere. When present with a dicentric, these derivatives were found
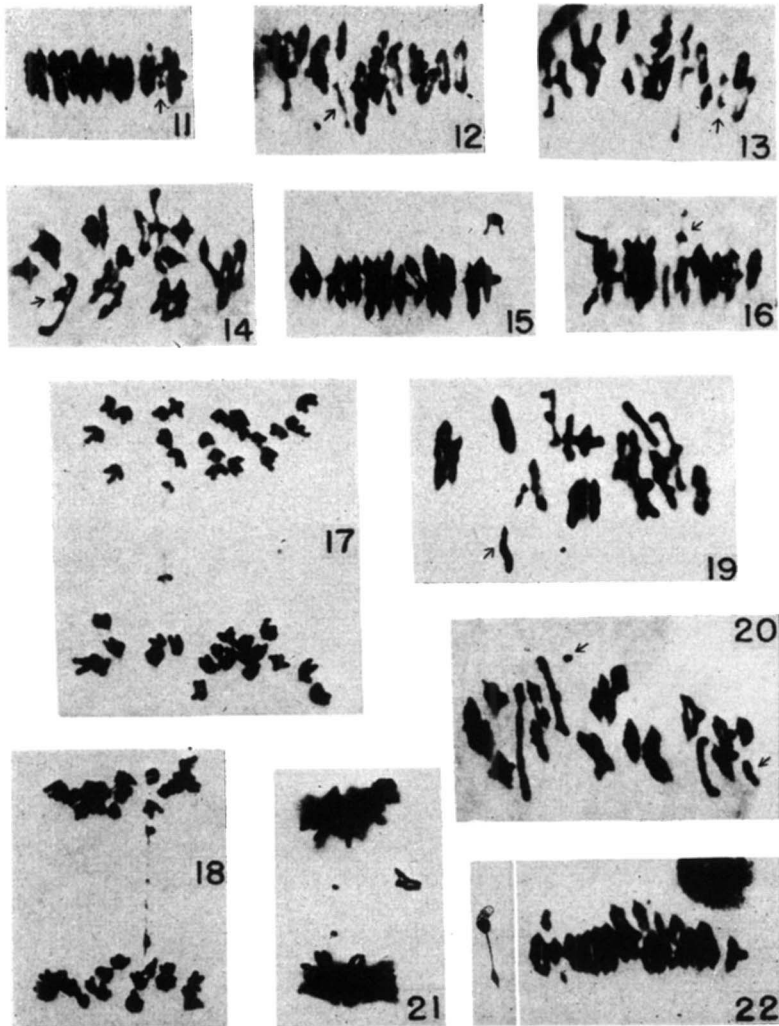
FIGURES 11–22.—First meiotic division. From aceto-carmine smears. × 790. FIGURES 11–16.—Pair of dicentrics at MI. In figures 11–14 the bivalents differ from the others only by their smaller size and, in 11–13, by shortness of the free arms. In figure 15 the two centromeres of each chromosome are opposing each other, attenuating the intercentromeric region to form the two vertical limbs of the configuration. The paired long arms are seen in optical cross section between the two limbs. Figure 16 shows an asymmetrical bivalent, in whose lower half the secondary centromere is the active one. FIGURES 17, 18.—Bridges at AI resulting from pairing of two dicentrics. The bridge in figure 17, and possibly that in 18, has already resolved itself. FIGURE 19.— Heteromorphic bivalent consisting of a dicentric chromosome and a normal chromosome VII. FIGURES 20–22.—Supernumerary heteromorphic pair consisting of a chromosome from *Aegilops umbellulata* and a telocentric for the short arm of this chromosome. The chromosomes are unpaired in figures 20 and 21, paired in figure 22. The heteromorphic bivalent in figure 22 is shown separately in the drawing to the left of the photograph.

paired with the dicentric in 87 percent of 300 microsporocytes. The large
majority of the bivalents formed were held together by chiasmata in the long
arm only. Interstitial as well as terminal chiasmata were found, as in pairs
of two dicentrics. Bridges were observed in 4.05 percent of 222 first telo-
phases. These bridges all involved the intercentromeric region.

When two derivative chromosomes were present, these were found paired
in 85.5 percent of 200 microsporocytes. Again, pairing was mostly or all in
the long arm, and chiasmata were interstitial as well as terminal. No bridges
were found in 93 first telophases.

THE DICENTRIC AT MITOSIS

Few observations have been made of the behavior of the dicentric chromo-
some at mitosis. There is little reason to suspect, however, that it behaves
much differently than at the second meiotic division, where it passes un-
changed through the large majority of divisions. Certainly it is true that in
plants carrying a dicentric the chromosome is found intact in most micro-
sporocytes. Sectors do occur, however, in which the terminal region contain-
ing the secondary centromere has been lost; and bridges have been observed
occasionally in telophases of root-tip mitoses.

TRANSMISSION OF THE DICENTRIC

Transmission data indicate that the dicentric chromosome, when unpaired,
is less subject to loss than are normal univalents. On the female side, where
no selection occurs, transmission was at least 41.7 percent (based on 60 off-
spring), whereas normal monosomes are transmitted through the egg to only
20 to 25 percent of offspring. This increased transmission is presumably due
to the fact that the dicentric usually goes through meiosis like a bivalent, and
hence is subject to loss at only the first division. As for male transmission,
gametes carrying the dicentric are clearly favored over those lacking it, but
no adequate data are available comparing its transmission with that of normal
VII or with monocentric derivatives of the dicentric.

BEHAVIOR OF AN EXTREMELY HETEROMORPHIC BIVALENT

Two possible explanations were offered by SEARS and CÂMARA for the
ability of the primary centromere to pull the unpaired dicentric off the plate
in spite of the pull in the opposite direction by the secondary centromere.
One explanation was that the primary centromere was intrinsically stronger
than the secondary; while the other theory held that the primary was stronger
by virtue of having a much larger portion of the chromosome under its con-
trol. If the latter explanation were correct, then heteromorphic bivalents,
composed of one normal and one telocentric chromosome, should also lie off
the metaphase plate, provided the disparity in size were great enough.

No chromosome in wheat is sufficiently heterobrachial to provide a critical
test of this theory, but a chromosome has been added to wheat from *Aegilops
umbellulata* (SEARS 1949) which has a marked disparity in arm length. Fol-

lowing misdivision of the unpaired Aegilops chromosome, a telocentric for the short arm was isolated and was then combined with the normal Aegilops chromosome. This provided a heteromorphic bivalent of which one member was at least six times as long as the other (figs. 20, 21)—approximately the same disparity as in the regions under the control of the two centromeres of the dicentric chromosome. Pairing of the telocentric with its normal homologue occurred in fewer than one percent of microsporocytes (3 in a sample of 460), but several good pairs were seen, of which some were definitely on the plate (fig. 22) and none was certainly off the plate. Therefore, it seems clear that the weakness of the secondary centromere cannot be attributed to its location, but must be considered an intrinsic characteristic.

<div align="center">DISCUSSION</div>

SEARS and CÂMARA suggested two methods, neither of which is entirely satisfactory, by which the dicentric chromosome may have been derived from iso-VII. The first theory postulated the origin of the dicentric through an inversion involving one break within the centromere of iso-VII (fig. 23 A).
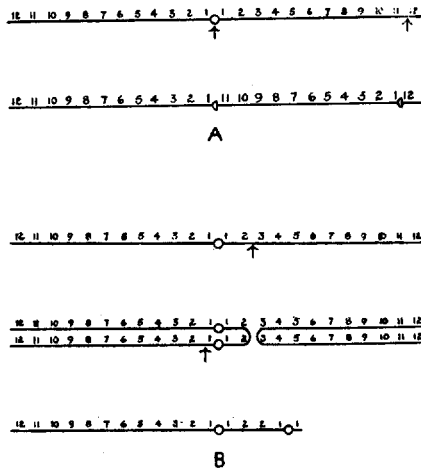

FIGURE 23.—Two possible methods of origin of a dicentric chromosome from an isochromosome: A. Through a simple inversion with one break in the centromere. B. Through a break, followed by union of sister ends, and then a second break.

This theory provides an explanation for the weakness of the secondary centromere, in that this centromere may consist of less than half of the original centromere. But the theory has the great weakness of not accounting for a chromosome with the proper relative lengths of its component parts. The long arm would be no longer than the intercentromeric region plus the short arm, whereas in both meiotic and mitotic divisions the long arm is seen actually to be about three times the length of the rest of the chromosome.

The second theory of the origin of the dicentric (fig. 23 B) has the isochromosome first losing most of one arm. Then the broken sister ends unite

to form a dicentric chromosome, and finally most of one of the free arms of this dicentric is lost. The resulting chromosome has, in order, one intact arm of the isochromosome; a centromere; a reversed duplication of the region adjacent to this centromere; a second duplication of this region, again reversed; a second centromere; and finally a smaller reversed duplication of the region adjacent to the second centromere. Thus one segment is duplicated three times, and a part of this segment is duplicated four times. An objection to the second theory is that it fails to explain the weakness of the secondary centromere. The theory may be modified so that it does explain the weak centromere, but before this is done, it will be well to compare the pairing behavior of the actual dicentric with that expected of the dicentric chromosome provided by the theory.

When present at meiosis as a monosome, the hypothetical dicentric shown in figure 23 B should frequently double back on itself to bring the two centromeres into opposition and thereby cause bivalent behavior. Often, however, the minute arm beyond the secondary centromere should pair with the identical region on the other side of this centromere, presumably preventing the secondary centromere from pairing with the primary, and thereby causing the dicentric to behave as a univalent. The fact that univalent behavior is seen in only about one percent of microsporocytes would seem to indicate either that the hypothetical chromosome does not correspond with the actual one, or that pairing of the minute arm tends to be suppressed.

Following loss of the region containing the secondary centromere of the hypothetical dicentric, the derivative monocentric chromosome would have homologous regions on the two sides of its centromere. Meiotic pairing and crossing-over between these regions would result in the two long arms of the divided univalent chromosome going to one pole and the two short arms to the other. In actual fact, such divisions are commonly seen. These figures resemble misdivision, but other types of misdivision figures, notably those with three arms going to one pole and one to the other, are not observed. Furthermore, in some figures one of the short arms can be seen disjoining from one of the long arms, as though the two had been paired.

When two hypothetical dicentrics are present, they should often pair with each other throughout their length and form a bivalent with all four centromeres active. That such bivalents occur rarely, if at all (those which obviously had all four centromeres active had not been paired with each other throughout their length), suggests that the two secondary centromeres are in some way prevented from pairing with each other. Intrachromosomal pairing of the minute arm with the region just across the secondary centromere might prevent these centromeres from pairing; but frequent occurrence of this type of intrachromosomal pairing is ruled out by the behavior of the univalent dicentric. The hypothetical dicentric provides a reasonable explanation of the asymmetrical bivalents observed, in that these could be the result of pairing of the intercentromeric region adjacent to the secondary centromere of one chromosome with the portion of the long arm of the other chromosome adjacent to the primary centromere.

It is in the pairing behavior with normal chromosome VII that the hypothetical dicentric is least satisfactory. According to the theory, the long arm of the dicentric is identical with the arm of chromosome VII (the short arm) which gave rise to the isochromosome. These two arms should therefore pair intimately. That they do not do so is indicated by the relatively low frequency of bivalents formed (about 60 percent) and by the absence of interstitial chiasmata in the bivalents that occur.

A modification of the theory could account for the weakness of the secondary centromere, by assuming a misdivision of the isochromosome. It is also possible to make the theory somewhat more plausible by (1) attributing the initial break to bridge formation following pairing between the two arms of the isochromosome, and (2) attributing the position of the second break to the tendency of a dicentric with one weak centromere to be displaced toward one pole. But detailed consideration of such refinements of the theory seems scarcely warranted when the final dicentric product has already been shown to be unsatisfactory. Development of an adequate explanation for the origin of the dicentric may not be possible until pachytene techniques are available for wheat which will reveal more fully the constitution of the dicentric.

Although the time of origin of the dicentric has not been definitely established, there is good reason to designate meiosis as the most probable occasion. At meiosis the parental isochromosome, which was present as a univalent, would have been undergoing misdivision with appreciable frequency. Under conditions leading to frequent misdivision, other abnormal processes would not have been unexpected. It is of course possible that the dicentric arose previous or subsequent to meiosis. If before meiosis, however, it could not have arisen long enough before to be present in all of one floret, for the zygote produced in the critical floret had the isochromosome as well as the dicentric. Neither could it have arisen long after fertilization, else a sector having the unchanged isochromosome.should have been detected in the plant concerned.

When a dicentric is involved in pairing with another chromosome, a bridge should result at AI every time there is a chiasma in the intercentromeric region, provided that each chiasma represents a cross-over, as is generally believed. Double crossovers could occur without leading to bridges, but these should be rare in this short region. In plants with two dicentrics, chiasmata were actually seen at MI involving the intercentromeric region, in a frequency which, while not precisely determined, was of an order comparable to that of the frequency of anaphase bridges (13 percent) in the same plants. It was not certain that all of the bridges were due to crossovers, since if there were activity of all four centromeres in one bivalent, and if the two centromeres of each chromosome moved toward opposite poles, a bridge-like configuration might be the result at AI. The " bridge " in figure 17 may well be of this sort.

It has been assumed that the primary centromere of the dicentric is of normal strength and the secondary weak. From the mere fact that the primary outpulls the secondary when they are opposed, it might equally well be argued

that the secondary is normal and the primary abnormally strong. But from the behavior of pairs between normal chromosome VII and the dicentric, where the secondary centromere remains inactive, and of pairs between normal VII and derivatives lacking the secondary centromere, it seems clear that the primary centromere is of normal strength. These bivalents show no tendency to be displaced from the metaphase plate toward the pole to which the primary centromere of the dicentric is directed.

Since the secondary centromere is demonstrably weak, derivative chromosomes with only this centromere might be expected to show abnormalities of behavior such as lagging during mitosis. Where such derivatives have been observed at second telophase, however, they had divided and passed to the poles with no apparent tendency to lag. It must be concluded that a weak centromere shows its weakness only when opposed by a stronger centromere.

What is responsible for the weakness of the secondary centromere? Does it perhaps have a reduced amount of centromere substance? If so, this might mean deficiency for one of the two centromeric chromomeres observed by LIMA-DE-FARIA (1949) in the closely related genus Secale. The centromeres of telocentric chromosomes produced by misdivision are of normal strength, although these too presumably contain but a single centromeric chromomere; but possibly the terminal position permits more efficient functioning of the defective centromere.

If the secondary centromere of the dicentric chromosome is a deficient centromere, a ready explanation for its origin is at hand in the phenomenon of misdivision. The isochromosome from which the dicentric arose was present as a monosome, and hence subject to misdivision in every sporocyte. If weak centromeres arise in this way in appreciable frequency, monocentric chromosomes with weak centromeres might also appear following misdivision. None have yet been demonstrated, but scarcely any data are available on the centromere strength of dibrachial chromosomes issuing from misdivision.

SUMMARY

A dicentric chromosome in common wheat originated from an isochromosome for the short arm of chromosome VII. Its three parts have a length ratio of about 30 : 10 : 1.

At meiosis the dicentric usually behaves as a bivalent, with the two centromeres opposing each other. Since the sub-terminal centromere is weak, the chromosome lies off the plate at MI, and is commonly included entire in one telophase group.

At the second meiotic division and in mitosis the dicentric usually divides normally, with the two centromeres of each chromatid being directed to the same pole.

When the dicentric pairs with another chromosome, only one of its centromeres is active, as a rule, and this is almost always the primary centromere.

An extremely heteromorphic bivalent was not displaced from the metaphase plate. Therefore, the weakness of the secondary centromere is believed

an intrinsic property rather than being due to the position of this centromere in the chromosome.

Of two possible methods suggested for the origin of the dicentric from iso-VII, neither is entirely satisfactory.

The defectiveness of the secondary centromere seems likely to have resulted from misdivision of the isochromosome. The weakness of this centromere is only expressed when it is opposed by a stronger centromere.

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