THE RELATION OF OXYGEN POSTTREATMENT AND
HETEROCHROMATIN TO X-RAY-INDUCED CHROMOSOME
ABERRATION FREQUENCIES IN CREPIS CAPILLARIS¹

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SEVERAL studies have now demonstrated that postirradiation oxygen treat-
ments can markedly increase the initial X-ray damage in barley seeds
(ADAMS, NILAN AND GUNTHARDT 1955; NILAN 1955; ADAMS AND NILAN 1958;
CALDECOTT et al. 1957; CURTIS, CALDECOTT AND KONZAK 1957). One of the more
striking of these after effects is the marked increase in the frequencies of chromo-
some aberrations (ADAMS, NILAN AND GUNTHARDT 1955; ADAMS AND NILAN
1958).

The present investigation was undertaken to determine if the influence of
oxygen after X-irradiation was random or differential in its action on the location
of chromosome breaks in seeds. In other words, does oxygen increase chromosome
aberrations in particular regions of the chromosomes?

Such a study required a detailed analysis of the chromosome aberrations that
was not possible with barley. Therefore, Crepis capillaris (2n = 6) with its
readily identified chromosome arms was chosen as the experimental organism.

In addition to providing data on the original objective, the study also disclosed
some new information concerning chromosome breakage in Crepis. Here the
increased chromosome rejoining in heterochromatic regions is of particular
importance.

MATERIALS AND METHODS

The experiments were conducted with resting seeds of Crepis capillaris (2n=6).
This species has only three pairs of chromosomes (A, C, and D) and each chromo-
some arm is readily identified. Chromosome A is the longest and its short arm is
longer than the short arm of either of the other two chromosomes; C is the short-
est and its short arm is medium in size; D is medium in length and its short arm
is very small and has a tiny satellite attached. The average lengths of the chromo-
some arms at mitotic metaphase are as follows: A1 6.0 μ, As 1.8 μ; Cl 3.0 μ, Cs 1.0
μ; Dl 4.9 μ, Ds 0.5 μ. These measurements and a structural alteration in the D
chromosome of the strain used in this investigation have been reported elsewhere
(NILAN and SIRE 1958).

Samples of seeds at eight percent moisture obtained by storage over dry CaCl₂

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Measure 171.
were X-irradiated at 1500 r and 3500 r in a single layer on a turntable which was centered 42 cm beneath a beryllium window of an AEG 50 Machlett X-ray tube. The tube was operated at 34 KV and 25 ma without filter. The high voltage supply produced full wave rectification smoothed out by a 0.2 microfarad condenser. The voltage was read on a meter having voltage resistance in series. Doses were given at the rate of 990 r per minute at the surface of the seed as measured by a Victoreen condenser r meter with a nylon walled 250 r chamber.

The oxygen gas for the postirradiation treatments was obtained from commercial cylinders (Industrial Air Products) and assayed as follows: 99.6 percent O₂, + 0.4 percent N₂. The X-irradiated seeds were immediately placed in specially constructed chambers under 100 lbs pressure and stored for 42 days after the irradiation. During the posttreatment the gas was changed every three days. Samples of the seeds were analyzed cytologically immediately after, and 3, 15, 28, and 42 days after the irradiation. Samples of nonirradiated seeds were analyzed before storage and after 42 days of storage in oxygen.

Following the various treatments the seeds were germinated on filter papers in petri dishes. When the root-tip cells were undergoing their first mitotic divisions, the seeds were placed in .002 M 8-hydroxyquinoline for 1^-2 hours. Aceto-orcein squash preparations were made of the root tips, and the slides were made permanent with Euparal. This technique has been described in greater detail by TJIO and LEVAN (1950).

The cytological analysis consisted of recording the numbers of normal and disturbed cells, frequencies of dicentric chromosomes, and frequencies of isodiametric and rod acentric fragments at metaphase. Except for a few chromatid breaks, all of the aberrations were of the chromosome type. Chromosome breaks which rejoined to form dicentric chromosomes were tabulated. These breaks were the easiest to identify and could be located with considerable accuracy. Translocations resulting from the rejoining of centric and acentric fragments were more difficult to identify. The total number of detectable breaks was determined by adding the dicentric breaks, other translocation breaks, and simple breaks (no rejoining). The majority of the simple breaks were identified and located from scoring rod fragments that had not restituted or rejoined and that were not associated with a translocation. This was a more reliable measure of simple breaks than scoring from isodiametric fragments. Nevertheless, these simple breaks could not be identified and located as easily as the dicentric breaks. Intrahomologous dicentrics and chromatid breaks were also recorded. The breaks in the various chromosome arms of many cells were located by drawing the chromosome aberrations resulting from the breaks. The normal chromosome complement of Crepis capillaris and some of the aberrations observed are shown in Figures 1 to 5.

RESULTS

Effects of oxygen

Chromosome aberration frequencies: Postirradiation treatment of 100 lbs pressure of oxygen increased the frequencies of abnormal cells, dicentric chromo-
FIGURES 1-5.—Photomicrographs of somatic metaphase chromosomes of *Crepis capillaris* showing X-ray induced aberrations. All figures are ca. × 2600.

**Figure 1.**—Chromosomes A, C, and D of a normal cell.

**Figure 2.**—Intrahomologous translocation involving the long arms of A; no fragments.

**Figure 3.**—Dicentric chromosome involving the long arm of A and the long arm of C; two pairs of rod fragments.

**Figure 4.**—Intrahomologous dicentric chromosome involving the short arms of the A chromosomes; one isodiametric fragment (arrow) and one pair of fragments just detached from the long arm of D.

**Figure 5.**—Chromatid break in the long arm of D.

Somatics and isodiametric and rod fragments (Table 1). During 42 days of post-irradiation treatment aberration frequencies gradually increased. The isodiametric fragment frequencies increased faster than the rod fragment frequencies. Seeds X-rayed at 3500 r and given the same posttreatments showed similar increases in abnormal cells, dicentrics, and fragments. Nonirradiated seeds stored for 42 days in oxygen showed considerably more aberrations than the control.

*Proportions of breaks in the chromosome arms:* Table 2 presents the total and dicentric breaks in each chromosome arm immediately following X-irradiation, and after postirradiation treatments of oxygen. Since X-ray dose did not alter
### TABLE 1

**Frequencies of abnormal cells, chromosome dicentrics, and rod and isodiametric fragments in X-irradiated (1,500r) seeds of *Crepis capillaris* stored in oxygen at 100 lbs pressure from 0 to 42 days**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells</th>
<th>Chromosome aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>Number</td>
<td>Abnormal percent</td>
</tr>
<tr>
<td>1500r</td>
<td>0</td>
<td>393</td>
</tr>
<tr>
<td>1500r</td>
<td>3</td>
<td>193</td>
</tr>
<tr>
<td>1500r</td>
<td>15</td>
<td>319</td>
</tr>
<tr>
<td>1500r</td>
<td>28</td>
<td>250</td>
</tr>
<tr>
<td>1500r</td>
<td>42</td>
<td>277</td>
</tr>
<tr>
<td>0r</td>
<td>0</td>
<td>172</td>
</tr>
<tr>
<td>0r</td>
<td>42</td>
<td>281</td>
</tr>
</tbody>
</table>

#### TABLE 2

**Proportion of total breaks and breaks leading to dicentrics in each chromosome arm of X-irradiated (1,500r and 3,500r) seeds of *Crepis capillaris* stored in oxygen at 100 lbs pressure from 0 to 42 days**

<table>
<thead>
<tr>
<th>On post treatment</th>
<th>Breaks</th>
<th>Al</th>
<th>As</th>
<th>Chromosome arms</th>
<th>Cl</th>
<th>Cs</th>
<th>Di</th>
<th>Ds</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Total no.</td>
<td>503</td>
<td>188</td>
<td>368</td>
<td>70</td>
<td>401</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>31.7</td>
<td>11.9</td>
<td>23.2</td>
<td>4.4</td>
<td>25.3</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Dicent. no.</td>
<td></td>
<td>198</td>
<td>81</td>
<td>209</td>
<td>48</td>
<td>182</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>26.3</td>
<td>10.7</td>
<td>27.7</td>
<td>6.4</td>
<td>24.1</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>Total no.</td>
<td>45</td>
<td>19</td>
<td>35</td>
<td>10</td>
<td>41</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>28.8</td>
<td>12.2</td>
<td>22.4</td>
<td>6.4</td>
<td>26.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Dicent. no.</td>
<td></td>
<td>27</td>
<td>10</td>
<td>22</td>
<td>8</td>
<td>29</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>27.0</td>
<td>10.0</td>
<td>22.0</td>
<td>8.0</td>
<td>29.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>15 days</td>
<td>Total no.</td>
<td>93</td>
<td>52</td>
<td>73</td>
<td>23</td>
<td>90</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>27.2</td>
<td>15.2</td>
<td>21.3</td>
<td>6.7</td>
<td>26.3</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Dicent. no.</td>
<td></td>
<td>41</td>
<td>24</td>
<td>49</td>
<td>15</td>
<td>48</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>22.3</td>
<td>13.0</td>
<td>26.6</td>
<td>8.2</td>
<td>26.1</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>28 days</td>
<td>Total no.</td>
<td>99</td>
<td>47</td>
<td>53</td>
<td>19</td>
<td>75</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>32.5</td>
<td>15.4</td>
<td>17.4</td>
<td>6.2</td>
<td>24.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Dicent. no.</td>
<td></td>
<td>54</td>
<td>24</td>
<td>35</td>
<td>10</td>
<td>47</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>30.0</td>
<td>13.3</td>
<td>19.4</td>
<td>5.6</td>
<td>26.1</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>42 days</td>
<td>Total no.</td>
<td>121</td>
<td>59</td>
<td>95</td>
<td>39</td>
<td>102</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>27.9</td>
<td>13.6</td>
<td>21.9</td>
<td>9.0</td>
<td>23.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Dicent. no.</td>
<td></td>
<td>70</td>
<td>42</td>
<td>73</td>
<td>30</td>
<td>63</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td>24.1</td>
<td>14.5</td>
<td>25.2</td>
<td>10.3</td>
<td>21.7</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>

$x^2$ on total breaks obtained: 36.06
$x^2$ on total breaks expected (25 d.f.): 37.65 at 5% level, 44.31 at 1% level
the proportion of breaks in each chromosome arm the data from the 1500r and 3500r treatments were combined.

By inspection it can be seen that the oxygen treatments did not alter the proportion of these breaks in each arm. This conclusion is supported by the fact that none of the differences were significant according to a chi-square test of independence in a row \( \times \) column heterogeneity table (Snedecor 1956).

**Rejoining of chromosome breaks:** The number of breaks that rejoined was determined in the following manner: The number of dicentrics in each treatment (Table 1) was scored with considerable reliability. However, translocations, other than dicentrics, were more difficult to score. If the two translocated segments were approximately the same length, it was impossible to know if an aberration was present. Thus, the total number of translocations (rejoined chromosomes) were considered to be double the number of dicentrics. It is assumed in this calculation that if two chromosomes break in a cell, chance determines which two ends come together to form the reconstituted chromosomes. If breaks occur in any two arms and rejoining takes places, the result can be either a dicentric chromosome plus one (Figure 4) or two (Figure 3) fragments or two translocated chromosomes with no fragments (Figure 2). According to Lewitsky and Sizova (1935) the “X-ray translocations in Crepis are for the most part, if not all, reciprocal.” The present study supports this conclusion since most dicentrics were accompanied by only one fragment. In addition, there was no evidence of preferential rejoicing between certain arms of the chromosomes in any of the treatments applied.

The breaks that did not rejoin were computed from the fragment number. This number was halved since each pair of fragments scored was the result of one break. Moreover, the fragments remaining from dicentric formation were subtracted. The breaks that remained were those leading to fragments that did not rejoin or become restituted.

The data in Table 3 which were computed in the above manner reveal that X-irradiation without oxygen posttreatment produced 4.3 breaks that rejoined to one break that did not rejoin. However, after 15 days of postirradiation treatment

<table>
<thead>
<tr>
<th>Or post treatment</th>
<th>Dicentric bridge no.</th>
<th>Breaks that rejoined</th>
<th>Fragment pairs</th>
<th>Simple breaks</th>
<th>Rejoined breaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>34</td>
<td>136</td>
<td>75</td>
<td>41</td>
<td>4.3</td>
</tr>
<tr>
<td>3 days</td>
<td>50</td>
<td>200</td>
<td>110</td>
<td>60</td>
<td>3.3</td>
</tr>
<tr>
<td>9 days</td>
<td>51</td>
<td>204</td>
<td>165</td>
<td>104</td>
<td>2.0</td>
</tr>
<tr>
<td>15 days</td>
<td>102</td>
<td>408</td>
<td>450</td>
<td>348</td>
<td>1.2</td>
</tr>
<tr>
<td>28 days</td>
<td>90</td>
<td>360</td>
<td>401</td>
<td>311</td>
<td>1.15</td>
</tr>
<tr>
<td>42 days</td>
<td>145</td>
<td>580</td>
<td>734</td>
<td>589</td>
<td>1.0</td>
</tr>
</tbody>
</table>
in oxygen the ratio was reduced to 2:1 and after 42 days it was further reduced to 1:1.

**Distribution of breaks in relation to chromosome length**

If the chromosome breaks induced by X-rays are random, then the frequency of breaks in each chromosome arm should be directly related to the length of the arm. To ascertain the expected distribution of the breaks in *Crepis capillaris*, the length of each arm was converted to a percentage of the total length (17.2 microns at mitotic metaphase) of all the chromosomes. The expected number of breaks in each arm was determined by multiplying the percentage figure by the total number of breaks in all chromosome arms. Chi-square tests were conducted to determine if the differences between the number of observed breaks and expected breaks in each arm were significant.

The observed number of breaks leading to dicentrics was much greater than expected in the long arm of C and less than expected in the long arm of A (Table 4). On the other hand, there were more simple breaks than expected in the long arm of A and less than expected in the short arm of C. These differences are significant at the one percent level. The proportions of breaks in the remaining arms were not significantly different from expected. This differential breakage in relation to chromosome length was not influenced by oxygen posttreatment.

**Relation to heterochromatin**

**Location of chromosome breaks:** After many chromosome breaks were observed, it became clear that a large majority of the breaks leading to dicentrics occurred in or near the heterochromatic regions of the chromosome arms. These regions can be determined in prophase chromosomes in root-tip cells treated with oxyquinoline (Tjio and Levan 1950). The entire short arm and the proximal

<table>
<thead>
<tr>
<th>Type of break</th>
<th>AI</th>
<th>As</th>
<th>Cl</th>
<th>Cs</th>
<th>DL</th>
<th>Ds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breaks leading to dicentrics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>198</td>
<td>81</td>
<td>209</td>
<td>48</td>
<td>182</td>
<td>36</td>
</tr>
<tr>
<td>Expected on basis of length</td>
<td>259</td>
<td>78</td>
<td>130</td>
<td>43</td>
<td>212</td>
<td>22</td>
</tr>
<tr>
<td>$x^2$</td>
<td>15.00**</td>
<td>.05</td>
<td>44.42**</td>
<td>.21</td>
<td>4.53*</td>
<td>6.50*</td>
</tr>
<tr>
<td>Simple breaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>77</td>
<td>13</td>
<td>21</td>
<td>0</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Expected on basis of length</td>
<td>52</td>
<td>16</td>
<td>26</td>
<td>9</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>$x^2$</td>
<td>12.02**</td>
<td>.56</td>
<td>.96</td>
<td>9.0**</td>
<td>.38</td>
<td>4.0*</td>
</tr>
</tbody>
</table>
and the very distal end of the long arm of each chromosome are heterochromatic. Furthermore, the heterochromatic areas in the long arms are approximately equal in size. Similar areas of heterochromatin in the chromosomes of *Crepis capillaris* have been pictured earlier (Kostoff and Arutunian 1938).

Two hundred dicentric breaks were analyzed, and the locations of the breaks were tabulated (Table 5). Sixty-five percent of the breaks were definitely located in heterochromatin, 2 percent in euchromatin; the remaining 33 percent could have been in either area. However, most of these questionable dicentrics were more likely formed from breaks in heterochromatin for usually the dicentric was of the same length as one or the other of the donor chromosomes. In other words, one donor chromosome had broken near the centromere and the other had broken near the distal end of the long arm.

One hundred simple breaks were scored for their distribution on the chromosomes (Table 5). Sixty-two percent were definitely in euchromatin, 24 percent were in heterochromatin, and 14 percent could not be distinguished.

**Frequencies of dicentric breaks:** The heterochromatic regions of the long arms are about equal in length. The dicentric breaks in each long arm occur with about equal frequency (Tables 2 and 4). This observation is substantiated by significantly low chi-square values (Table 6). The frequencies of dicentrics involving the short arms (Tables 2 and 4) also indicate a positive relation to heterochromatin. As indicated earlier, the entire short arms are heterochromatin and as shown in Table 6 the frequencies of breaks are also related to length.

**Frequencies of chromatid breaks**

There were 1587 chromosome breaks and 35 chromatid breaks (Figure 5) in the various treatments. The chromatid breaks thus comprised 2.16 percent of the total breaks observed. These breaks appeared to be nonrandomly distributed, because 25 were in the long arm of D chromosome, six in the long arm of A, and four in the long arm of C. This distribution was not influenced by the oxygen treatments.

**Frequency of intrahomologous exchanges leading to dicentrics**

On the basis of the six Crepis chromosomes, 15 combinations or exchanges leading to dicentrics are possible. Of these, three (20 percent) can be exchanges
between homologous chromosomes. Table 7 shows that the number of intra-homologous dicentrics fits the expected value very well. The oxygen treatments did not influence the frequency of intrahomologous exchanges.

**DISCUSSION**

The present study clearly shows the pronounced after effect of oxygen treatment on X-ray induced chromosome aberration frequencies in seeds of *Crepis capillaris*. Thus, similar results found earlier in barley are confirmed (Adams, Nilan and Gunthardt 1955; Nilan 1955; Adams and Nilan 1958; Caldecott et al. 1957).

It is shown in the present study that an oxygen posttreatment does not influence differentially the induced chromosome breakage pattern in *Crepis capillaris*. Apparently oxygen is quite random in its influence and uniformly increases aberrations in all the chromosome arms.

Earlier reports (Adams, Nilan and Gunthardt 1955; Adams and Nilan 1958) have attempted to arrive at a suitable hypothesis to account for increased chromosome aberration frequencies during post-X-ray treatments. Two hy-
hypotheses were considered appropriate. One of these concerned the effect of oxygen on the restitution of induced chromosome breaks (cf. Schwartz 1952; Swanson and Schwartz 1953). The second relates to the effect of oxygen on the conversion of latent to primary breaks (cf. Swanson 1954). However, from previous data it was impossible to distinguish between these two hypotheses.

Evidence has now been presented in this paper that suggests that a major effect of oxygen during the postirradiation treatments is to inhibit the restitution of chromosome breaks. As the storage period progresses, more and more breaks fail to rejoin. Furthermore, many of the breaks do not rejoin to form dicentrics (Table 3). This is shown by the finding that the ratio of breaks that rejoined decreased from 4.3:1 immediately after irradiation to 1:1 42 days after irradiation. This interpretation can account for the striking increase in fragments as compared to dicentric chromosomes found during oxygen storage of X-irradiated seeds.

Support for this interpretation can be found in the cytological studies of the after effects in barley seeds (Adams and Nilan 1958). In these seeds, also, there is a very marked increase of fragments during the postirradiation period and a much smaller increase of dicentric chromosome bridges. Furthermore, differential rejoining and restitution has been considered a factor in the increased chromosome sensitivity to X-irradiation in certain stages of mitosis in microspores of Tradescantia (Bishop 1950; Sparrow and Maldawer 1950; Deschner and Sparrow 1955).

In order to determine if the inhibition of restitution was due to oxygen or the pressure, an experiment was conducted using nitrogen as the storage atmosphere. Seeds in this experiment were irradiated at 1500r and stored separately under 100 lbs pressure of nitrogen (Industrial Air Products—99.6% N+ 0.4% O₂ and CO₂) and 100 lbs pressure of oxygen for three weeks. The seeds were maintained at four percent moisture throughout the experiment.

It can be seen in Table 8 that the frequencies of aberrations after three week storage in nitrogen are somewhat higher than those in the nonstored seeds but much lower than those found in the seeds stored in oxygen. Presumably the slight increase in the nitrogen stored seeds is due to the oxygen already present in the seeds. Similar results have been obtained in this laboratory with barley

<table>
<thead>
<tr>
<th>X-ray Treatment</th>
<th>Posttreat.</th>
<th>Cells Number</th>
<th>Abnormal percent/ cell</th>
<th>Chromosome aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500r</td>
<td>no storage</td>
<td>276</td>
<td>12</td>
<td>.04</td>
</tr>
<tr>
<td>1500r</td>
<td>N₂ storage</td>
<td>279</td>
<td>32</td>
<td>.10</td>
</tr>
<tr>
<td>1500r</td>
<td>O₂ storage</td>
<td>306</td>
<td>86</td>
<td>.56</td>
</tr>
</tbody>
</table>

TABLE 8
Frequencies of abnormal cells, chromosome dicentrics, and rod and isodiametric fragments in X-irradiated (1,500r) seeds of Crepis capillaris stored in oxygen and nitrogen at 100 lbs pressure from 0 to 21 days (summary of two experiments)
(Adams and Nilan 1958; Nilan and Kronstad 1958). Apparently, then, oxygen and not the pressure is chiefly responsible for the increase in aberrations and hence the inhibition of restitution in the X-rayed stored seeds.

Especially interesting is the finding that oxygen under pressure can induce chromosome aberrations (Table 1). Preliminary studies (unpublished) in barley have also indicated that oxygen per se can break chromosomes in seeds. This result supports the data of Conger and Fairchild (1952) which showed that oxygen can cause chromosome breaks in Tradescantia pollen.

A number of reports have presented evidence for non random chromosome breakage and many of these have been summarized by Sparrow (1951). Nava-shin and Gerassimova (1936) concluded that all the chromosomes of the set of Crepis capillaris broke with a frequency dependent upon their length. Their data, on the other hand, indicated that the frequency of chromosome breaks in the long arm of chromosome C was higher than would be expected on the basis of its length. This finding agrees with the results of the present work.

Lewitsky and Sizova (1935) reported increased break frequency in the proximal regions of the long arms of chromosomes A, D, and C and in the central region of the long arm of C. They indicated that the "liability to fragmentation" in these arms is associated with a lesser contraction of the chromonema in these particular regions. However, these conclusions were reached from a small number of breaks, and the differences indicated may not be significant.

A large majority of the breaks that rejoin to form dicentrics are located in or near the heterochromatin. The breaks that do not rejoin (simple breaks), on the other hand, are most frequently found in the euchromatic regions (Table 5). This fact suggests that heterochromatin and euchromatin have different effects on chromosome rejoining and restitution.

The different influences of heterochromatin and euchromatin on the behavior of chromosome breaks can explain the apparent non randomness of the dicentric breaks in the long arms of A and C and of the simple breaks in the long arm of A and the short arms of C and D (Table 4). As the long arm of the A chromosome is the longest, one might have expected that it would have more dicentric breaks than the long arms of C or D. Such was not the case. Dicentric breaks occurred with equal frequency in the long arms of A, C, and D. Thus, though the breaks per chromosome arm were equal, breaks per unit of length were not. On this basis, the long arm of C had more breaks and the long arm of A fewer than expected. However, when compared by amounts of heterochromatin the observed breaks equaled the expected (Table 6). The frequencies of dicentric breaks in the short arms were proportionate to the lengths (Table 6). Here it should be recalled that the short arms are entirely heterochromatic.

The data pertaining to the simple breaks (Table 4) are not very extensive but nevertheless appear to fit into the general scheme. The increase of simple breaks over the expected in the long arm of A could be due to the longer region of euchromatin in relation to total length. Since the short arms of C and D are entirely heterochromatic no simple breaks would be expected and none were found. The
appreciable frequency of simple breaks in the short arm of A cannot be accounted for at this time.

The finding that breaks tend to rejoin more in heterochromatin than in euchromatin is difficult to understand. One clue may be obtained, however, from the reports that pairing between non-homologous chromosomes is initiated between heterochromatic segments in haploid rye (Levan 1942) and haploid Triticum monococcum (Kostoff 1938). Such an affinity of heterochromatin between non-homologous chromosomes of Crepis capillaris would possibly provide greater opportunity for rejoining between heterochromatic than between euchromatic segments of the chromosomes.

The data and above conclusions suggest that the apparent non randomness of chromosome breaks by X-rays in Crepis capillaris seeds is a consequence of the rejoining and restitution processes rather than the breakage process. In other words, the initial chromosome breakage in Crepis chromosomes is actually random, but through differential influence of euchromatin and heterochromatin on rejoining and restitution the breaks in certain arms appear to be non random. This hypothesis was suggested earlier by Sax (1940), Sparrow (1951), and Swanson (1957). Furthermore, Sparrow (1951) summarized several reports which indicate that in chromosomes of certain species euchromatin and heterochromatin differ in their sensitivity to X-ray damage.

Navashin and Gerassimova (1936) reported that the X-ray induced intra-homologous exchanges in Crepis capillaris occurred at the expected frequency. The present paper supports this finding (Table 7) and further shows that the proportions of these exchanges are not altered by oxygen posttreatment.

The occurrence of chromatid breaks in supposedly monopartite chromosomes (cf. Caldecott and Smith 1952) of resting seeds of Crepis capillaris cannot be accounted for at this time. Nor is there a ready interpretation for the high frequency of these breaks in the long arm of D.

CONCLUSION

From the above data and hypotheses it may be concluded that the initial X-ray induced breaks in chromosomes of resting seeds of Crepis capillaris are at random. The apparent non randomness of breaks by X-rays in certain arms of the chromosomes is due to the differential influence of euchromatin and heterochromatin on the rejoining and restitution of the chromosome breaks.

Oxygen applied after X-irradiation has no influence on the proportion of aberrations which occur in each chromosome arm. Nevertheless, through its influence on restitution and rejoining of chromosome breaks it uniformly increases the frequencies of chromosome aberrations in all arms.

During the oxygen posttreatments the broken ends in the euchromatin "heal," restitution and rejoining are inhibited, and an increasing number of fragments result. In the heterochromatin, on the other hand, the oxygen is not as effective in "healing" and some rejoining does occur. This can account for the much more rapid increase of fragments (from inhibition of restitution in the euchromatin)
than dicentric chromosomes (from some rejoining in the heterochromatin) during the postirradiation oxygen treatments.

**SUMMARY**

Resting seeds of *Crepis capillaris* (2n = 6) were X-irradiated at 1500r or 3500r and then stored under 100 lbs oxygen pressure for periods up to 42 days. The frequencies of abnormal cells, dicentric chromosomes, and acentric rod and isodiametric fragments increased during the postirradiation oxygen treatment. Non-irradiated seeds exhibited an appreciable frequency of chromosome aberrations after 42 days of the oxygen treatment. Oxygen had no influence on the proportion of breaks in each chromosome because it uniformly increased the aberrations in all six chromosome arms. However, oxygen did appear to inhibit the restitution and rejoining processes since the ratio of breaks that did rejoin to those that did not rejoin decreased during the postirradiation treatment.

In both X-irradiated and stored X-irradiated seeds, certain arms exhibited frequencies of breaks leading to dicentric chromosomes and simple breaks (those that do not rejoin or restitute) that significantly differed from the frequencies expected on the basis of length of arms. The cause of this apparent non randomness was found in the relation of euchromatin and heterochromatin to chromosome breaks. Breaks leading to dicentrics occurred in or near the heterochromatic areas whereas the simple breaks occurred in the euchromatic areas of the chromosome arms. When the observed frequencies of breaks were compared with the lengths of heterochromatin and euchromatin in each arm, they equaled the expected frequencies. It is concluded that X-ray induced breakage probably occurs at random in *Crepis capillaris* chromosomes, and the apparent non randomness of these breaks is due to the differential influence of euchromatin and heterochromatin on the rejoining process.

The frequency of intrahomologous exchanges observed equalled the expected frequency. However, higher than expected frequencies of chromatid breaks were found; most of these breaks occurred in the long arm of D.

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**LITERATURE CITED**


