Repetitious Production of Similar Karyotypes in Different Plants of *Haplopappus gracilis*, an Annual Asteraceae, Following Exposure to Ionizing Radiation

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Summary  Following exposure to X-rays or reactor radiation at the early germinating stage, seedlings of *Haplopappus gracilis* (Nutt.) Gray were inspected for karyotypic changes in the meristems of lateral roots 2 months later. Among 210 plants studied in total, 12 were carriers of aberrant karyotypes in the root systems. One of them was a whole-body variant of spontaneous origin, showing a complete change of karyotype not only in the root system but also in the shoot system. The remainders were mosaics, showing a complete karyotypic change in one or more lateral roots but not in the whole root system. Of the 11 cases of aberrant karyotypes detected as inter-root mosaics, 5 were unique to the respective plants, each characterized by a specific kind of non-reciprocal translocation, reciprocal translocation or complex exchange; 2 were commonly characterized by a complex exchange; 3 were by a non-reciprocal translocation; one was characterized by a fragment chromosome accompanied with a shortened chromosome and was not distinguishable from the aberrant karyotype of spontaneous origin. These results support the conclusion that the chromosomes of *H. gracilis* possess the plasticity to rearrangements after irradiation. The repetitious occurrence of specific rearrangements in different plants and the recovery of fragment-bearing karyotype are discussed as suggestive evidence for the existence of preferential sites for breakage and rearrangement in *H. gracilis* genome.

Key words  Chromosome healing, Chromosome rearrangement, Fragment chromosome, *Haplopappus gracilis*, Irradiation, Neocentromere activation.

With the low chromosome number of 2n=4 (Jackson 1957) and the documented evidence of the karyotype evolution (Jackson 1973), *Haplopappus gracilis* (Nutt.) Gray, Asteraceae, has provided unique opportunities for studying karyotype variation and chromosomal evolution. And a body of relevant information is already available in the literature. To summarize briefly, the karyotype is polymorphic in natural populations, showing a variation of the chromosome number from the standard of 2n=4 chromosomes (Jackson 1965) and structural variation of a specific chromosome by centromere transposition in the 2n=4 races (Jackson 1973); it is also variable in laboratory populations by translocations (Jackson 1985, Yonezawa 1991), centromere transposition (Yonezawa 1981) and heteromorphism of a chromosome arm (Jackson 1963, Kamra 1963). The present study was carried out to examine the variability of karyotype in the laboratory populations following exposure to ionizing radiation, an agent that can efficiently induce the various types of chromosome rearrangements in higher plants.

Shigenobu and Kojima (1994) and Kurasawa and Shigenobu (1996) have reported that novel karyotypes could be successfully detected as large clones of rearrangement-bearing cells in the...
shoot systems of mature plants of *Zebrina pendula*, Commelinaceae, after irradiation as young plants with X-rays, and that the karyotypes were distinguishable from each other by the types of rearrangements involved. In the present study, we inspected the root meristems of *H. gracilis* plants for evidence of clonal expansion of rearrangement-bearing cells following irradiation at the early germinating stage with X-rays or reactor radiation and found several kinds of novel karyotypes as large clones. By analogy with the results reported by Shigenobu and Kojima (1994) we expected unique rearrangement for each of the detected karyotypic changes. This was not the case; aberrant karyotypes from two or three plants were characterized commonly by particular kinds of rearrangements. Furthermore, a $2n = 5$ karyotype associated with fragment chromosome was detected independently in two different plants, one as an inter-root mosaic and the other as a whole-body variant. In this report, we describe cytological results pertaining to these unusual phenomena and discuss the implications in relation to the genome structure of *H. gracilis*.

Material and methods

**Material and irradiation**

A reserved strain of *Haplopappus gracilis* (Nutt.) Gray, Kansas-Hiroshima Strain No. 1 (KH-1), was used in the present study. Prof. K. Kondo kindly supplied seeds of this strain, which had been reserved at the Laboratory of Plant Chromosome and Gene Stock in Hiroshima University since 1962 (cf. Tanaka 1967). The seeds were stored in a refrigerator under dark and dry conditions until use. Before irradiation, the seeds were sterilized firstly with 1% solution of benzalkonium chloride and then with 1% solution of sodium hypochlorite, for 5 min each, and immersed in water with aeration for 24 h at 20°C (Yonezawa and Tanaka 1973). Hydrated seeds were placed on a moistened blotting paper in Petri dishes, and the covered dish was irradiated with X-rays or neutron-gamma mixed radiation available inside a nuclear reactor installed at Kinki University.

For X-irradiation, a Hitachi X-ray generator was used at 140 kV and 4.5 mA with a 1.0 mm Al plus 0.2 mm Cu filter. The dose rate was 0.65 Gy/min as measured by a Victreen chamber. Seeds were irradiated at a dose of 18, 36 or 72 Gy. The reactor used is called the University Teaching and Research Reactor of Kinki University, UTR-KINKI, and manufactured in 1958 by Advanced Technology Laboratory (Mountain View, CA, USA). For irradiation with neutron-gamma mixed radiation, the reactor was operated at 1 W and the Petri dishes containing seeds were placed at the center of the reactor’s core for 2, 4 or 6 h during the operation. Dose rate of neutron-gamma mixed radiation was 0.4 Gy/h as measured by a pair of ionizing chambers. Dose-rate ratio of neutrons to gamma rays was 1 : 1.

**Culture of irradiated seeds and identification of grown plants**

At 48 h after irradiation, the seeds with the hypocotyl of 4–6 mm length were sown on nursery beds. At 6 weeks after irradiation, when 4 or 5 leaves sprouted out, the seedlings were transplanted into pots in a green house and allowed to grow there for 2 weeks. Each group of plants grown was designated according to the type of radiation and exposure time or dose used. Thus, N2, N4 and N6 were given to groups exposed to reactor radiation for 2, 4, and 6 h, respectively. To plant groups irradiated with X-rays, X18, X36 and X72 were given according to the dose applied in Gy unit. In each of the irradiated groups, individual plants were numbered so as to distinguish from each other and the year of investigation that was 1991, 1994, 1996 or 1997. The starting numbers of 1, 71, 101 and 201 were given to plants studied in these years, respectively.

**Karyotype analysis**

Four or more lateral roots were sampled from each plant 2 months after irradiation and subjected to karyotype analysis. Sampled roots were pretreated with 0.1% colchicine solution for 1 h at
20°C and fixed with 45% acetic acid for 15 min at 5°C. Fixed roots were macerated in a mixture of 1 part of 45% acetic acid and 2 parts of 1 M HCl for 15 s at 60°C; the terminal 1–2 mm was cut off from each root on a slide glass and stained with 2% aceto-orcein solution. The stained root tips were squashed under cover slips.

The preparations were microscopically examined at a magnification of ×1000 for the presence of chromosome rearrangements in the metaphase spreads. When 5 or more metaphase spreads on a preparation showed no evidence of rearrangements, the lateral root was recorded as cytologically normal. When all lateral roots examined from a plant showed no evidence of karyotype change, the plant was recorded as a non-carrier of aberrant karyotype in the root system. Once a metaphase spread showed evidence of abnormal karyotype, all the other metaphase spreads in the preparation were examined for possible coexistence of normal cells and/or other kinds of aberrant karyotypes; thereafter 30–36 metaphase spreads with clear chromosome figures were photographed for the measurements of arm lengths in the chromosome complements. Some prometaphase spreads were also photographed to examine the distribution of early condensing regions (ECRs) in chromosomes.

On each of the photographed metaphase figures, all chromosome arms were separately measured for the length, and totaled arm length was used as total chromosome length for the metaphase chromosome complement. The length of each arm relative to the total length of the chromosome complement was calculated, and the relative length was obtained for each chromosome as the sum of the relative length of short arm and long arm (Kagawa 1927). The relative chromosome length was averaged for all photographed metaphase figures from the same plant, since, with no exception, all metaphase spreads with rearrangements from one plant were karyotypically indistinguishable from each other. The longest chromosome in each chromosome complement was designated as chromosome 1, followed by chromosome 2, chromosome 3 and chromosome 4, according to the relative chromosome length. The terms metacentric, submetacentric, telocentric and subtelocentric were used to describe these chromosomes based on the ratio of the length of long arm to that of short arm (i.e., the arm ratio of Levan et al. 1964). From the averaged chromosome length and the arm ratio idiograms of aberrant karyotypes were drawn, where information on the distribution of ECRs was incorporated.

Results

Normal karyotype of H. gracilis

Fig. 1 shows the normal karyotype of H. gracilis at mitotic metaphase and prometaphase consisting of 2 chromosome pairs; the larger was metacentric, and the shorter subtelocentric with satellite. These chromosomes were designated, respectively, as 1g and 2g, according to the description of Tanaka (1967). As shown by the diagram of prometaphase chromosomes in Fig. 1, chromosome 1g had ECRs in the proximal regions of both arms and the interstitial and subdistal regions of long arm; chromosome 2g had ECRs in the whole region of short arm including satellite and in the proximal region of long arm (see also Fig. 3). These results confirm the reports by Tanaka (1967) and Yonezawa (1981).

Frequency and type of chromosome rearrangements after irradiation

As shown in Table 1, we examined 210 plants in total and found 12 plants (6%) among them as carriers of aberrant karyotypes in the root systems. Ten of the 12 plants were from X-irradiated series, where the frequency of plants with karyotype change in the root systems increased in a dose-dependent manner. Each of the 12 plants had one kind of aberrant karyotype in one or more of lateral roots examined. Among 12 aberrant karyotypes detected, 10 were characterized by chromosome exchanges and the remainder by chromosome fragment, as will be described later in detail.

In Table 2, the 12 plants with aberrant karyotypes in the root systems are listed, and the ratios
of affected roots to total lateral roots examined for respective plants are shown in the last column. The ratio value of lower than unity can be taken as evidence of inter-root mosaicism for aberrant karyotype, because, in any of the affected lateral roots from those plants, we found no evidence of intra-root mosaicism after inspection of several tens of metaphase figures for possible coexistence of normal cells and other kinds of abnormal karyotypes. That was the case for all plants, except for Plant X18-38, for which the ratio value was 1 (Table 2). Plant X18-38 had a fragment chromosome accompanied with a shortened chromosome in all metaphase figures from 8 roots examined. Based on this finding and other data (see Fig. 4), we registered this plant as a whole-body variant.

Hereafter, we use the plant numbers to nominate aberrant karyotypes, e.g., X36-11 for the karyotype from Plant X36-11. Metaphase and prometaphase figures of the 12 karyotypes are shown in Fig. 2. From the diagrammatic representations of prometaphase figures shown in Fig. 2, the ECR distribution could be determined along all chromosomes involved in the aberrant karyotypes, except for X36-29 and X36-107 where chromosome sticking had masked ECRs in one or two chromosomes (see Fig. 3).

Table 1. Frequency of H. gracilis plants with aberrant karyotypes in the root systems 2 months after irradiation at the early stage of germination and the type of rearrangements that characterized the aberrant karyotypes

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Exposure (Gy)</th>
<th>No. of plants examined</th>
<th>No. of plants with affected roots (%)</th>
<th>Rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor</td>
<td>0.8</td>
<td>45</td>
<td>1 (2)</td>
<td>Exchange (1)</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>17</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>15</td>
<td>1 (7)</td>
<td>Exchange (1)</td>
</tr>
<tr>
<td>X-rays</td>
<td>18</td>
<td>62</td>
<td>2 (3)</td>
<td>Fragment (2)</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>61</td>
<td>5 (5)</td>
<td>Exchange (5)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10</td>
<td>3 (30)</td>
<td>Exchange (3)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>210</td>
<td>12 (6)</td>
<td>Exchange (10), Fragment (2)</td>
</tr>
</tbody>
</table>

a) Neutron-gamma mixed radiation from UTR-KINKI. b) The number of plants whose aberrant karyotypes were characterized by the indicated rearrangement.
Characteristics of aberrant karyotypes

Table 3 shows the compiled data for the relative length of chromosomes and chromosome arms in the complement of the 12 aberrant karyotypes. Normal 1g and 2g chromosomes shown in this table were determined based on the chromosome length, the arm ratio and the presence or absence of satellite, using the description for normal chromosome complement by Tanaka (1967) as criteria. As can be seen in Table 3, the length of normal chromosomes, 1g or 2g, from different chromosome complements agreed, within the limits of experimental errors, with each other, suggesting that there were no marked differences in the amount of chromosome material among the aberrant karyotypes detected. This result enabled close comparison of idiograms among the 12 cases of aberrant karyotypes with respect to the size of rearranged chromosomes and chromosome arms.

Idiograms, which diagrammatically represented the length data from Table 3 and the ECR data from Fig. 2, are shown in Fig. 3. It was found from the comparison of idiograms that, among the 12 aberrant karyotypes, five (i.e., X36-11, X36-29, X36-40, X36-53 and X36-107) were unique to the respective plants; the remainders could be classified into 3 groups consisting of similar karyotypes from 2 or 3 plants. That is to say that 2 karyotypes X72-201 and X71-205 or X18-38 and X18-49, and 3 karyotypes N6-5, N2-44 and X72-106 were indistinguishable from each other within the limit of resolution of the method used.

In the following paragraphs, characteristics of the 8 classes of aberrant karyotypes are described with heading of the karyotype nomination(s) and the chromosome complement:

1) X36-11 (2n=4, 1g+2g+two rearranged chromosomes, i.e., chromosomes 2, 3): Chromosome 2 was a telocentric chromosome with apparently intact short arm of 2g; the long arm was longer than that of 2g. Chromosome 3 was metacentric with apparently intact short arm of 1g; the long arm was shorter than that of 1g. The rearrangement appeared to be a non-reciprocal translocation of the distal quarter of long arm of 1g to the end of long arm of 2g.

2) X36-29 (2n=4, 1g+2g+two rearranged chromosomes, i.e., chromosomes 2 and 3): Chromosome 2 was metacentric. Chromosome 3 was a subtelocentric chromosome with apparently intact short arm of 2g; the long arm was longer than that of 2g and had ECRs in the proximal and subdistal regions. The rearrangement appeared to involve a non-reciprocal translocation of the distal portion of long arm of 1g to the end of long arm of 2g, as it was the case for aberrant karyotype...
Table 3. Relative length of each chromosome in the complements with aberrant karyotypes found in *H. gracilis* plants 2 months after irradiation as germinating seeds

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Chromosome 1</th>
<th>Chromosome 2</th>
<th>Chromosome 3</th>
<th>Chromosome 4</th>
<th>Chromosome S^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13.3+16.4=29.7^1z</td>
<td>13.0+16.4=29.4^1z</td>
<td>2.8+1.9^1n+16.0=20.7^1z</td>
<td>2.8+1.9^1n+15.4=20.1^1z</td>
<td></td>
</tr>
<tr>
<td>X36-53</td>
<td>18.3+23.6=41.9 (3.6)^1i</td>
<td>2.7+2.8^1d+16.1=20.8^1e (2.0)^1g</td>
<td>2.6+2.0+15.3=19.9^1k (1.8)^1f</td>
<td>6.9+11.5=18.4 (1.1)^1f</td>
<td></td>
</tr>
<tr>
<td>X36-107</td>
<td>16.0+19.3=35.3 (2.7)</td>
<td>2.9+2.6+16.4=21.9^2z (1.8)</td>
<td>3.0+2.6+16.1=21.7^2k (1.5)</td>
<td>7.2+14.2=21.4 (2.2)</td>
<td></td>
</tr>
<tr>
<td>X36-11</td>
<td>12.7+18.2=30.9^2e (4.0)</td>
<td>2.0+1.0+22.2=25.2 (3.6)</td>
<td>11.7+12.9=24.6 (2.6)</td>
<td>1.9+1.2+17.1=20.2^2k (3.1)</td>
<td></td>
</tr>
<tr>
<td>X36-29</td>
<td>12.2+17.2=29.4^2i (2.8)</td>
<td>10.9+15.7=26.6 (2.5)</td>
<td>2.8+2.2+18.7=23.7 (2.9)</td>
<td>2.6+1.6+16.0=20.2^2k (2.2)</td>
<td></td>
</tr>
<tr>
<td>X36-40</td>
<td>16.2+17.7=33.9 (2.4)</td>
<td>12.5+17.1=29.6 (2.1)</td>
<td>2.2+1.8+15.6=19.6^2k (1.8)</td>
<td>5.1+1.7+9.6=16.4 (1.6)</td>
<td></td>
</tr>
<tr>
<td>N6-5</td>
<td>17.1+21.1=38.2 (3.9)</td>
<td>12.5+16.6=29.1^1z (2.4)</td>
<td>2.5+1.8+15.7=20.0^2z (2.5)</td>
<td>2.4+2.3+8.3=13.0 (1.7)</td>
<td></td>
</tr>
<tr>
<td>N2-44</td>
<td>17.0+21.4=38.4 (3.9)</td>
<td>12.6+16.9=29.5^2s (3.0)</td>
<td>2.5+1.5+16.4=20.4^2k (3.3)</td>
<td>2.6+1.6+8.2=12.4 (3.5)</td>
<td></td>
</tr>
<tr>
<td>X72-106</td>
<td>17.2+21.2=38.4 (2.6)</td>
<td>11.4+16.1=27.5^1v (1.5)</td>
<td>2.2+1.9+17.5=21.6^2k (1.5)</td>
<td>2.1+1.9+8.8=12.8 (1.8)</td>
<td></td>
</tr>
<tr>
<td>X72-201</td>
<td>2.7+10.2^1i+24.6=37.5 (3.6)</td>
<td>12.3+16.6=28.9^2d (1.9)</td>
<td>4.7+16.3=21.0 (1.5)</td>
<td>2.7+1.7+8.4=12.8 (1.8)</td>
<td></td>
</tr>
<tr>
<td>X72-205</td>
<td>2.5+10.4+24.8=37.3 (3.7)</td>
<td>12.2+17.1=29.3^1z (2.6)</td>
<td>4.2+16.6=20.8 (2.1)</td>
<td>2.4+1.6+8.3=12.3 (1.8)</td>
<td></td>
</tr>
<tr>
<td>X18-38</td>
<td>12.6+17.3=29.9^1z (2.7)</td>
<td>11.7+15.7=27.4 (2.3)</td>
<td>2.7+1.8+15.1=19.6^2k (2.6)</td>
<td>2.6+1.6+13.8=18.0^2k (2.4)</td>
<td></td>
</tr>
<tr>
<td>X18-49</td>
<td>12.3+16.6=28.9^2z (3.3)</td>
<td>11.6+15.9=27.5 (3.1)</td>
<td>2.7+1.6+14.8=19.1^2k (3.1)</td>
<td>2.8+1.5+13.7=18.0^2k (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

1g=normal 1g chromosome; 2g=normal 2g chromosome. a) Measured length adjusted to unity for totaled arm length in the chromosome complement (see Materials and Methods). b) Used to rank chromosomes in each of the chromosome complements, giving the number 1 to the longest, 2 to the second to the longest and so on. c) Fragment chromosome with centromere. d) Data from Tanaka (1967). e) Length of satellite+length of the main part of short arm. f) Standard deviation calculated for the total chromosome length based on 36 measurements on average.
Fig. 2. Aberrant karyotypes detected in the root systems of *H. gracilis* plants 2 months after irradiation as germinating seeds with X-rays or reactor radiation. The italicized characters indicate each karyotype detected in each plant. The leftmost panel, the middle panel and the rightmost panel for each karyotype show metaphase chromosomes, prometaphase chromosomes and diagrammatic representation of the prometaphase figure, respectively. The number attached to each chromosome shows the chromosome number in the complement. Letters (1g) and (2g) indicate normal 1g and 2g chromosome, respectively. Bar is 10 μm for all panels.
However, the satellite-bearing chromosome was chromosome 3, the third to the longest, in the complement of this karyotype, but it was chromosome 2 in that of \textit{X36-11}, indicating that the translocated segment was relatively shorter in this karyotype.

3) \textit{X36-53} (2\textit{n}=4, two 2g+two rearranged chromosomes, \textit{i.e.}, chromosomes 1 and 4): Chromosome 1 was a metacentric chromosome with ECRs in the proximal, interstitial and subdistal regions of both arms. Chromosome 4 was a submetacentric chromosome with ECRs in the pericentric regions. The rearrangement was a reciprocal translocation between the distal two-thirds of the short arm of one 1g and the distal half of the long arm of the other 1g.

4) \textit{X36-107} (2\textit{n}=4, two 2g+two rearranged chromosomes, \textit{i.e.}, chromosomes 1 and 4): This karyotype was similar to \textit{X36-53}, with respect to the ECR distribution along chromosomes 1 and the shape of chromosomes 4. However, chromosomes 1 and 4 in this complement were shorter and longer than the corresponding chromosomes in \textit{X36-53}'s complement, respectively.

5) \textit{X36-40} (2\textit{n}=4, 1g+2g+two rearranged chromosomes, \textit{i.e.}, chromosomes 1 and 4): Chromosome 1 was metacentric with short arm, which appeared to be intact long arm of 1g; the long arm was longer than the short arm of 1g. Chromosome 4 was metacentric with satellite on the short arm; as compared with the corresponding arms of normal 2g chromosomes, the long and the short arms were shorter and longer, respectively. The rearrangement was a complex exchange, involving translocations of two segments from a distal portion of the long arm of 2g to two positions, one to the end of satellite in the short arm of 2g and the other somewhere in the short arm of 1g.

6) \textit{N6-5}, \textit{N2-44} and \textit{X72-106} (2\textit{n}=4, 1g+2g+two rearranged chromosomes, \textit{i.e.}, chromosomes 1 and 4): In any one of the 3 cases of aberrant karyotypes, chromosome 1 was metacentric; the short arm appeared to be the long arm of 1g; the long arm was longer than the short arm of 1g. Chromosome 4 was subtelocentric with apparently intact short arm of 2g; the long arm was shorter than that of 2g. The rearrangement appeared to be a non-reciprocal translocation of a distal portion of the long arm of 2g to the end of short arm of 1g.

7) \textit{X72-201} and \textit{X72-205} (2\textit{n}=4, 1g+three rearranged chromosomes, \textit{i.e.}, chromosomes 1, 3 and 4): In either case, chromosome 1 had satellite on the short arm; the long arm of chromosome 3 appeared to be the intact long arm of 2g; the short arm of chromosome 4 was similar to the short arm of 2g. That is, the rearrangement involved both arms of one 1g, the short arms of one 2g, and the long arm of the other 2g. The complex exchange appeared to involve 2 reciprocal translocations.
(one between the distal portion of short arm of 1g and the satellite in the short arm of 2g, and the other between the long arm of the other 2g and the distal portion of long arm of the affected 1g) and an interstitial deletion of chromosome segment containing the subdistal ECR from the affected long arm of 1g.

8) \textit{X18-38} and \textit{X18-49} (2n=5, 1g+two 2g+two rearranged chromosomes, \textit{i.e.}, chromosomes 2 and 5): In either case, chromosomes 2 was a shortened 1g chromosome and chromosome 5 a fragment chromosome consisting of ECR in almost the whole region; the mean length of the fragment chromosome was several-fold longer than the length of lost portion in the shortened chromosome, \textit{e.g.}, 5.7 vs. 2.2 for \textit{X18-38}. Since the shortened chromosome did not have the subdistal ECR present in the long arm of 1g, the ECR fragment was judged to have derived, at least partly, from a distal portion of the long arm of 1g.

Plant \textit{X18-38}, the complete variant in the root system, was reexamined at the flowering stage for the presence of the fragment chromosome in cells of the anther wall and the pollen mother cells. In both types of cells, a fragment chromosome could be observed (Fig. 4). In cells of the anther wall, the 2n=5 chromosome complement was confirmed (Fig. 4A). In the pollen mother cells, the fragment chromosome was present as a univalent at metaphase I (Fig. 4B-1) and as laggard chromosomes at anaphase II (Fig. 4B-2). These data support the conclusion that Plant \textit{X18-38} is a whole-body variant for the fragment-bearing karyotype \textit{X18-38}.

\textbf{Discussion}

\textit{Monoclonal origin of each aberrant karyotype}

In the present study, we irradiated the seeds of \textit{H. gracilis} at the early germinating stage with X-rays or reactor radiation and examined them for the presence of abnormal karyotypes in the meristems of lateral roots 2 months after irradiation. Among total 210 plants examined, 12 (6\%) were carriers of abnormal karyotypes in the root systems (Table 1). One of them (\textit{i.e.}, Plant \textit{X18-38}) was a whole-body variant, showing complete karyotype change not only in the root system but also in the shoot system (Table 2, Fig. 4). This case may well be due to chromosome aberration that had arisen spontaneously some time before the onset of embryogenesis for the plant development, probably in the preceding generation. All the remainders were mosaics, showing complete karyo-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig_4}
\caption{Chromosomes at anaphase in mitosis of an anther wall cell (A), at metaphase I (B-1) and at anaphase II (B-2) in meiosis of pollen mother cells of Plant X18-38, a whole-body karyotypic variant of \textit{H. gracilis} found in an X-irradiated group. Bar in A is 5 \(\mu\)m and that in B-2, 10 \(\mu\)m for B-1 and B-2.}
\end{figure}
type changes in one or more lateral roots but not in the whole root systems (Table 2). We assume that the majority of aberrant karyotypes detected as inter-root mosaics were induced at the early stage of germination after irradiation, because the frequency of mosaic plants increased from a low level of 3% to a high level of 30% as X-ray dose was increased from 18 Gy to 72 Gy (data from Table 1). When taken at the face values, this dose-response relation nicely fits to an exponential curve as usual for chromosome aberrations induced by X-rays in plants (e.g., see Evans 1962). From the averaged ratio of affected roots to total roots examined in the mosaic plants (Table 2), we estimate that the number of initial cells per radicle existing during irradiation is 3 or 4 on average. We thus may explain the origin of inter-root mosaicism as follows: One of a few initial cells had suffered karyotypic change in the radicle of germinating seed after irradiation and then undergone clonal expansion and tissue differentiation in concert with normal initial cells during the growth from the radicle to mature root system, resulting in inter-root mosaicism for the aberrant karyotype.

It is self-evident from the monoclonal origin of aberrant karyotypes that rearrangements involved are stable ones, a category of chromosome changes that can persist through many mitotic divisions after the formation. Exchange and fragment were the 2 classes of stable rearrangements found in the mosaic plants (Table 1). The former was the predominant one, accounting for 10 out of 11 cases of aberrant karyotypes from the mosaic plants. As described in the preceding section, reciprocal translocation, non-reciprocal translocation and complex exchange were the types of stable exchanges that characterized, respectively, 2, 5 and 3 of the 11 cases of aberrant karyotypes detected as mosaics. The fragment chromosome was accompanied by a shortened chromosome, and this combination characterized the remainder case of aberrant karyotypes detected as mosaics. These data support the conclusion that the H. gracilis karyotype is highly variable by rearrangements following exposure to radiation at the early stage of germination.

Characteristics of chromosome rearrangements and breakage points

Of the four types of rearrangements detected in the present study, reciprocal and non-reciprocal translocations are the types reported previously as spontaneously arisen rearrangements in the laboratory populations of H. gracilis. The reciprocal translocation described by Yonezawa (1991) is an exchange between 1g and 2g. In the present study, both of the two reciprocal translocations, which characterized aberrant karyotypes X36-53 and X36-107 (Fig. 3), were exchanges between two 1g. And simple exchanges involving non-homologous chromosomes were detected as non-reciprocal translocations in aberrant karyotypes X36-11, X36-29, N6-5, N2-44 and X72-106, any one of which appeared to be a translocation of a terminal segment from one chromosome arm to the end of another chromosome, namely, terminal translocation, according to the nomenclature used by Xiao et al. (1999). The non-reciprocal translocation reported by Jackson (1985) is also a terminal translocation, namely one arm from 1g is translocated to the end of long arm of 2g. This kind of terminal translocation was not detected in the present study. We thus may register all of the aberrant karyotypes detected in the present study as novels.

To our surprise, novel karyotype due to a specific rearrangement was independently found in two or three different plants irradiated with different doses and in different years (Fig. 3). This can hardly be interpreted as a fortuitous event because the repetitious occurrence was seen not only for karyotypes due to terminal translocation (i.e., N6-5, N2-44 and X72-106), but also for karyotypes due to complex exchange (i.e., X72-201 and X72-205). Such exchanges require multi-breakage events for their formation and, therefore, a heavy dose of radiation for induction. As a matter of fact, two of the three aberrant karyotypes detected after X-irradiation at 72 Gy, the highest tolerable dose used, were due to complex exchanges and indistinguishable from each other. These data suggest that there are preferential sites for breakage and rearrangement in the H. gracilis genome. When DNA damage is introduced in or near the site and not repaired in an error-free manner, the damage may trigger rearrangement in a site-depending manner. Otherwise, the affected site may be
involved in the formation of unstable rearrangements, a category of chromosome changes that cannot persist through successive mitotic divisions.

According to Tanaka (1967), but Ikeda (1987) has proposed another order, 1r chromosome of *H. gracilis* is composed of 1r chromosome, a part of 2r chromosome and 3r chromosome of *H. ravenii*, a closely related species of *H. gracilis*, in tandem order of 1r-3r-2r; 2g chromosome is composed of 4r chromosome plus a euchromatic segment of 2r chromosome of *H. ravenii*. As a consequence of the reconstruction, the *H. gracilis* chromosomes would have gained one or more blocks of centromeric and telomeric sequences in the interstitial regions in a suppressed state (Yonezawa 1981). This possibility is worthy of scrutiny in seeking potential candidates for the above-specified preferential sites for breakage and rearrangement in the *H. gracilis* genome. In support of this proposal, some studies on the distribution of radiation-induced breakage points along the chromosomes of mammalian cells have shown the preferential involvement of interstitial telomeric blocks in the formation of exchange-type aberrations (Alvarez *et al.* 1993, Bouffler *et al.* 1993, Fernandez *et al.* 1995, Dominguez *et al.* 1996).

**Neocentromere activation and chromosome healing**

Another point of interest in the present data concerns the fragment-bearing karyotype detected either as a whole-body variant or as a mosaic. For the whole-body variant, we reasonably assume the presence of centromere-like structure in the fragment; the cytological evidence of anaphase separation of chromatids was seen in both mitotic and meiotic cells (Fig. 4). Since the fragment was accompanied with a shortened chromosome in the 2n=5 complement, it would have certainly originated as structurally acentric and required the formation of centromere-like structure to be stabilized. The assumed centromere-like structure may be related to reactivation of the suppressed centromere (Yonezawa 1981) rather than neocentromere activation (cf. Choo 1997).

Two mechanisms are considered for the production of fragment, namely, by a simple breakage event in the long arm of 1g or by incomplete intra-chromosomal exchange in 1g. If the former was the case, the fragment and the affected chromosome both should have required *de novo* formation of telomeres at the broken ends. If the latter were the case, both ends of the affected chromosome should have healed (cf. Kipling 1995, Slijepcevic and Bryant 1998), while the signals of telomere sequence have been not detected on the interstitial regions of both chromosomes in *H. gracilis* (Cox *et al.* 1993, Fuchs *et al.* 1995). In association with the stabilization, amplification of some DNA sequence may have occurred, resulting in the fragment larger than the lost portion in the shortened chromosome.

A similar scenario can be considered for the fragment-bearing karyotype of mosaic type, independently of its similarity to the karyotype of whole-body variant. If the fragment was acentric, the affected cells would have been eliminated during the growth of the root system or failed to form a clone of appreciable size due to partial aneuploidy resulting from mitotic loss of the fragment. This was not the case. The observed mosaicism accounted for as large as 50% of the root system as a fraction affected by the fragment-bearing karyotype (Table 2). This fact demonstrates mitotic stability of the fragment.

From these arguments, we are inclined to believe that efficient mechanisms are operating in the *H. gracilis* genome to stabilize broken chromosomes, acentric or centric, and reconstruct the genome structure. The possible existence of interstitial blocks of centromeric and telomeric sequences in the *H. gracilis* genome may merit serious consideration in this context, too.

In summary, the present study has shown a high variability of *H. gracilis* karyotype after irradiation. As far as we were aware of, none of the karyotypes described in this report had been known. Another aspect of the variability has been revealed as repetitious occurrence of specific rearrangements in different plants and the recovery of fragment-bearing 2n=5 karyotype. Molecular cytogenetic studies on the architecture of *H. gracilis* genome are needed in order to unravel mecha-
isms involved in these unusual phenomena.

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References


