5. The synthesis of two-chromosome double interchanges.

Two-chromosome double interchange stocks were synthesized in maize by intercrossing single interchange stocks, each with breakpoints that involved the same two chromosomes. Combinations that combined both of the single interchanges into the two pairs of chromosomes were established presumably by simultaneous crossovers that occurred in the two differential segments.

A series of interchanges involving chromosomes 9 + (1 through 10) were employed along with 1-5°s and 2-6°s. Nine different opposite-arms double interchange stocks were obtained in maize involving the following chromosomes: 1-9, 2-9, 4-9, 5-9, 6-9, and 9-10 from 9 + (1 through 10) series, one 2-6, and two different 1-5°s. The series involving 9 covers all chromosomes except 3, 7, and 8. Five different I-IV same-arms double interchange stocks were also synthesized in Neurospora crassa.

Several of the synthesized two-chromosome double interchange stocks in both maize and <u>Neurospora</u> were tested for their effectiveness as linkage detectors by crossing them with multiple marker stocks. It appears that such stocks can be more effective than other interchange techniques in locating unplaced genes.

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1. A rapid screening technique for photosynthetic mutants.*

Photosynthetic mutants have provided a valuable approach to the resolution of questions concerning photosynthetic mechanisms (Levine, R. P., 1969, Ann. Rev. Pl. Physiol. 20:523-540). As the use of mutants necessitates their rapid identification, an <u>in vivo</u> screening technique was developed to locate photosynthetic mutants that have normal pigment

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and structural appearance. This technique has been used to locate Zea mays mutants of the above type.

A screening technique was developed by Levine for algal mutants which compared levels of chlorophyll fluorescence. Colonies which were blocked in photosynthetic electron transport, either by chemical inhibition or by mutation, showed higher visual fluorescence than normal types (Bennoun, P. and R. P. Levine, 1967, Plant Physiol. 42:1284-1287; Levine, R. P., 1971, In: A. San Pietro, ed. Methods of Enzymology, XXIII, Part A. Academic Press, N.Y., pp. 119-129). The feasibility of a similar technique for higher plants was suggested since in vivo fluorescence of whole plant leaves could be observed and increased with photosynthetic inhibitors (Stokes, G. G., 1852, Phil. Trans. Roy. Soc. London 1852: 463-562; Kantsky, J. and A. Hirst, 1931, Naturwissenchaften 19:964).

Young leaves of tomato, spinach, the plastome mutant of Oenothera hookeri (Stubbe, W., 1959, Z. Vererbungslehre 90:288-298) and suspect mutants of Zea mays were used.

Whole leaf fluorescence could be observed and photographed in the dark through a Corning 2030 red filter which transmits wavelengths above 640 nm. Fluorescence was excited by a tungsten light (2 x 10 ergs/cm² sec.) passed through a 6 mm heat absorbing glass (or 10 mm water) and a Corning 4305 blue filter. This filter had a peak transmission of 473 nm and a range from 320 to 640 nm. Leaves were placed directly on the blue filter and covered by the red. An alternate method employed a spectroline SL-3660 long wave ultraviolet lamp for excitation of fluorescence. This lamp had a peak output at 360 nm and a range from 315 to 420 nm at an intensity of 1 x 10 ergs/cm² sec. The plant leaves were suspended 7 mm from the lamp by a thin polyethylene sheet and viewed through a red acrylic cutoff filter (Rohm and Hass 2444) that transmits light above 605 nm.

In order to show that a variation in fluorescence was indeed correlated with functionality of the photosynthetic apparatus, a comparison of the level of fluorescence was made between normal leaves and those treated with photosynthesis inhibitors. Quite striking differences were noted in the visual fluorescence when plants were treated with these

inhibitors. As a further proof, the plastome mutants of <u>Oenothera</u>, in which variegated (mutant-normal) leaves are produced, were also examined. Mutant sections fluoresced intensely compared to normal sections. When treated with electron transport inhibitors normal leaf parts fluoresced as brightly as the mutant sections, but the fluorescence of the mutant areas was no further increased by chemical inhibition. These plants were reported to be PS-II mutants (Fork, C. D. and U. W. Heber, 1968, Pl. Physiol. 43:606-612).

These results indicate that when electron transport is blocked between the two photosystems, whether by chemical inhibition or mutation, the affected plant tissue can be identified visually by increased fluorescence. As further proof that the fluorescence technique is useful for screening higher plants, we have isolated suspected PS-I and PS-II mutants of Zea mays seedlings due to their high in vivo leaf fluorescence. A more detailed report has been submitted to Plant Physiology and additional work is now underway.

As a practical note, care must be taken that there is little variation in chlorophyll content or in thickness of leaves which are scanned. These variations will alter fluorescence since more pigment or a thicker cross section would allow more re-absorption by chlorophyll.

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1. Two new B-type translocations involving chromosome 10.

X-ray-induced A-B translocations involving both arms of chromosome 10 have been identified. TB-10b, located on the long arm, is proximal to $\underline{\text{li}}$, $\underline{\text{g}}_1$, $\underline{\text{r}}$ and $\underline{\text{sr}}_2$. The TB-10S mentioned in MNL 45:144 is