through glass bead columns (*7*) or by Ficoll Hypaque gradient separation (*8, 11*) bear insulin receptors. In contrast, human peripheral blood lymphocytes (*17*) or rat spleen lymphocytes (*11*) prepared by passage through a nylon wool column do not bear insulin receptors; however, receptors emerge upon the nonadherent effluent cells during lymphocyte transformation induced by concanavalin A (*18*). Since the primary consequence of passing mononuclear cells through nylon wool columns is deletion of B lymphocytes (*19*) and macrophages (*20*), integration of these data would indicate that cells adherent to nylon wool bear insulin receptors (*9*) whereas nonstimulated splenic and peripheral blood T lymphocytes lack insulin receptors unless the nylon wool column damages insulin receptors. The present data indicate that immunoglobulin-negative cytotoxic lymphocytes (almost certainly T lymphocytes) bear functional insulin receptors. The mononuclear cells of patients with adult onset diabetes (*21*) exhibit deficient insulin receptors. Since insulin profoundly augments LMC, the propensity of diabetic patients to suffer from recurrent and severe microbial infection may be due in part to altered lymphocyte metabolism resulting in suboptimal lymphocyte responsiveness.

TERRY B. STROM
ROBERT A. BEAR
CHARLES B. CARPENTER
Department of Medicine,
Peter Bent Brigham Hospital,
Boston, Massachusetts 02115

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22. We gratefully acknowledge the help of W. Chick of the Joslin Research Foundation, who performed the insulin determinations, and the excellent technical assistance of R. Kostick, S. Washington, R. Price, and K. George. Supported by NIH grant AM15579 and the Leukemia Research Foundation, Inc. C.B.C. is an investigator of the Howard Hughes Medical Institute.

23 August 1974

Permanent Translocation Heterozygosity and Sex Determination in East African Mistletoes

Abstract. Viscum fischeri *has* 2n = *23 chromosomes in male plants. These form 7 bivalents and a translocation chain of 9 chromosomes during meiosis. Pollen with 11- and 12-chromosome genomes is thus produced. Female plants have* 2n = *22 chromosomes and produce 11 bivalents during meiosis. Sex determination is technically a rare multiple X–multiple Y type, but more importantly it provides the mechanism whereby permanent translocation heterozygosity is maintained in the system. In a second species,* Viscum engleri, *male plants have* 2n = *28 chromosomes, associating as 11 bivalents and a ring of 6 chromosomes at meiosis.*

Recently we described an unusual case of translocation heterozygosity in an East African mistletoe, *Viscum fischeri* Engl. (*1*). A chromosome number of $2n = 23$ was determined in male plants undergoing meiosis. In first meiotic metaphase a configuration of 7 bivalents and an open, multivalent chain of 9 chromosomes was consistently observed (Fig. 1). A regular, alternate (zigzag) orientation of the chain resulted in 4 : 5 disjunction at first anaphase, so that 11- and 12-chromosome genomes were transmitted via the pollen. The 11-chromosome set characteristically was comprised of 10 metacentrics or submetacentrics and 1 acrocentric, while the 12-chromosome set had 9 metacentrics or submetacentrics and 3 acrocentrics [for additional photographic documentation, see (*1*)]. The exceptionally large chromosomes in *Viscum* greatly facilitate analysis (*1*).

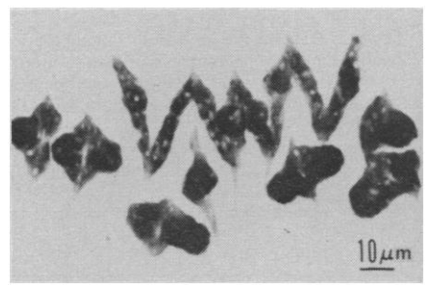

Fig. 1. First meiotic metaphase of a male plant of *V. fischeri* illustrating the chain of 9 chromosomes and the 7 bivalents. The chain is oriented in typical zigzag fashion prior to 4 : 5 disjunction at first anaphase; chiamata are visible as the rounded enlargements along the chain.

We also pointed out that if the translocation heterozygosity in *V. fischeri* is permanent, then the system differs significantly from other permanent translocation heterozygotes such as species of *Oenothera* and *Isotoma* (*2*). In *V. fischeri* the system involves structurally and numerically different genomes in which some chromosomes are acrocentric, and an open (chain) multivalent, whereas in other translocation heterozygotes even-numbered ring-forming genomes with metacentric chromosomes are the rule. It was also noted that *V. fischeri*, being dioecious, is an obligate outcrosser, whereas inbreeding is characteristic of other permanent translocation heterozygotes.

Since the species is dioecious we also noted that the numerically different genomes were possibly related to the sex-determining system. One essential aspect for further study, therefore, was the karyological structure and meiotic behavior of female plants, which could not be determined from the original materials. A study of mitotic cells from shoot apices in female plants from two geographically diverse populations in

Kenya (Nairobi and Nyeri) has now been made. In both cases the somatic chromosome number is $2n = 22$. The female genome is composed of 14 metacentric, 6 submetacentric, and 2 acrocentric chromosomes (Fig. 2). Meiosis in the female plant has also been observed and 11 bivalents are formed. The female plants thus possess two 11-chromosome sets structurally identical with the 11-chromosome set identified in the male plants (*1*). Meiosis has now been studied in male plants from a total of ten populations from throughout highland Kenya. All possessed the typical 7 bivalents and chain of 9 chromosomes previously reported, leaving little doubt that the translocation heterozygosity is permanent.

Viscum fischeri, however, is not a permanent translocation heterozygote in the classical sense. The translocation hybridity is, in fact, linked to the sex-determining mechanism of the species. The female plants are presumably homogametic, with two sets of 11 chromosomes, while the males are heterogametic with one set of 11 and one of 12 chromosomes. The chain multivalent which occurs in the male plants is therefore functionally a sex multivalent (novemvalent), and the male therefore has $2n = 14A + X_1X_2X_3X_4$-$Y_1Y_2Y_3Y_4Y_5$, where A stands for autosome. Similarly the female presumably has $2n = 14A + X_1X_1X_2X_2X_3X_3X_4X_4$; the sex-determining system is thus of the multiple X–multiple Y type.

Multiple X–multiple Y sex-determining systems are apparently rare. Among insects they are known in *Drosophila*, the beetle *Blaps*, and the centipede *Otocryptops* (*3*) and in flowering plants only in *Humulus*, hops (*4*). Other multiple systems such as X_nX_n-Y_nY and X_nX_n-X_n0 are more frequent. If the sex-determining system of *V. fischeri* is interpreted as a multiple X–multiple Y system then it is most unusual in plants, especially since hops only reach the level of $X_1X_1X_2X_2$-$X_1X_2Y_1Y_2$. Male and female plants of *V. fischeri* have been analyzed to determine the geographical constancy of the system so far identified (*5*).

Earlier we also reported translocation heterozygosity in *Viscum engleri* Tiegh. ($n = 14$) from the western Usambara Mountains of Tanzania (*1*). Study of additional materials of male plants has shown that all but 1 of the 20 plants now examined have the ring of 6 chromosomes previously reported (Fig. 3). The other plant examined had 10

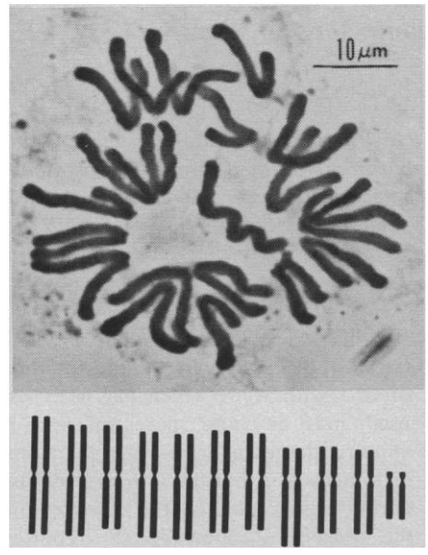

Fig. 2. Mitotic chromosomes and karyotype of a female plant of *V. fischeri*.

bivalents and two rings of 4 chromosomes. The ring of 6 chromosomes thus appears to be fixed, at least in most male plants of the populations studied, and may represent another case of sex-associated translocation heterozygosity. If this is the case it would appear that this phenomenon has some special adaptive value in the dioecious *Viscum* species of the East African highlands.

In *V. fischeri* all the chromosomes comprising the sex multivalent are surely not sex chromosomes in the restricted sense. Most elements of the sex multivalent must represent autosomes brought into the sex-determining system fortuitously through reciprocal translocations. In general usage, however, all members of sex multivalents are considered to be sex chromosomes even though they lack such features as heterochromatin, which is typical of many sex chromosomes, especially the Y. Where sex multivalents of at least quadrivalent complexity become established through reciprocal translocations, the sex-determining system, per se, is probably incidental. The central issue should be the adaptive value of the fixed translocation system which happens to contain the gene, genes, or chromosomes that determine sex.

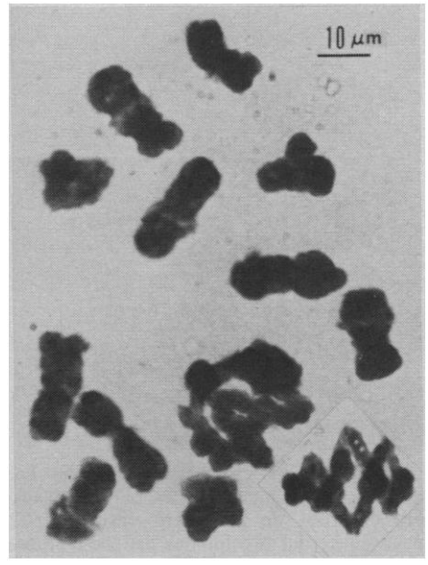

Fig. 3. First meiotic metaphase in a male plant of *V. engleri* illustrating the ring of 6 chromosomes and the 11 bivalents. The inset in the lower right depicts a nondistorted ring of 6 chromosomes oriented in the typical zigzag configuration.

The maintenance of permanent translocation heterozygosity requires a system in which the identity of two gametic sets is preserved during meiosis. In groups with fixed translocation heterozygosity, such as *Oenothera*, this is accomplished by balanced lethals. The incorporation of translocation heterozygosity into the sex-determining system, however, would offer an alternative strategy of maintaining nonrandom segregation of the translocated genomes. The adaptive significance of the translocation system in *V. fischeri* is especially difficult to reconcile because heterozygosity is apparently preserved only in the male plants, since the female plants are homozygous for translocations. Further studies show that this phenomenon occurs in other African mistletoes (*5*).

DELBERT WIENS
Department of Biology,
University of Utah,
Salt Lake City 84112

BRYAN A. BARLOW
School of Biological Sciences,
Flinders University of South Australia,
Bedford Park 5042

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