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*INTERSPECIFIC HYBRIDIZATION OF SOMATIC CELLS**

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Hybridization of somatic cells *in vitro*, first described in 1960 by Barski, Sorieul, and Cornefert,[1] has since been shown to occur in mixed cultures of many different pairs of cultured mouse cells. A recent review[2] lists 12 different "crosses" of mouse cells resulting in the formation of viable hybrids. This list includes crosses between cells of permanent lines as well as crosses between the latter and freshly explanted normal diploid cells. In this paper we describe the first interspecific cross giving rise to rapidly multiplying mononucleate somatic hybrids.[3]

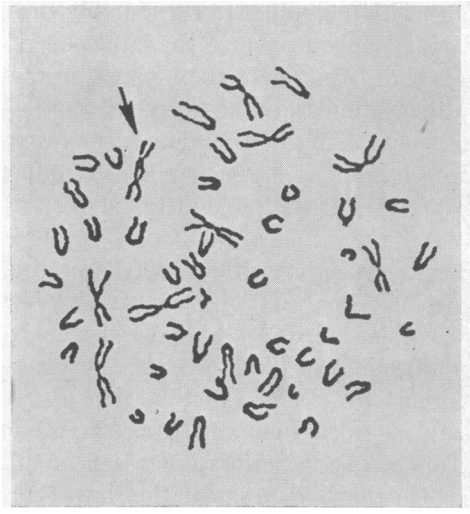

FIG. 1.—Metaphase of a cell of mouse cell line LM (TK$^-$) 1D with 53 chromosomes. Note the presence of 9 long biarmed chromosomes with median or submedian centromeres; the arrow points to the "D chromosome" with a characteristic secondary constriction.

The two cell types involved are: (1) clone LM(TK$^-$)1D, a 5-bromodeoxyuridine-resistant thymidine kinase-deficient subclone of *mouse* L cells, kindly given to us by Dr. S. Kit; (2) recently explanted Wistar *rat* embryo diploid cells.

The detection and identification of the hybrids was facilitated by the use of a recently established selective technique for the isolation of hybrids between biochemically deficient L cells and normal mouse cells[5] and by the characteristically different karyotypes of the "parental" cells. The karyotype of the mouse cells (Fig. 1) is characterized by a modal number of 53 chromosomes, 7 to 11 of which are long biarmed chromosomes, which have median or submedian centromeres, and are clearly different from those of rat cells. Ninety per cent of the mouse cells carry one biarmed chromosome presenting a characteristic secondary constriction. The karyotype of the rat ($2n = 42$) is characterized by the presence of 20 small and 4 long biarmed chromosomes, the latter with subterminal centromeres (Fig. 2).

Trypsinized suspensions of the two cell types are prepared and mixed to give a 1:1 mouse/rat cell ratio. Two million cells of this mixed population are inoculated

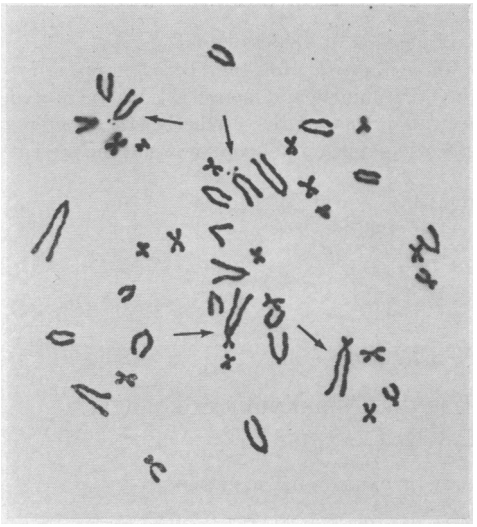

FIG. 2.—Metaphase of a diploid rat cell (42 chromosomes). Note the small biarmed chromosomes and the characteristic long ones, indicated by arrows.

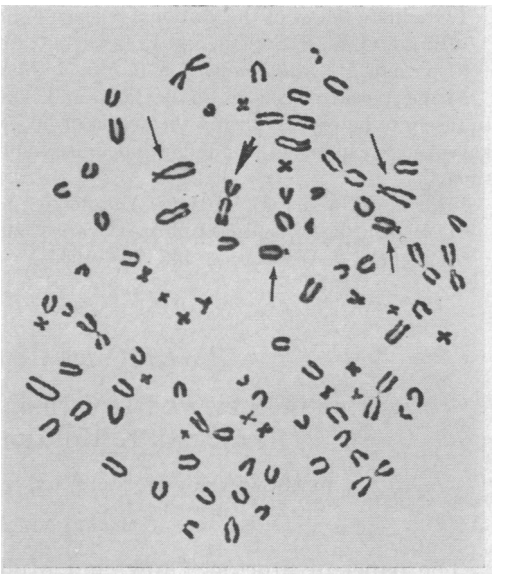

FIG. 3.—Metaphase of a hybrid cell with 89 chromosomes. Note the presence of 10 long biarmed mouse chromosomes (among them the "D chromosome," indicated by a large arrow) and of 4 long rat biarmed chromosomes (small arrows). Many small biarmed rat chromosomes can also be recognized.

into a 20-ml plastic bottle containing 5 ml of modified Eagle's minimal medium, supplemented with 5 per cent calf serum, and incubated for 24–48 hr at 37°C. During this period the cells attach to the bottom of the flask. The medium is then replaced by the selective medium, which differs from the above by the addition of 1×10^{-4} *M* hypoxanthine, 4×10^{-7} *M* aminopterin, and 1.6×10^{-5} *M* thymidine, and the culture is further incubated at 37°C.[6]

In the selective medium, the LM cells degenerate (because they lack thymidine kinase and therefore cannot utilize exogenous thymidine which must be used since the *de novo* synthesis of thymidine is blocked by aminopterin) while the rat cells grow and form a monolayer. On this background, the hybrid cells, unhampered by the selective medium, form, in the course of the next few days, discrete, multilayered colonies which can easily be isolated and subcultured. Karyological examination of the subcultured hybrid cells clearly shows the presence of chromosomes characteristic of each of the parents (Fig. 3).

At the present date, the hybrid cells have been maintained under conditions of continuous multiplication for 1 month, going through at least 25 cell generations. The evolution of their karyotype and some of their enzymatic characteristics are being investigated.

We wish to thank Dr. L. J. Scaletta and Mr. M. C. Yoshida for their help in karyological analysis and for preparing the microphotographs, and Miss Patricia Menéndez for her able technical assistance.

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[3] It has recently been shown by Harris and Watkins[4] that human and mouse cells can be caused to fuse into heterokaryons by the action of UV-inactivated Sendai virus. Whether these heterokaryons are capable of (limited or indefinite) multiplication appears to be unknown at the present time.

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*GENETIC LOAD IN TRIBOLIUM**

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The paper of Morton, Crow, and Muller,[1] which presented a method designed to differentiate between mutational and balanced (segregational) loads, stimulated a great deal of research on the subject, and generated considerable controversy. Both the validity of the technique (see Crow[2] and Levene[3] for references), as well as the interpretation of the results in different species (Neel[4] summarizes the extensive human material; Malogolowkin-Cohen, Levene, Dobzhansky, and Solima Simmons[5] may be consulted for literature on Drosophila), have been questioned.

Much of the dispute on the utility of the method proposed by Morton, Crow, and Muller sprang from theoretical considerations of both mathematical and biological nature. But an even more important source of disagreement existed in the comparative dearth of extensive and accurate data. Some of the information on man suffers from unreliability, partly as a result of intrinsic inaccuracies of field material and partly because of scant numbers.[4] Inferences from studies on domestic animals[6] are complicated by their previous history of inbreeding. Even the Drosophila material reported upon in the early investigations was, relatively speaking, limited.

It was hence thought that species of the flour beetle, Tribolium, which possess many advantages as experimental material for population genetics studies (Sokoloff and Shrode[7]), could be profitably utilized in a diversified mass test of the Morton, Crow, and Muller model.

Essentially, the experimental procedure in such a test involves comparison of survival rates from egg to larva and from larva to adult of the offspring from matings between full sibs, half sibs, and unrelated parents. Experiments were undertaken on two species, *T. castaneum* (hereafter designated as CS) and *T. confusum* (to be referred to as CF), in each of two environments differing in relative humidity. The different kinds of strains investigated included, for each species, two natural populations, a stock derived from a cross between them, a synthetic laboratory population constituted from a number of wild strains, a heterozygous population reconstituted from a four-way cross of inbred lines, and a highly inbred line.