Pacific Science (1988), vol. 42, nos. 1–2

Identification of Mullerian Chromosomal Elements in Hawaiian *Drosophila* by in situ DNA Hybridization[1]

DUANE E. JEFFERY, JAMES L. FARMER, AND MICHAEL D. PLILEY[2]

ABSTRACT: We have hybridized *Drosophila melanogaster* DNA sequences to polytene chromosomes of *D. silvestris*. The results support Muller's hypothesis that the chromosomal elements have been largely conserved in the evolution of the genus *Drosophila*. As originally suggested by Carson, *D. melanogaster* elements X, 2L, 2R, 3L, and 3R appear to correspond to chromosomes X, 3, 2, 5, and 4, respectively, in *D. silvestris* and the Hawaiian picture-winged species.

AS EARLY AS 1940, H. J. Muller proposed that chromosomal arms (elements) in *Drosophila* have remained essentially intact throughout evolutionary history. The primitive karyotype of the genus, he proposed, consisted of five rod-shaped chromosomes (which he designated A–E) and a small dot chromosome (F). The subsequent evolution of the genus seems to have been accompanied by many paracentric inversions and a number of centric fusions, but relatively few translocations or pericentric inversions. Although inversions occurring through evolutionary time would shuffle the order of syntenic genes along an element, genes on a specific element in one species would generally be expected to be on the same element in all other species.

Muller's hypothesis has been tested by several types of interspecific studies: linkage relationships among genes thought to be homologous, banding patterns of salivary gland chromosomes, chromosomal location of nucleolar-organizer regions, and chromosomal locations of rDNA (e.g., Stuart et al. 1981, Sturtevant and Novitski 1941). It is often equivocal, however, whether inferred homologies are real; e.g., is the gene that is mapped as the white locus in one species the same gene that causes white eyes in other members of the genus? In situ DNA hybridization can establish homologies with much less ambiguity.

We have hybridized recombinant plasmids carrying *Drosophila melanogaster* DNA from each of the five large chromosomal elements to polytene chromosomes of *D. silvestris*, one of the Hawaiian picture-winged species. Since the polytene banding pattern homology of elements in the picture-winged species has been described exhaustively (e.g., Carson and Yoon 1983), the use of *D. silvestris* allows us to tie the *D. melanogaster* elements to that entire large Hawaiian series.

MATERIALS AND METHODS

The recombinant plasmids containing *Drosophila melanogaster* DNA were obtained from G. Rubin (pm12.8, white locus, polytene chromosome position 3C), T. Barnett (pYP3, a yolk protein locus, 12BC), J. E. Natzle (DTB1 and DTB3, beta-tubulin loci, 97EF and 60C, respectively), M. Higgins via R. MacIntyre (p7R6, a putative vitelline membrane gene, 26A), and J. Lis (aDm63BC.1, *hsp83* locus, 63BC). Probes and chromosomes were prepared, hybridized, and stained as described by Pliley et al. (1986).

RESULTS

The results of the hybridizations are summarized in Table 1. Each probe hybridized to

[1] This work was supported by Brigham Young University research funds.

[2] Brigham Young University, Department of Zoology, Provo, Utah 84602.

TABLE 1

HYBRIDIZATION SITES OF PROBES IN *D. melanogaster* AND *D. silvestris*

PROBE	MULLER ELEMENT	*D. melanogaster*	*D. silvestris*
pm12.8	A	X	X
pYP3	A	X	X
p7R6	B	2L	3
DTB3	C	2R	2
aDm63BC.1	D	3L	5
DTB1	E	3R	4

a precise site on an element. This suggests that in situ hybridization is very specific and demonstrates genic homology. Further, there seems to have been neither deletion nor obvious duplication of the loci involved. No element in *Drosophila silvestris* hybridized with probes from more than one *D. melanogaster* element. This is consistent with the hypothesis that there have been no translocations involving the genes represented by these probes.

DISCUSSION

Gene homologies between *Drosophila melanogaster* and several other congeneric species were studied by Sturtevant and Novitski (1941). Their work compared mutant alleles that displayed similar phenotypes, and suggested that chromosomal elements are indeed conserved in evolution as Muller (1940) proposed.

A number of studies using in situ DNA hybridization have added additional evidence of element conservation. A notable early study is that of Wimber and Wimber (1977), involving the hybridization of 5S RNA to polytene chromosomes of six species of the *virilis* group. Kubli (1984) and Steinemann et al. (1984) provide both basic data and references to other work. Research to date has centered primarily on the *melanogaster*, *virilis*, and *obscura* groups. As a general summation (to this point), these studies have demonstrated that single-copy genes are linked in ways that support Muller's original proposal of linkage conservation. Genes involving repeated sequences, however, appear to have undergone rearrangements (e.g., translocations) that are not consistent with Muller's hypothesis. The present study has employed only single-copy sequences.

Based on his extensive experience with polytene banding patterns of the Hawaiian picture-winged species, Carson (personal communication, 1982) tentatively suggested that Hawaiian elements X, 3, 2, 5, and 4 correspond to *Drosophila melanogaster* elements X, 2L, 2R, 3L, and 3R, respectively. The *D. silvestris* homologies we have determined are indeed in precise agreement with this suggestion. The identification is strengthened by the finding of Brennan et al. (1984) that the *Adh* locus, known to be on 2L in *D. melanogaster* (Lindsley and Grell 1968) is, as would be predicted, on chromosome 3 in the Hawaiian picture-winged species.

ACKNOWLEDGMENTS

We thank K. Y. Kaneshiro and W. J. Dickinson for supplying us with *D. silvestris* larvae and helpful suggestions.

LITERATURE CITED

BRENNAN, M. D., R. G. ROWAN, L. RABINOW, and W. J. DICKINSON. 1984. Isolation and initial characterization of the alcohol dehydrogenase gene from *Drosophila affinidisjuncta*. J. Mol. Appl. Genet. 2:436–446.

CARSON, H. L., and J. S. YOON. 1983. Genetics and evolution of Hawaiian *Drosophila*. Pages 297–344 *in* M. Ashburner, H. L. Carson, and J. N. Thompson, Jr., eds. The

genetics and biology of *Drosophila*. Vol. 3b. Academic Press, New York.

KUBLI, E. 1984. *Drosophila melanogaster* (fruitfly), transfer RNA and U-RNA in situ hybridization data. Pages 300–303 *in* S. J. O'Brien, ed. Genetic maps 1984. Vol. 3. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

LINDSLEY, D. L., and E. H. GRELL. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Institute of Washington Pub. No. 627.

MULLER, H. J. 1940. Bearings of the "*Drosophila*" work on systematics. Pages 185–268 *in* J. Huxley, ed. The new systematics. Clarendon Press, Oxford.

PLILEY, M. D., J. L. FARMER, and D. E. JEFFERY. 1986. In situ hybridization of biotinylated DNA probes to polytene salivary chromosomes of *Drosophila* species. Dros. Inf. Serv. 63:147–149.

STEINEMANN, M., W. PINSKER, and D. SPERLICH. 1984. Chromosome homologies within the *Drosophila obscura* group probed by in situ hybridization. Chromosoma 91: 46–53.

STUART, W. D., J. G. BISHOP, H. L. CARSON, and M. B. FRANK. 1981. Location of the 18/28S ribosomal RNA genes in two Hawaiian *Drosophila* species by monoclonal immunological identification of RNA–DNA hybrids in situ. Proc. Nat. Acad. Sci. U.S. 78:3751–3754.

STURTEVANT, A. H., and E. NOVITSKI. 1941. The homologies of the chromosome elements in the genus *Drosophila*. Genetics 26:517–541.

WIMBER, D. D., and D. R. WIMBER. 1977. Sites of the 5S ribosomal genes in *Drosophila*. I. The multiple cluster in the *virilis* group. Genetics 86:133–148.