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Crossing-over in the female hybrids between *Drosophila simulans* and *Drosophila mauritiana*[1]

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Summary. The cross between *D. simulans* and *D. mauritiana* yields fertile hybrid females. Crossing-over occurs in such female hybrids. This system allows the transfer of pieces of chromosomes from one species into another, thus providing an experimental tool to analyze the genetic basis of evolutionary problems, as well as the regulation of gene expression during development.

Speciation is the process by which one species splits into 2 or more species. Species are defined as Mendelian populations whose members share a common gene pool, and between which gene exchange is prevented by reproductive isolating mechanisms (RIMs). Subsequently, 2 gene pools (species) evolve independently.

What are the genetic changes that underlie the development of reproductive isolation, and hence lead to the formation of new species? How many genes are involved in the establishment of the different kinds of RIMs? Do RIMs arise as a result of changes in a relatively small number of genes, or are they the by-product of accumulated changes in many loci?

It has been recognized that hybrids between different species might constitute a good system for studying evolutionary problems. Hybrids are formed between species that have diverged recently in evolution. A genetic analysis of such hybrids should help to reveal the genetic differences required to separate 2 species. Limitations arise because of the impossibility of obtaining certain hybrids due to RIMs. Furthermore, when 2 different species can be crossed and give rise to adult hybrids, the genetic analysis can usually not be taken beyond the 1st generation, either because the hybrids are sterile, or because genetic markers in the parental species are not known.

David et al.[3] reported that the cross between *D. simulans* and *D. mauritiana* yields fertile females and sterile males. Both species belong to the *melanogaster* subgroup. Several genetic markers are available in *D. simulans* and a few in *D. mauritiana*, so that it is possible to test whether crossing-over occurs in the female hybrids *simulans-mauritiana*. I have analyzed the 3 major chromosomes; the X, the 2nd and the 3rd chromosomes, for which genetic markers are available. *D. simulans* females homozygous for certain genetic markers were crossed with wild-type *D. mauritiana* males and the hybrid females of such a cross were backcrossed to *D. simulans* males homozygous for the same genetic markers as the parental females. The results are

Crossing-over between the X chromosomes, between the 2nd chromosomes and between the 3rd chromosomes in the female hybrids *simulans-mauritiana*. The numbers in parentheses represent percentages. The frequency of crossovers in female hybrids for the different chromosomal intervals were: yellow-forked *(y-f²)*: 42%; net-polychaete *(net-py)*: 49%; polychaete-plum *(py-pm)*: 31%; javelin-scarlet *(jv-st)*: 35% and scarlet-peach *(st-p)*: 41%. The flies were kept at 25 °C on standard food. Special care was taken to culture the flies in uncrowded conditions, so that competition between the different recombinant genotypes was practically eliminated. The marker mutations are described by Sturtevant[4]. *net* and *jv* are mutations homologous to the mutations described in *D. melanogaster*. *st p* homozygous flies have a phenotype clearly distinguishable from *st* or *p* alone. Some of the stocks of *D. simulans* were provided by Dr J. Puro (Turku) and the *D. mauritiana* flies were provided by Dr R.C. Woodruff (Bowling-Green).

Chromosome	Genotype of hybrid female	Genotype of *D. simulans* male	Total progeny scored	Phenotype and number of recombinants					
X	$\frac{yf^2}{++}$	$\frac{yf^2}{Y}$	1129	y 263 (23.3)			f^2 212 (18.7)		
2nd	$\frac{net\,py\,pm}{+\,+\,+}$	$\frac{net\,py\,pm}{net\,py\,pm}$	1175	net 238 (20.2)	py pm 172 (14.6)	net py 99 (8.4)	pm 104 (8.8)	net pm 72 (6.1)	py 93 (7.9)
3rd	$\frac{jv\,st\,p}{+\,+\,+}$	$\frac{jv\,st\,p}{jv\,st\,p}$	1037	jv 119 (11.4)	st p 109 (10.5)	jv st 119 (11.4)	p 162 (15.6)	jv p 73 (7)	st 70 (6.7)

described in the table. I found that crossing-over takes place in all 3 chromosomes and all possible recombinants were produced. The frequencies of recombination in the hybrid females are not significantly different ($p > 0.30$) from the ones observed by Sturtevant[4] in *D. simulans* females heterozygous for the same genetic markers. In addition, the banding pattern of the polytene chromosomes of *D. simulans* and *D. mauritiana* is homosequential in both species[5]. Therefore, it seems reasonable to assume that the frequency of crossing-over in hybrid females *simulans-mauritiana* and in *D. simulans* females might be the same for any chromosomal interval. The existence of crossing-over in the female hybrids *simulans-mauritiana* might furnish an experimental system for studying some specific problems, such as: How many genes are responsible for the male hybrid sterility? Where are they located in the genome? Also evolutionary questions of a more general kind can be studied in those hybrids, e.g. how much genetic information can we transfer from one species into another, and what is the effect on viability of such recombinant hybrids, which carry a certain part of the genome of one species in combination with a majority of genes from the other species.

The *simulans-mauritiana* hybrid males of the first generation are sterile, but after 3 generations of backcrossing the female hybrids with *D. mauritiana* males, I was able to get fertile males which had, in large part, a *mauritiana* genome, in combination with the yellow marker mutation or the white marker mutation from the parental *D. simulans* females (*y w*: yellow and white). It was possible to construct stocks from such males.

Recently, the potential of hybrids for studying regulation of gene expression during development has been pointed out[6]. It has been demonstrated, by means of interspecific hybrids between some species of Hawaiian Drosophilidae[6,7], that cis-acting and trans-acting genetic elements control the pattern of tissue specificity for certain enzymes. However, the mapping of such elements, as well as an estimate of their number could not be done, due to the sterility of the hybrids. Backcrosses between the female hybrids *simulans-mauritiana* with the parental species might constitute a useful tool for such a genetic analysis, and at the same time might yield information about the evolution of regulatory genetic systems.

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Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*[1]

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Summary. Hypocotyls, cotyledons and leaves from 6 cultivars of *Solanum melongena* were cultured to induce callus formation and subsequently plantlet regeneration. Differences were observed among explants and cultivars. These differences were explained assuming that the explants reached different degrees of dedifferentiation in callus persisting also after numerous subcultures.

Regeneration of plantlets by cell cultures is a major goal when the interest is focused upon the genetic aspects. Since the initial reports on this subject[3,4] a great deal of work on regeneration has been devoted to finding an equilibrium between the components of the medium. Numerous studies indicate that it is difficult to make extensive generalizations; in fact, an inductive treatment developed for a particular culture is not necessarily successful in other cultures. Consequently there is no doubt that organogenesis in vitro depends on a complex system of endogenous and

Table 1. Responses in callus induction from hypocotyl, cotyledon and leaf explants to different auxins; observations after 2 weeks

Media	Hypocotyl	Cotyledon	Leaf
LS + 2,4-D 0.4 mg/l	Soft yellowish friable callus	Scarce development of yellowish friable callus	Scarce development of slightly compact callus
LS + 2,4-D 2 mg/l	Scarce callus development	Scarce callus development and root formation	Scarce callus growth and profuse roots
LS + NAA 1 mg/l	Friable callus, occasional development of roots and leaf regeneration from explant	Scarce callus production and occasional development of roots	Nodular callus and many hairy roots
LS + IAA 2 mg/l	Rapid development of callus and roots	Rapid development of callus and roots	Rapid development of callus and roots