tivity of donor cells against rat RBC could be detected, whereas donor type antigenicity was found in the spleen of the tolerant mice. On the basis of these findings it was proposed that the definition of tolerance given for the whole animal could also be applied to its immune system.

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*ILLEGITIMACY AND SPECIFIC FACTOR TRANSFER IN SCHIZOPHYLLUM COMMUNE**

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In *Schizophyllum commune*, a tetrapolar basidiomycete, the dikaryotization of a homokaryon by a dikaryon is expected if the dikaryon contains a nucleus that is compatible with the nucleus of the homokaryon, i.e., $(A^1B^1 + A^2B^2) \times A^3B^3 \rightarrow (A^1B^1 + A^3B^3)$ or $(A^2B^2 + A^3B^3)$, $(A^1B^1 + A^2B^2) \times A^2B^3 \rightarrow (A^1B^1 + A^2B^3)$.[1-3] The mating, $(A^1B^1 + A^2B^2) \times A^1B^2$, is considered noncompatible, however, since each of the two nuclei of the dikaryon contains one incompatibility factor in common with one in the nucleus of the homokaryon (either common A factors or common B factors). Occasionally, however, genetic recombination of mating type factors between the two nuclei of the dikaryon produces a nucleus that is compatible with the nucleus of the homokaryon, i.e., $(A^1B^1 + A^2B^2) \times A^1B^2 \rightarrow (A^2B^1 + A^1B^2)$,

an "illegitimate" dikaryotization. A number of such illegitimate dikaryotizations have been analyzed with respect to the mechanism of recombination.[4] The method for analysis was based on the recombination of several biochemical mutations in nuclei in which recombination for mating type factors had occurred. Two classes of recombinants were observed. Class I was characterized by possessing some combination of nonselective markers, biochemical mutations, present only in the original dikaryon. The recombinational event, therefore, involved only the two nuclei of the original dikaryon and was distinguished from the parasexual cycle described by Pontecorvo[5] by the high frequency of recombination between linked markers. Reduction was considered to be a meiotic event as the linkage relationships were very similar (with exceptions of two small subgroups) to those obtained via the standard sexual cycle. Class II individuals possessed, aside from mating type factors from the two nuclei of the original dikaryon, only genetic markers from the original homokaryon. A mating type factor specificity had apparently been transferred from each of the two nuclei of the original dikaryon into the nucleus of the homokaryon. Obviously, such an event involved all of the three original nuclear types.

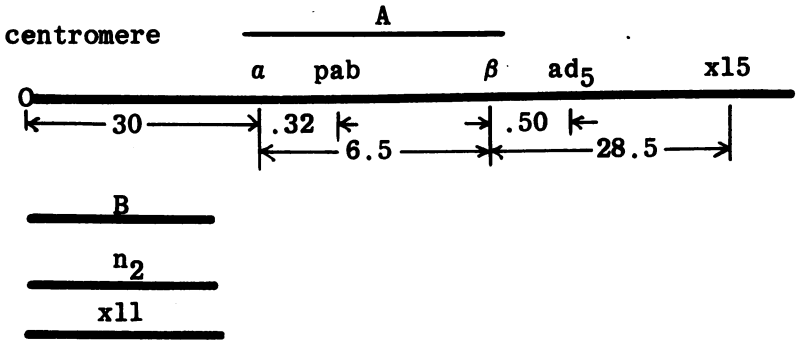
Three hypotheses were offered to explain the origin of Class II individuals.[4] (1) A triploid nucleus was formed from the three original nuclear types, underwent haploidization, and haploids with the same genetic markers as the original homokaryon were selected to dikaryotize the homokaryon. (2) Two successive recombinational events occurred, the first in the original dikaryon and the second in the derived dikaryon, and were followed by the selection of a nucleus homozygous, except for mating type, with the nucleus of the homokaryon. (3) There is a mechanism of genetic recombination that is specific in that only the incompatibility factors are recombined.

The first two hypotheses assume that there is strong internuclear selection based on different biochemical mutations and their wild-type alleles, an assumption that is not substantiated in studies of fully compatible dikaryotic-homokaryotic matings.[3] To obtain internuclear selection, there must have been a burst of recombinational events to provide a population of recombinants from which to select. No evidence is available that recombinational events produce populations of different types of recombinants within a single thallus. Support for the third hypothesis requires a demonstration that *only* the incompatibility factor specificities are transferred from the two nuclei of the dikaryon to the homokaryon.

The experiments reported herein were designed (1) to study the origin of Class II recombinants with the aid of two mutations, *pab* (para-aminobenzoic acid requiring), located between the two subunits of the *A* factor, and ad_5 (adenine requiring), located distal to *A* but very closely linked to $A\beta$, and (2) to prove conclusively the genotypes of the recombined nuclei incorporated into the derived dikaryons by several methods of analysis.

Materials and Methods.—All cultures used in this study were highly isogenic except for mating type factors (A^{41}, A^{42}, A^{47}, A^{d}, B^{41}, B^{42}, B^{47}) and biochemical mutations n_2 (nicotinic acid requiring), *x11* and *x15* (two unlinked mutations with unknown requirements), *pab*, and ad_5. Strain 699 was the background genome.[4]

A linkage map of the mutants used in this study is represented as follows:[6, 7]



All matings were made on migration-complete media.[8] Tests for specific nutritional requirements were made on minimal and appropriately supplemented media[9] or for the heterokaryotic allelic tests on minimal medium and migration-complete medium supplemented with para-aminobenzoic acid.

The illegitimate dikaryotic-homokaryotic matings were made as follows: The two homokaryotic cultures ($A^{41}B^{42}ad_5n_2$ and $A^{42}B^{41}pab$) that were used to synthesize the dikaryon were mated upon agar plates and incubated at 33°C; a dikaryon was synthesized within 72 hours. The homokaryon ($A^{41}B^{41}$ *x11 x15*) in the illegitimate matings was inoculated onto 5 plates and incubated at 22°C for 72 hr. The newly formed dikaryon ($A^{41}B^{42}ad_5n_2 + A^{42}B^{41}$ *pab*) was macerated with sterile distilled water, and a small aliquot of macerate was pipetted onto the growing edge of each of the five colonies of the homokaryotic culture, $A^{41}B^{41}$ *x11 x15*. The total process was repeated 240 times to make a total of 1,200 dikaryotic-homokaryotic matings. Forty-eight hours after the dikaryotic-homokaryotic matings were made, the dikaryon was removed by cutting out that portion from the plate. Seven days after the dikaryotic-homokaryotic matings were made, the plates were examined for the presence of dikaryotic sectors in the homokaryon. Hyphal tips of 1–3 cells were cut from dikaryotic sectors and placed upon migration-complete medium plus 0.2% yeast extract. The hyphal tips produced small colonies in five days at 22°C; the cultures were then stored at 1°C to prevent fruiting so that analysis of genetic constitution could be made later.

Experimental Results.—Eighty-six cultures were obtained from the isolation of hyphal tips from dikaryotic sectors that arose in the homokaryon in illegitimate dikaryotic-homokaryotic matings.

The first analysis of the 86 cultures consisted of mating each culture with four tester strains to determine the mating factor constitution of each of the two nuclei in each culture. The reaction patterns observed with the four testers, the frequency of occurrence of each pattern, and the genetic constitution necessary in the dikaryotic culture to elicit each pattern were as follows:

Genetic constitution of dikaryon	No. of cultures	Testers			
		$A^{41}B^{41}$	$A^{41}B^{42}$	$A^{42}B^{41}$	$A^{42}B^{42}$
$A^{41}B^{41} + A^{42}B^{42}$	22	+	−	−	+
$A^{41}B^{42} + A^{42}B^{41}$	42	−	+	+	−
$A^{x}B^{41} + A^{42}B^{42}$	4	+	+	−	+
$A^{41}B^{41} + A^{x}B^{42}$	2	+	−	+	+
$A^{42}B^{41} + A^{x}B^{42}$	8	+	+	+	−
$A^{x}B^{41} + A^{41}B^{42}$	3	−	+	+	+
$A^{x}B^{41} + A^{y}B^{42}$	3	+	+	+	+
$A^{41}B^{41} + A^{41}B^{42}$ (common A) ?	1	− (?)	−	+	+
$A^{42}B^{41} + A^{42}B^{42}$ (common A) ?	1	+	+	−	− (?)
Total	86				

+ = compatible reaction; − = noncompatible reaction.

Twenty-two cultures were $A^{41}\ B^{41} + A^{42}\ B^{42}$ and should represent dikaryons formed between the $A^{41}\ B^{41}$ nuclei of the homokaryon and the $A^{42}B^{42}$ nuclei resulting from recombination between the two types of nuclei of the original dikaryon, i.e., $A^{41}B^{42} + A^{42}B^{41} \rightarrow$ nuclear fusion (2n) $\rightarrow$ haploidization $\rightarrow$ haploid $A^{42}B^{42}$. Forty-two cultures were $A^{41}B^{42} + A^{42}B^{41}$ and represent the two nuclei of the original dikaryons that migrated through the homokaryon and displaced the homokaryon on its periphery. Twenty dikaryotic cultures possessed recombinant A factors in one or both nuclei; the scoring of two cultures was questionable.

The 22 dikaryons that were recombinant for mating type factors—and were not intra-A factor recombinants—were analyzed by heterokaryotic allelic tests for the presence of nonselective markers, the biochemical mutations present in the recombinant nucleus $A^{42}B^{42}$. Each of the dikaryons was mated with a series of five $A^{41}B^{41}$ testers, each carrying a different biochemical mutation. The $A^{42}B^{42}$ nuclei migrated into the $A^{41}B^{41}$ tester strains, and the new dikaryons were plated on minimal medium and on migration-complete medium supplemented with para-aminobenzoic acid. The procedure is exemplified in the analysis of dikaryon *63 × 11253:*

		Media	
		Minimal	Complete
$(A^{42}B^{42} + A^{41}B^{41}) + A^{41}B^{41} \rightarrow A^{42}B^{42} + A^{41}B^{41} \rightarrow$ $n_2?\ ad_5?\quad x11\ x15 \qquad n_2 \rightarrow \qquad n_2$ $pab?\qquad x11?$ $x15?$		+	+
"	$\times\ A^{41}B_{x11}{}^{41} \rightarrow A^{42}B^{42} + A_{x11}{}^{41}B^{41} \rightarrow$	−	+
"	$\times\ A^{41}B_{x15}{}^{41} \rightarrow A^{42}B^{42} + A_{x15}{}^{41}B^{41} \rightarrow$	−	+
"	$\times\ A^{41}B_{pab}{}^{41} \rightarrow A^{42}B^{42} + A_{pab}{}^{41}B^{41} \rightarrow$	+	+
"	$\times\ A^{31}B_{ad5}{}^{41} \rightarrow A^{42}B^{42} + A_{ad5}{}^{41}B^{41} \rightarrow$	+	+

+ = growth; − = no growth.

The recombinant nucleus with mating type factors, $A^{42}B^{42}$, possessed, therefore, biochemical mutations, *x11* and *x15*, and the wild-type alleles of n_2, *pab*, and ad_5.

The genotypes for the recombinant nuclei and the frequency of their occurrence were:

Genotype of recombinant nuclei	No.
$A^{42}B^{42}\ n_2\ pab$	2
$A^{42}B^{42}\ x11\ x15$	20

Two of the 22 recombinants possessed biochemical mutations from the original dikaryon (Class I recombinants).[4] Twenty possessed, aside from mating type factors, only nonselected markers from the original homokaryon (Class II).[4] Though *pab* was located between the 2 subunits of $A_{3-5}^{42\ 6}$ in the nucleus of the original dikaryon, the A_{3-5}^{42} factor specificity was transferred, in the 20 Class II recombinants, to the basic genome of the original homokaryon without transferring either the mutant *pab* allele or the $x15^+$ allele distal to the A factor in relation to the centromere.

A basic assumption in the mating of the 22 dikaryons, $A^{42}B^{42} + A^{41}B^{41}$, with the five tester strains, $A^{41}B^{41}$, each with a different biochemical mutation, was that only the $A^{42}B^{42}$ nuclei migrated into the homokaryons. There is now good evidence that the $A^{41}B^{41}$ nucleus from the dikaryon will also migrate into the homokaryon although the two nuclei are noncompatible.[10] There was, therefore, a possibility

of mis-scoring the recombinant nucleus by the method, but reconstruction experiments indicate the probability of mis-scoring to be very small.

As a further test of the genetic constitution of the 22 dikaryons, each of the 22 was fruited and a sample of 16 spores/dikaryon was collected.

Sixteen haploid progeny of each of nine dikaryons (Class II) were plated on four different media: minimal supplemented with (1) niacin + adenine, (2) adenine + para-aminobenzoic acid, (3) niacin + para-aminobenzoic acid, and (4) niacin + adenine + para-aminobenzoic acid. None of the progeny of any of the nine dikaryons grew on either of these four media. The dikaryons thus appeared to be homozygous for either *x11*, *x15*, or both *x11* and *x15*.

The 16 haploids from each of the 22 dikaryons were analyzed for mating type factors by mating with four testers, $A^{41}B^{1}$, $A^{42}B^{1}$, $A^{1}B^{41}$, and $A^{1}B^{42}$. A^{41} segregated from A^{42}, and B^{41} segregated from B^{42} among the progeny of each of the 22 dikaryons, and segregation approximated a 1:1 ratio for both *A*'s and *B*'s. There was no evidence for trisomic ratios or segregation of "modifier" mutations.

A heterokaryotic allelic test was also applied to the haploid cultures in order to determine what mutations each possessed. Each of the 16 haploids from each of the 22 dikaryons was mated with five testers, $A^{47}B^{47}n_2$, $A^{47}B^{47}$ *x11*, $A^{47}B^{47}$ *x15*, $A^{d}B^{47}$ *pab*, and $A^{d}B^{47}ad_5$, and six days later the newly synthesized dikaryons were put on minimal and complete media. The procedure is diagrammed as follows:

Haploid mutations?	Testers	Media: Minimal	Media: Complete
"	× $A^{47}B^{47}$ n_2 → dikaryon →	+	+
"	× $A^{47}B^{47}$ *x11* → " →	−	+
"	× $A^{47}B^{47}$ *x15* → " →	−	+
"	× $A^{d}B^{47}$*pab* → " →	+	+
"	× $A^{d}B^{47}$ ad_5 → " →	+	+

The haploid culture, therefore, contained mutations, *x11* and *x15*.

The haploids from each of the two dikaryons of Class I segregated alleles at the four loci: n_2, *x11*, *x15*, and *pab*. All haploid progeny from the 20 dikaryons of Class II possessed only *x11* and *x15*. All 20 dikaryons were, therefore, homozygous wild-type for n_2, *pab*, and ad_5 and mutant for *x11* and *x15*, in full agreement with previous tests.

The two dikaryons, $A^{41}B^{41} + A^{x}B^{42}$, were tested for growth on minimal medium because of the possibility they represented transfer of only one of the subunits of the *A* factor. Growth should have occurred on minimal medium if they were Class I recombinants and not if they were Class II recombinants. Both grew on minimal media.

Discussion.—The results presented here conclusively demonstrate that the Class II recombinants do possess the nonselective markers from the original homokaryon, and only those markers. From the results presented here and from a previous study,[4] with but one exception (which could possibly have been an error in scoring), there is no evidence that any genetic material other than the incompatibility factors is transferred to the background genome of the original homokaryon. This fact is particularly significant since *pab* is located between the two subunits of the *A* factor.

To explain the origin of Class II individuals on the basis of one or two typical recombinational events,[4] it is necessary to assume (1) a significant number of mul-

tiple crossovers, precisely located in the genome; (2) multiple crossing-over occurring in restricted portions of the total number of recombinational events; (3) a population of recombinational events; and (4) an eventual internuclear selection, based on biochemical mutations, that is absolutely specific for the biochemical mutations that were present in the original homokaryon. Such a system seems highly improbable, particularly since a single recombinational event that reassociates the *A* and *B* factors from the original dikaryon is all that is necessary to satisfy the minimal requirements for the dikaryotization of the homokaryon.

Mutation of the incompatibility factors as a possible explanation has been ruled out on the basis of three arguments:[4] (1) Mutations of the *A* and *B* factors exhibit a loss of function rather than a change in specificity,[11-13] (2) there would have to be simultaneous mutations at both subunits of the *A* factor, and (3) the mutations would have to be directed.

Quintanilha[14] had suggested that the exchange of mating type factors could be achieved if mitotic spindles coalesced as the nuclei divided and migrated through the homokaryon. Such a system would involve exchange of whole chromosomes and, as such, would give no explanation why other genetic markers on the same chromosome as the *A* factor were not transferred.

The origin of the Class II recombinants obviously involves all three nuclear types present in the dikaryotic-homokaryotic mating. If the transfer of genetic material is nonreciprocal, two steps would be postulated, namely, the release of genetic material from two nuclei and incorporation of specific genetic units into the third nucleus.

The normal genetic stability of the incompatibility factor specificities suggests that very special conditions must be necessary to effect specific factor transfer. Once the appropriate physiological state is achieved, both subunits of the *A* factor are always, or almost always, simultaneously incorporated into the third nucleus. Only one subunit specificity, however, need be transferred to effect a compatible reaction with the nucleus of the homokaryon. The incorporation of specific genetic material into the nucleus would have to assume a specificity of incorporation analogous to the incorporation of a temperate phage into a bacterium and the prophage's association with a specific site on a bacterial chromosome.[15-18]

An experimentally testable model for the transfer process is not immediately available.

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*THE DEPENDENCE OF VIABILITY EFFECTS CAUSED BY IRRADIATION ON THE TYPE OF MATING**

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Experiments to determine the spectrum of induced viability mutations in the X-chromosome of *Drosophila melanogaster* and their effects in heterozygotes have been carried out in this laboratory for the last year. The daughters of irradiated males were mated individually to either of two kinds of males. The progeny of these matings were scored and the proportions of the classes obtained for each sex indicated the viability of flies hemizygous and heterozygous for irradiated chromosomes. It was found that the penetrance of the induced mutations in the heterozygotes depended on the type of mating. It could be shown further that the differences were due to intraculture competition and to the partial "replacement"[1] of one class of flies by another.

Material and Methods.—Two stocks were used throughout these experiments: (*a*) A wild-type (+) stock, Qiryat Anavim, originating from flies collected in nature by Dr. E. Goldschmidt. This stock was moderately inbred but has been kept for some time in mass cultures. (*b*) A marked-balancer stock, Binscty, "*y* sc^{81} *B In49* ct^{n8} sc^{8}," which was kindly supplied to us by Dr. H. J. Muller of the University of Indiana. Both stocks were kept in mass cultures throughout the experiments with an occasional passage through a culture of 3–5 pairs of parents.

Flies were grown on medium prepared according to the formula of E. B. Lewis, seeded with Wagner's Y–2 yeast strain.[2] The cultures were kept in a constant temperature room at 25°C. On hot days, however, the temperature rose occasionally to 30°C.

Irradiations were performed at the Department of Physical Chemistry with a G. E. Maximar–200 X-ray machine. The machine was operated at 200 KVP 15 mA, with a 1 mm Al, 0.25 mm Cu filter at a dose rate of approximately 250 r/min for 8 min.

The experimental procedure was essentially identical in all repeats carried out, with only minor changes between them. Binscty females were mated to +-males and *vice versa*. The heterozygous females were backcrossed to both Binscty and