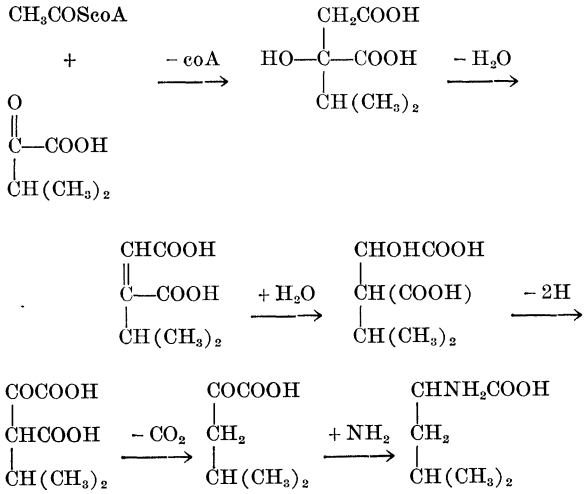
keto analog of leucine. The present results confirm and extend this postulation.

By analogy with the condensation of oxalacetate with acetate to yield citric acid, the following detailed mechanism is proposed for the synthesis of the leucine carbon chain.



α-Ketoisovalerate is presumed to condense with the methyl carbon of acetyl coA to yield α-hydroxy-α-isopropylsuccinic acid. By the same series of reactions undergone by citric acid to yield α-ketoglutaric acid, this hydroxy acid would be converted to α-keto-γ-methylvaleric acid, which, by transamination with glutamic acid (*7*), would yield leucine. The possible participation of these hypothetical intermediates in leucine biosynthesis is now under study (*8*). A similar reaction sequence was suggested by us to account for the synthesis of α-aminoadipic acid in connection with the biosynthesis of lysine (*9*).

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* Postdoctoral fellow of the National Institutes of Health, U.S. Public Health Service.

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22 November 1954.

Segregation of Sex Factors in a Diploid Line of *Ustilago zeae* Induced by Alpha Radiation

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During investigations of the effect of naturally radioactive heavy metals on microorganisms, a phenomenon that appears to be somatic segregation and recombination has been induced repeatedly in solopathogenic, diploid lines of *Ustilago zeae,* the common corn smut fungus, by alpha radiation. This report (*1*) on the solopathogenic line 410qq compares the segregation and recombination of the factors for sexual compatibility that occurred during meiosis in chlamydospore germination with that which was induced by the irradiation of vegetative cells.

U. zeae is normally heterothallic, and galls and chlamydospores (zygotes) are produced when corn is inoculated with pairs of compatible haploid lines (*2, 3*). Solopathogens were described by Christensen (*4, 5*) and Eddins (*6*) as monosporidial lines that by themselves produced galls and chlamydospores in inoculated corn. The cultural, sexual, and pathogenic segregants obtained during germination of the chlamydospores produced by these lines (*4, 5*) are identical to those recovered from chlamydospores of a cross of haploid lines. Since cells of solopathogens are uninucleate (*5*), they are considered to be diploid, although cytological proof of the presence of $2n$ chromosomes has not been obtained.

The solopathogenic character of monosporidial lines of *U. zeae* is not always stable. Some of the solopathogenic lines characterized by Christensen (*5*) and Stakman *et al.* (*7*) were found to be avirulent in later tests. Chilton (*8*) isolated a variant from a solopathogen that was compatible with certain haploid lines but nonpathogenic when inoculated alone in corn. The solopathogenic line 410qq used in this study was isolated in 1943 by Stakman *et al.* (unpublished) from a cross of lines 10A4 × 17D4, and the character of solopathogenicity has remained unchanged in stock cultures since that time. However, variants that were compatible with 10A4 but not with 17D4 were isolated by Gattani (*9*) from line 410qq grown on mediums containing uranyl nitrate. During the current investigation, 349 monosporidial isolates from suspensions of cells of line 410qq that were not exposed to alpha radiation were tested as controls, and all were solopathogenic.

Vegetative sporidia of the diploid line 410qq that were exposed to alpha radiation were harvested from young monosporidial cultures grown in potato-dextrose-broth (PDB) by shake culture. In the original trials, washed sporidia were exposed to alpha radiation by suspension in a solution of 1 μc/ml of polonium-210 after the method of Rowell *et al.* (*10*). For later exposures, however, the alpha radiation emitted from a 10-mc source of polonium-210 plated

Table 1. Comparison of the segregation and recombination in sex factors resulting from meiosis in germinating chlamydospores and from exposure of vegetative sporidia of a diploid line (410qq) to alpha radiation.

Sex genotype	Mating reaction of nonsolopathogenic isolates with tester lines				No. of isolates for each genotype				
					From germinating chlamydospores		From treated sporidia of 410qq		
	17D4 (a^2b^4)	822Cb (a^2b^1)	NyCe (a^1b^4)	10A4 (a^1b^1)	10A4 × 17D4	410qq	I	II	III
a^1b^1	+*	–	–	–	9	6	3	7	10
a^1b^4	–	+	–	–	1	10	3	3	4
a^2b^1	–	–	+	–	2	8	0	11	5
a^2b^4	–	–	–	+	1	6	0	0	2
$a^1a^2b^1$	+	–	+	–	12	1	3	17	6
$a^1a^2b^4$	–	+	–	+	2	1	3	15	15
$a^1a^2b^1b^4$	No mating reaction†				26	1	75	197	72

* The symbol + indicates combinations of lines that are sexually compatible, that is, produce galls and chlamydospores in inoculated corn. The symbol – indicates no reaction.

† Solopathogenic lines that by themselves produce galls and chlamydospores in inoculated corn.

on a 1-in. brass square and coated with a thin layer of gold and plastic lacquer was used to irradiate a dried, monocellular layer of sporidia on the surface of 2-percent water agar. Isolates from the survivors on dilution plates were tested for solopathogenicity and sexual compatibility by inoculating seedling corn by the partial-vacuum method of Rowell and DeVay (*11*).

The sex alleles of monosporidial lines from a cross can be determined by matings in corn seedlings with lines representing each of the four haploid combinations of the parental sex factors. Very detailed studies previously reported in a symposium paper (*12*) have established that sexual compatibility in *U. zeae* is governed by genes at two loci, one of which has only two known alleles (a^1 and a^2), while the other has multiple alleles (b^1, b^2, b^3, . . . b^n). Mated haploid lines produce galls and chlamydospores in corn only if different alleles of *a* and *b* are brought together (see mating reactions in Table 1). In addition to the haploid lines, there are amphisexual lines (*12*) that have the alleles $a^1a^2b^1$ or $a^1a^2b^4$, and these lines are compatible with any haploid line having a different *b* allele. The four tester lines used to identify the mating types of isolates in this study were 10A4 (a^1b^1), 822Cb (a^2b^1), NyCe (a^1b^4), and 17D4 (a^2b^4) in which the complement of sex alleles had been established by inheritance studies (*12*).

The number of monosporidial isolates for each sex genotype obtained by meiosis from germinating chlamydospores of the cross 10A4 × 17D4 ($a^1b^1 \times a^2b^4$) and the solopathogenic progeny of this cross, 410qq ($a^1a^2b^1b^4$), are listed in Table 1. The last three columns of the table list the number of isolates of the same genotypes found in tests of survivors from three separate irradiation trials with vegetative sporidia from line 410qq. Only cultural "mutants" were tested in trial I. Trial II was a test of a random sample of the survivors after exposure to alpha radiation, and it was found that most avirulent lines were isolated from initially slow-growing colonies with distorted cell shapes (Fig. 1). The isolates in trial III were all made from dilution plates of broth subcultures of such characteristically abnormal colonies. Thus, the changes in sex factors induced by alpha radiation in the vegetative sporidia of line 410qq were similar to those obtained during segregation and recombination from chlamydospores.

The abnormal cells were studied to determine if the observed changes in sex factors were induced immediately by the alpha radiation or were produced during subsequent growth of these cells. Ten entire young colonies of abnormal-cell survivors of irradiation were transferred to PDB. Suitable control cultures were made from colonies of untreated cells. The isolates with abnormal cells produced approximately 1000 times less growth in broth than did the control isolates. After 3 days' growth by shake culture, the resulting cell populations were sampled on dilution plates. A majority of the colonies developing from the lines with abnormal cells resembled the initial abnormal colony; in the minority were colonies of various cultural types and with the normal cell shape and growth rates of the untreated 410qq. The same results

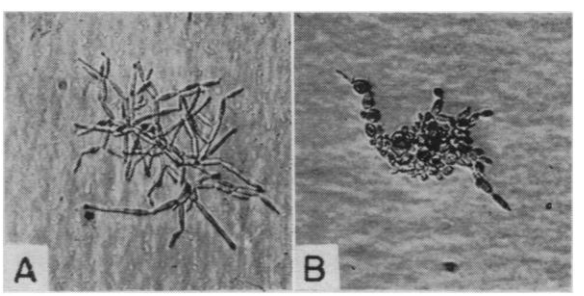

Fig. 1. Young colonies of *Ustilago zeae* developing from single sporidia of the diploid line 410qq. (*A*) Typical sporidial colony produced in 24 hr by a single, nonirradiated cell. (*B*) Colony of distorted cells produced in 72 hr by a single abnormal cell obtained after the line was exposed to alpha radiation.

were obtained when this process was repeated through each of nine successive cultural generations with subtransfers made to PDB from colonies with abnormal cells.

Representatives of the many variants with normal cell characters were isolated from the dilution plates for each of the 10 abnormal cell lines and tested for sex type by inoculating corn seedlings. As many as six different sex genotypes were found among the descendants from each original abnormal cell. However, no mutations were found in the genes for sexual compatibility. Thus, it is apparent that exposure to alpha radiation induced an unstable condition in vegetative cells of this diploid line that resulted in the segregation and recombination of the factors for sexual compatibility during multiplication of the affected cell.

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Number and Size of Radial Resin Ducts in Slash Pine

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Rate of flow of oleoresin from freshly made wounds on slash pine (*Pinus elliottii* Engelm.) is determined in part by the number and size of radial resin ducts. In this study, the relationships of these two anatomical factors with age of tree and width of growth ring were determined as part of a study on inheritance of factors that determine oleoresin yield.

Microtome sections were prepared at five-ring intervals from the vascular cambium to the pith of 10 parent trees. Wood samples, approximately ½ by 1½ in., were removed from the selected trees at breast height by boring two holes to the pith, one above the other, and sawing out the connecting wood. The samples were separated at every fifth ring, and tangential sections, 25 to 30μ thick, were cut with a sliding microtome. These were stained with haematoxylin and safranin that sharply differentiated the resin ducts and accompanying cells (*1*).

A complete count was made of the horizontal resin ducts within each section with the aid of a mechanical stage. The ducts were counted in parallel strips, using the diameter of the field of vision as the width of each strip. Counts were made under 100× magnification. The area of each section was determined from measurements with a graduated mechanical stage. The average area for each measured sample was 3.03 cm^2. Radial resin canals in tangential section appear elliptical; hence, the major and minor axes were measured to include thickness of the epithelial cells (Fig. 1). Ten ducts per section were measured with an ocular micrometer under a magnification of 440×.

Results of the relationships between number and size of ducts and age of ring, and between number and size of ducts and average ring width, are presented in Fig. 2. Inspection of the data showed the following relationships.

1) The number of radial ducts formed per unit area was highest during the early age (rings near the pith) and decreased rapidly until about the 20th year, after which it leveled off. In the wood close to the pith, most of the ducts are found in those vascular rays initiated contiguously with medullary rays. As the diameter of the tree increases, there is a decrease in the number of these rays per unit area of tangential surface. This decrease in number of resin ducts is partially offset by formation of additional vascular rays containing resin ducts.

2) A similar relationship existed when number and size of resin ducts were plotted against average width of ring. The gradual decrease in ring width with age contributed to the stabilization in the number of ducts.

3) Average size of resin ducts decreased linearly with age of ring. Since the size of tracheids is reflected by age of ring, this effect is probably associated with resin-duct size.

With the measurements taken it was not possible

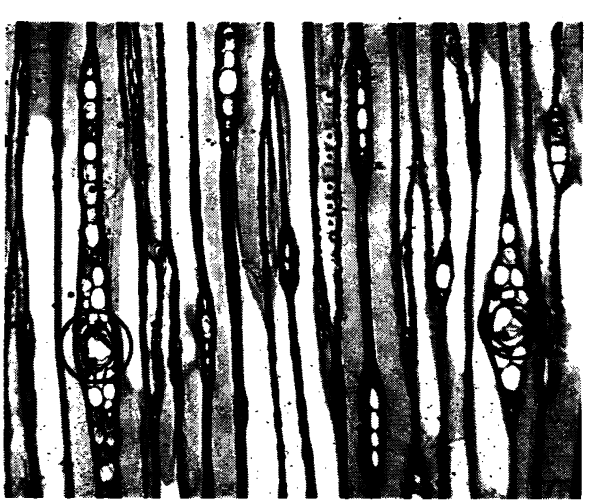

Fig. 1. Photomicrograph of two radial resin ducts on a tangential surface. The resin ducts (within circles) are contained in fusiform rays.