Table 1. Races of *Oenothera* which have been shown to possess incompatibility alleles.

strigosa	*biennis* group 2	*biennis* group 3	*parviflora*
Brookston	Indian River	Coudersport III	muricata
Fargo	Victorini	Linville	
Granger	Waterbury	Mountain Lake	
Heber		Newfound Gap	
Iowa 6		Smethport	
Iowa 12			
Palmer Lake			

lack S_I alleles. These races were previously interpreted as hybrids between the structurally homozygous, self-fertile *grandiflora* group and the *biennis* group 1 (*4*). The absence of incompatibility alleles in the complexes *neoacuens* (*grandiflora* de Vries) and alpha *Beaufort* confirm the earlier interpretation.

The above data indicate that incompatibility alleles may well be characteristic of the alpha complexes of most complex-heterozygotes. With the exception of the *grandiflora* de Vries and the Beaufort collections, all races which are complex-heterozygotes and which have so far been tested possess incompatibility alleles.

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Fractionation of Stable Isotopes of Sulfur by Thiobacilli

Tudge and Thode (*1*) have calculated thermodynamically that H_2S in equilibrium with elementary sulfur in aqueous solution shows no fractionation of the stable sulfur isotopes S^{32} and S^{34}. This can be interpreted to mean that during the oxidation of H_2S to sulfur, both isotopes react with equal rapidity. It was also found by Jones and Starkey (*2*) that *Thiobacillus thioxidans,* when it was grown on sulfur (it oxidizes sulfur to sulfate), likewise produced no fractionation.

In experiments carried out by our group with *Thiobacillus concretivorus* obtained in pure culture by C. D. Parker of the Melbourne and Metropolitan Board of Works (Australia) and grown on elementary sulfur, results not unlike those of Jones and Starkey (*2*) were obtained. The sulfur used was commercial sulfur and sulfur (of volcanic origin) from White Island, New Zealand. Very little fractionation was obtained in these experiments, and there certainly appeared to be no definite enrichment of the light isotope. Enrichment of S^{32} varied from $+0.05$ to -0.16 percent. Thus, if the results are to be taken as significant, there is a small enrichment of S^{34} during the oxidation

$$S \rightarrow SO_4^{--}$$

In preliminary experiments with *T. concretivorus* grown on H_2S as the sulfur source, enrichment of the lighter isotope could be detected when the products of the oxidation, sulfur and sulfate, were analyzed. Cultures of *T. concretivorus* were grown by C. D. Parker in an atmosphere containing approximately 200 ppm of H_2S at room temperature (20° to 25°C). After 8 to 10 days the sulfur which had formed as a pellicle was filtered from the culture medium, and the sulfate formed in the culture was precipitated as barium sulfate.

The results represented in Table 1 demonstrate that there is a significant enrichment of S^{32} both in sulfur and sulfate. In experiment 3, the sulfate was not precipitated in the culture solution but was separated by washing the filter papers, on which the sulfur was filtered, with water and then precipitating this solution with barium chloride. This may be the reason for the reduced enrichment.

In order to test whether fractionation occurs during abiological oxidation of H_2S, a Kipps apparatus was left standing for some weeks at approximately 23°C, and the sulfur formed at the top of the apparatus due to oxidation of H_2S was separated out. The sulfur showed a depletion of 0.3 ± 0.1 percent of the S^{32}/S^{34} ratio with respect to that of the H_2S, a change in the opposite direction to that of biological oxidation. This indicates that the biological oxidation of H_2S by *Thiobacilli* leads to an enrichment of S^{32} in the final products.

In addition to the afore-mentioned laboratory experiments, measurements were carried out on samples collected in natural environments where a sulfur cycle was in progress. The results represented in Table 2 indicate a definite enrichment of S^{34} in gypsum and sulfates with respect to the associated sulfur.

Although the sulfate in the concrete of sewers may be formed as a result of the biological and abiological oxidation of H_2S as well as of the microbial oxidation of sulfur (Parker, *3*), the gypsum crystals surrounding the sulfur nodules at Lake Eyre (South Australia) appear to be formed by the microbial oxidation of sulfur only (Baas Becking and Kaplan, *4*). It is difficult at this stage to interpret the results in order to establish with certainty whether an enrichment of S^{34} does take place, since part of the sulfate may redissolve and a physical fractionation may occur. Further, it is conceivable that a proportion of the gypsum found under these natural conditions may be of secondary origin, having at some stage entered a cycle in which sulfate reduction was taking place and so being enrichced in S^{34}. We draw attention to these factors as complications which deter us from drawing a definite conclusion. From field observations and under the conditions in which the samples were collected and analyzed, we consider, however, that the enrichment

Table 1. Fractionation of sulfur isotopes during the oxidation of H_2S by *Thiobacillus concretivorus.*

Sample	S^{32}/S^{34}	Relative enrichment of S^{32} (%)
Experiment 1		
Sulfide used	22.12	0.0
Bacterial sulfur	22.18	0.3 ± 0.1
Bacterial sulfate	22.29	0.8 ± 0.1
Experiment 2		
Sulfide used	22.14	0.0
Bacterial sulfur	22.27	0.6 ± 0.1
Bacterial sulfate	22.33	0.9 ± 0.1
Experiment 3		
Sulfide used	22.14	0.0
Bacterial sulfur	22.26	0.5 ± 0.1
Bacterial sulfate	22.28	0.6 ± 0.1

Table 2. S^{32}/S^{34} isotope ratios of sulfur and sulfate formed by the oxidation of sulfur.

Sample and locality	S^{32}/S^{34}	Relative enrichment of S^{32} (%)
Melbourne sewer		
Sulfur from concrete surfaces	22.29	0.7 ± 0.1
Sulfate from concrete surfaces	22.14	0.0
West side, Sulphur Peninsula, Lake Eyre		
Sulfur nodule	22.49	0.6 ± 0.1
Gypsum crystals around sulfur	22.36	0.0
East side, Sulphur Peninsula, Lake Eyre		
Sulfur nodule	22.34	0.4 ± 0.1
Gypsum crystals around sulfur	22.25	0.0

of S^{34} illustrated in Table 2 as a result of the biological oxidation of sulfur to sulfate is significant. This may be considered as further evidence confirming results on laboratory experiments that S^{32} is not enriched, but rather, depleted, during the oxidation of sulfur to sulfate by *Thiobacilli* (*5*).

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A New Strain of the Mouse Mammary Tumor Virus

The mammary tumor virus, transmitted from generation to generation through the mother's milk, is an important factor in the development of mammary tumors in mice. Although it has been postulated that this virus should show genetic autonomy and variability (*1*), this has not been established. The possibility that the mammary tumor viruses possessed by different inbred strains of mice may not be identical has been indicated by reports of differences in characteristics of tumor development (such as tumor incidence or mean age of tumor development) which occur when two such strains are crossed reciprocally (*2*). However, evidence of stable alterations in the activity of the virus within an inbred strain, in which the virus is detectable both before and after the change, has not been reported. To verify the assumption that the appearance of a new characteristic within a strain is the result of a change in the mammary tumor virus, it is necessary to compare the new and the original stocks in situations wherein the virus is the only variable, both in the test mice and in the females supplying virus to the test mice. In addition, the altered activity of the virus must be observed through several generations of mice to insure the stability of the change.

In our laboratory a fortunate combination of circumstances has resulted in a situation in which a change in a characteristic of an inbred strain of mice can be traced to an alteration in the activity of the mammary tumor virus. A line of Heston A mice (A/HeCRGL), inbred for over 85 generations, has been maintained in this laboratory since 1950. The mean age of mammary tumor development in the breeding females has consistently been about 12 months. In 1953 it was discovered that females in one branch of the stock were developing mammary tumors at about 8 months of age. This latter group was separated out, and it has been maintained separately as the A/viCRGL subline. It is at the present time in its 13th generation.

The experiments described below, in which all mice have been maintained as breeding females, were set up to determine whether this change in mean age of tumor development was the result of a change in the mouse or in the virus (*3*). In all experimental groups, some mice are still alive. Therefore, the final mean ages of tumor development may be slightly different from those reported here. However, the remaining mice are either so old or so few in number that their deaths (due to mammary tumors) will not affect the significance of the tumor age differences.

In the generations of stock mice concurrent with the experimental groups discussed below, 90 females of the A/He strain had a mean age of tumor development of 12.7 months; 111 females of the A/vi subline had a mean age of tumor development of 8.6 months (Table 1, experiment 1).

Reciprocal hybrids (34 A/He × A/vi hybrids, 51 A/vi × A/He hybrids) of the two stocks were collected, and their mean ages of tumor development were determined (Table 1, experiment 3). Each group of hybrid mice developed mammary tumors at a mean age similar to that of the strain to which their maternal parents belonged. These two groups of mice were identical genetically but differed in maternal influences.

Newborn mice of the A/vi subline were transferred to and nursed by females of the A/He strain and vice versa. Fourteen animals that received A/He milk either had a late age of tumor development or are alive and more than 13 months old, despite the fact that they are otherwise A/vi females; 25 females that received A/vi milk had an early mean age of tumor development despite the fact that they were otherwise A/He females (Table 1, experiment 2). Thus, the difference in mean age of tumor development is evidently mediated by factors carried in the milk.

The activity of the mammary tumor virus has been followed for two generations beyond the reciprocal hybrids by fostering females of the A/vi stock upon both groups of hybrids (Table 1, experiment 4) and by collecting the offspring of these fostered females (Table 1, experiment 5). In both of these generations, groups of 22 and 25 mice whose mammary tumor virus was originally from A/He mice are developing mammary tumors at a later age than did groups of 21 and 34 mice whose mammary tumor virus was originally from the A/vi stock. Thus, the two viruses have retained their difference in activity through these generations, despite the fact that the mice carrying each of them were similar in genotype in each generation. This eliminates the possibility that the difference in mean age of tumor development reported here is the result of a change in the genotype of the host which is expressed in the activity of the mammary tumor virus passed to the offspring.

Table 1. Summary of experiments involving the mammary tumor viruses of the A/He strain and the A/vi subline and their influence on the mean age of mammary tumor development in breeding female mice. Symbols designate genotype (in hybrids the female parent is mentioned first); numbers in parentheses indicate mean age, in months, of tumor development; arrows indicate transfer of the virus.

Expt.	Transfer of virus from A/He strain	Transfer of virus from A/vi subline
1	A/He (12.7)	A/vi (8.6)
2	↓ A/vi (> 12.8)	↓ A/He (9.6)
3	F_1* (13.1)	F_1† (9.4)
4	↓ A/vi (11.9)	↓ A/vi (9.6)
5	↓ A/vi (> 11.4)	↓ A/vi (9.4)

* A/He × A/vi. † A/vi × A/He.

The various experimental situations have shown the stability of the difference between the two viruses. Possible differences in the response of other strains of mice to these two strains of virus are being tested in a series of current experiments. In addition, the possibility of there being serological differences between the two viruses is being investigated.

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