each other in skeletal status at any other ages included in our study.

The most extensive study of the skeletal development of children in Japan is that reported by Sutow (*3*) and by Sutow and Ohwada (*4*), who assessed hand radiographs made of 1220 boys and 1150 girls of Hiroshima during 1951–52. These were apparently healthy children who served as controls in the Atomic Bomb Casualty Commission's study of the children who survived the atomic bombing of that city. Sutow and Ohwada reported that, on the basis of the Greulich-Pyle radiographic standards, the Hiroshima children were less advanced skeletally than the Cleveland children at every age considered in their study—that is, from 6 to 19 years. Their relative retardation ranged from 6 to 24 months in boys and from 9 to 24 months in girls.

Sutow suggested that the observed retardation in the skeletal development of the Japanese as compared with the Caucasian children might be due, at least in part, to some racial difference in the rate at which normal growth and development proceed in the two groups. However, he stressed as important factors contributing to their relative retardation in skeletal development the deprivations in food and other essentials which the Japanese children had experienced during World War II and the years immediately following its close.

Our findings on the American-born Japanese children do not support the view that the less advanced skeletal status of the children in Japan is attributable to some racial difference between Japanese and Caucasians. It seems more probable that, like their smaller average stature and their relatively shorter legs during childhood, the skeletal retardation of the children in Japan results from a less adequate diet and from other environmental conditions which are not so conducive to optimal growth as those existing in this country.

These findings, especially the observed similarity in skeletal status between the California Japanese and the Cleveland Caucasian children, indicate the need for caution in interpreting the relatively retarded growth and development of children in less favored parts of the world as the expression of some basic genetic difference between them and our own children (*5*).

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## Distribution of Incompatibility Alleles among the Complex-Heterozygotes of Oenothera

In earlier papers (*1*), evidence was presented indicating the presence of an incompatibility allele mechanism in oenotheras of the *biennis* group 1, a phylogenetic group of true-breeding translocation heterozygotes. This is a preliminary report (*2*) of the occurrence of incompatibility alleles in other phylogenetic groups of the genus *Oenothera*.

The oenotheras of *biennis* group 1 are composed of two genomes or "complexes" of seven chromosomes each. These are designated as the alpha and beta complexes and are characterized by completely different arrangements of their chromosome ends. As a result, at meiosis a circle of 14 chromosomes occurs, and only two kinds of spores are produced, one carrying the alpha, the other the beta, complex. Such plants, which are naturally self-pollinating, produce only alpha · beta combinations among the offspring. The absence of alpha · alpha or beta · beta combinations among the progeny is attributed to a balanced lethal system. In outcrossing these forms, it becomes apparent that one complex is transmitted to the offspring predominantly through the egg and the other largely through the pollen. This serves as a basis of distinguishing the alpha and the beta complexes, the latter coming through the pollen, the former through the egg.

In the *biennis* group 1 races, the alpha complex carries an incompatibility allele ($S_I$), while the beta complex carries an allele which has no incompatibility effect ($S_f$). The $S_I$ allele of the alpha complex serves as a pollen lethal, because in self-pollinations development of pollen carrying the alpha complex will be inhibited by the presence of the same $S_I$ allele in the style. The incompatibility allele mechanism thus acounts for one-half of the balanced lethal system so well known to *Oenothera*.

The incompatibility allele mechanism suggests an attractive hypothesis explaining the origin of the complex-heterozygotes. In two isolated populations showing orthodox cytogenetic behavior, the occurrence of random segmental interchange can, over a period of time, result in the differentiation of the two populations with regard to the arrangement of their chromosome ends. If interchange is extensive enough, the two populations may come to be characterized by completely different segmental arrangements. Let us suppose that one of these populations possesses an incompatibility allele system, while the other does not. If the two populations now come into contact and hybridization occurs between them, the hybrids will be complete translocation heterozygotes, producing only two kinds of spores. One of these will carry an $S_I$ allele; thus, pollen of this type will not develop in a self-pollination, and one type of segregate (alpha · alpha) which is structurally homozygous cannot occur. If megaspore competition occurs, and the complex carrying the $S_I$ allele is the one which succeeds in producing the embryo sacs, the other segregate which is structurally homozygous (beta · beta) will also be eliminated. Thus, such a hybrid will be true-breeding from its inception. In this way, the highly specialized oenotheras could have arisen simply through a single hybridization. Once established, of course, the nature of the cytogenetic mechanism allows the accumulation of recessive detrimental and lethal genes which reinforce the original balanced lethal system of incompatibility alleles and megaspore competition.

The strength of this hypothesis depends to a large extent upon the demonstration of the existence of incompatibility alleles in the other phylogenetic groups of complex-heterozygotes in the subgenus. If incompatibility alleles are found in the alpha complexes of most complex-heterozygotes, the hypothesis would seem to have general significance in explaining the origin of the complex-heterozygote.

Largely through the work of Cleland and his associates, several hundred collections of *Oenothera* from North America have been cytogenetically analyzed and classified accordingly into eight different phylogenetic groups (*3*). A study has been undertaken to survey the complex-heterozygotes among these collections for the presence of incompatibility alleles in their alpha complexes. The particular phylogenetic groups with which work has begun are the *strigosa*, the *biennis* group 2, the *biennis* group 3, and the *parviflora*. Some unforeseen difficulties in obtaining the critical hybrid combinations in many of the crosses has so far limited the collection of data regarding the distribution of incompatibility alleles in these groups. However, certain races (listed in Table 1) have been shown to possess incompatibility alleles.

It is interesting to note that the races *grandiflora* de Vries and Beaufort both

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 $0.3 \pm 0.1$ 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 | $0.3 \pm 0.1$ |
| Bacterial sulfate | 22.29 | $0.8 \pm 0.1$ |
| *Experiment 2* | | |
| Sulfide used | 22.14 | 0.0 |
| Bacterial sulfur | 22.27 | $0.6 \pm 0.1$ |
| Bacterial sulfate | 22.33 | $0.9 \pm 0.1$ |
| *Experiment 3* | | |
| Sulfide used | 22.14 | 0.0 |
| Bacterial sulfur | 22.26 | $0.5 \pm 0.1$ |
| Bacterial sulfate | 22.28 | $0.6 \pm 0.1$ |

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 | $0.7 \pm 0.1$ |
| Sulfate from concrete surfaces | 22.14 | 0.0 |
| *West side, Sulphur Peninsula, Lake Eyre* | | |
| Sulfur nodule | 22.49 | $0.6 \pm 0.1$ |
| Gypsum crystals around sulfur | 22.36 | 0.0 |
| *East side, Sulphur Peninsula, Lake Eyre* | | |
| Sulfur nodule | 22.34 | $0.4 \pm 0.1$ |
| Gypsum crystals around sulfur | 22.25 | 0.0 |