REVIEW

PROFESSOR MUKAI: THE MAN AND HIS WORK*

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ABSTRACT

Professor Mukai was my postdoctoral associate for more than two years, from June 1965 through July 1967. In this article, I discuss mainly the work done during that period.

Mukai's best known accomplishment was to demonstrate that the total deleterious mutation rate is considerably higher than was previously thought, due to the very high rate of mutation of viability-depressing polygenes. These are at least 25 times as frequent as lethals. These experiments, utilizing a mutation-accumulation scheme, were clever in design, meticulous in execution, and heroic in scope. In related experiments he showed that the typical viability-reducing mutation is deleterious in the heterozygous as well as homozygous state. From the viability experiments he was able to infer that there is strong heterozygous selection for fitness, comparable to that for lethals.

All of Mukai's research was characterized by a willingness to undertake problems that involved enormous numbers of Drosophilae and many hours of work. His ability to plan such experiments and to see that they were carried out carefully was a rare gift, one that the genetics community will sorely miss.

INTRODUCTION

The world of genetics suffered a great loss with the sudden, unexpected, and untimely death of my dear friend Terumi Mukai. I am honored to have this opportunity to discuss the man and his work.

Professor Mukai's outstanding research attribute was the ability, courage, and enthusiasm to undertake difficult, time-consuming problems. He also had the personal discipline and organizing capacity to plan such projects and see them to completion. He worked hard and he worked carefully; and he was able to inspire hard and careful work from his associates. His enormous accomplishment is all the more remarkable, for throughout much of his adult life his health was not good. But he didn't let this deter him; he worked as hard as he could, perhaps

* This paper is adapted from a talk given at a symposium in honor of Professor Mukai during the meeting of the Genetics Society of Japan on October 17, 1991.
too hard. His work was of the greatest importance to him. He thought about it continuously and enjoyed discussing it.

Dr. Mukai was a person of great scientific integrity. He followed the scientific paradigm of letting the research speak for itself. He did careful experiments and accepted the results, however much these might depart from conventional wisdom. I shall mention some of these later.

We were all shocked and saddened by his untimely death. When the great American composer, George Gershwin, died, a friend said: Death can be just and death can be kind, but why did it have to take him at this time. I feel this way about Terumi Mukai, whose life ended in the midst of a productive career.

We have one consolation. Professor Mukai lived long enough to learn of a very high award, the Duke of Edinburgh Prize from the Japan Academy. He had previously received the Annual Prize from the Genetics Society of Japan and a Prize from the Foundation for the Promotion of Genetics.

I am happy that many of his papers have been collected and reprinted by his colleagues. Re-reading them has brought back many poignant memories, and renewed respect for his productivity.

I first learned of Mukai from my friend Allan Burdick of Purdue University. Mukai was at that time a graduate student, working with Professor Burdick. They had discovered a recessive lethal on chromosome 2 of *Drosophila melanogaster* that persisted for a long time in populations. The mutation quickly came to the same equilibrium frequency in the population regardless of the starting frequency or genetic background, clearly a case of balancing selection (Mukai and Burdick, 1959).

It looked very much like an example of single-gene heterosis, or overdominance, although they could not completely rule out pseudo-overdominance brought about by two favorable dominant alleles closely linked in the trans position. Interestingly Mukai and Burdick showed that, over very long time periods—70 generations and more—the lethal frequency became less. It appeared as if the lethal would eventually be eliminated from the population (Mukai and Burdick, 1960).

Mukai and Burdick attributed this to the accumulation of modifiers that decreased the amount of overdominance, and suggested that for this reason most overdominant loci are transitory. This interesting example was the basis of Mukai’s Ph. D. thesis, and he received the Ph. D. degree in 1958. Later, in 1964, he received the Doctor of Science degree from Tokyo Metropolitan University.

He remained at Purdue University for two more years as an instructor. He then returned to Japan and started work at the National Institute of Genetics in July 1960. There he was closely associated with his friend, Yuichiro Hiraizumi. The two were greatly interested in the debate then raging between the proponents of the “classical” and “balance” schools of population genetics. Hiraizumi favored the classical viewpoint and Mukai the balanced, so they agreed to cooperate and discuss problems in this area. They thought that bringing two
points of view to the problem would help in its resolution.

In 1964 I learned of Dr. Mukai’s interest in coming to work with me at the University of Wisconsin. I was eager to have him join my group for two reasons: First, I had already had three Japanese students in succession: Motoo Kimura, Yuichiro Hiraizumi, and Takeo Maruyama and, I need not say, they were all outstanding. So I welcomed another person in this tradition of excellence. The second reason was that I was very interested in the impact of mutation on the population, and for this the spontaneous mutation rate was crucial.

Mukai had already done one mutation-accumulation experiment suggesting a very high rate of mutation of viability-reducing polygenes (Mukai, 1964), and I was very eager to test the generality of the conclusion by having the experiment repeated with a different group of chromosomes. He arrived in Wisconsin in June 1965 and remained there until July 1967, a little over two years. During this time he did a prodigious amount of work. And he established once and for all that the spontaneous rate of mutation of slightly deleterious genes is very high.

In July 1967 he accepted a position as Associate Professor of Genetics at North Carolina State University, USA, and was soon promoted to Professor. He remained there until March 1975, when he became Professor of Genetics at Kyushu University.

The work for which Mukai is best known is his study of the mutation rate of viability polygenes. It is these experiments that I shall emphasize. Perhaps his later work at Kyushu University will be reviewed by others.

MUKAI’S MUTATION RATE EXPERIMENTS

Geneticists had long suspected that mutations with minor effects occurred in high frequency, but there were no convincing data. Because of the mutation load principle (Haldane, 1937), mutations with minor effect have the same ultimate effect on fitness as more deleterious ones, so it was important to find out how frequent such mutations are. H. J. Muller was especially concerned from the standpoint of human welfare. In a letter written to J. B. S. Haldane in 1946, he noted that the frequency of mutations with minor effects is at least three times as great as the frequency of recessive lethals. He went on to write: “I hope that some day a more elaborate experiment of the sort can be undertaken to extend the range of detectability of these mutations.” This is exactly what Mukai did.

It is impractical to measure the frequency of spontaneous mutations arising in a single generation. Muller was the first to suggest that recessive mutations could be accumulated in heterozygous condition, thereby amplifying their number so the rate could be more accurately measured. Dobzhansky adopted this method and used it in some of his experiments. The analytical techniques were suggested by Bateman (1959), who used them for analyzing radiation-induced mutation rates.

Although several geneticists talked about such experiments for measuring the
spontaneous mutation rate, Mukai alone had the courage and determination to actually do such an experiment, involving careful observations on millions of flies. It is one thing to talk about doing such an experiment, it is quite a different thing actually to do one. The total number of flies in Mukai's 1964 paper was more than 1.7 million and a similar number were involved in the later experiments done in Wisconsin (Mukai, 1964; Mukai et al., 1972).

Experimental methods

Mukai's basic idea was to permit mutations to accumulate heterozygously with minimum selection for several generations. Therefore only one male was used each generation, and the flies were grown under optimal conditions of Drosophila husbandry. The original chromosome was free of mutations, or at least had a high homozygous viability. This chromosome was then multiplied and the accumulation replicated 104 times. This was the experiment done in Japan. In the repeat experiment done in Wisconsin, three other chromosomes were used for a grand total of 4 replicate experiments.

Figure 1 shows the mating scheme for accumulating mutations. Cy stands for curly wings and Pm for plum colored eyes. Both are dominant markers. Recombination is not a problem, since there is no crossing over in Drosophila males. In the Wisconsin experiment each chromosome was replicated 50 times, 25 with 3

![Diagram](image)

Fig. 1. The mating scheme for accumulating heterozygous mutations with minimum selection and measuring the homozygous viability of chromosomes that have accumulated these mutations. From Mukai (1964).
females and 25 with 10 as a test of the effect of crowding on mutation accumulation. There was no selection for male fertility in either experiment, since only a single male was used. Any sterile male was replaced by a brother reared in a small mass mating, used for insurance against loss of the strain. Actually a substitute male was required only a very few times. Since there was no significant difference in the crowded and uncrowded conditions, the results were pooled for analysis.

After accumulation for a specified number of generations the matings shown at the bottom of the Figure 1 were made. The Cy chromosome carried complex inversions, so crossing over in this generation was minimized. Since homozygous Cy flies are lethal, the expected ratio is 1 wild type to 2 Curly-winged. The index of viability, I, is twice the ratio of wild type to Cy progeny, with an expected value of 1.

That the experiment succeeded in its aim of accumulating mutations with a minimum of heterozygous selection is shown in Figure 2 (Mukai, 1969b). By about 30 generations the viability had decreased to its value in chromosomes extracted from a natural population and made homozygous, implying that the typical viability-reducing mutation persists in the population for about 30 generations. This graph shows one other thing. There is clearly synergistic epistasis, as indicated by the downward curvature, but significant curvilinearity does not appear until after about 40 generations. So to avoid the complications of epistasis

![Graph showing viability of homozygotes for chromosomes with accumulated mutations]

Fig. 2. The viability of homozygotes for chromosomes that have accumulated mutations for many generations. From Mukai (1969).
and to stay within the viability range of chromosomes in natural populations, the analysis was restricted to the results of the first 40 generations.

Since there were many replications, the variance could also be measured. The variance gives an indication of whether the reduction in viability is due to a few mutations with large effect or to many with small individual effects. Figure 3 shows the change in mean and variance for the three different chromosomes in the Madison experiments. It is clear that there is good agreement among the three experiments in the reduction in viability. It is also clear, as expected, that the variances are less reliably determined.

During the course of the experiment, lethal mutations occurred. These have been eliminated from the calculations. There were also non-lethal chromosomes in which there is a severe reduction in viability, but these are very rare. See Figure 3 in Mukai et al. (1972).

![Graph](image)

Fig. 3. The change of mean viability (upper) and the accumulated variance between chromosome lines (lower) for three experiments. From Mukai et al. (1972).

**Analytical methods**

The decrease in viability per generation is a function of the mutation rate and the amount of decrease per mutant, and the two quantities are completely confounded. Only their product is known. But, by using the variance estimate, Mukai was able to separate them.

Let \( \mu \) be the mutation rate at a single locus and \( s \) the viability reduction in flies homozygous for this mutation. Severe and lethal mutations are excluded and
linearity is assumed, justified by the linearity of the regression.

Summing over all relevant loci in Chromosome 2, the mean reduction in viability per generation is

\[ M = \sum \mu s = \bar{s} \sum \mu, \quad \bar{s} = \frac{\sum \mu s}{\sum \mu}. \]  

(1)

Note that it is not necessary for \( \mu \) and \( s \) to be independent, and they are very unlikely to be so. In this calculation, \( \bar{s} \) is the mean value of \( s \), appropriately weighted by the mutation rate of each mutation.

Likewise, the variance in homozygous viability introduced by one generation of mutation at a specific locus is

\[ \mu s^2 - (\mu \bar{s})^2 = \mu(1 - \mu)\bar{s}^2. \]

Summing over all relevant loci, the mean increase in variance per generation is

\[ V = \sum (1 - \mu)\bar{s}^2 = \sum \mu s^2 - \bar{s}^2 \sum \mu, \quad \bar{s}^2 = \frac{\sum \mu s^2}{\sum \mu}. \]

But \( \bar{s}^2 = \bar{s}^2 + V_n \), where \( V_n \) is the variance of the individual \( s \) values. Therefore

\[ V = (V_n + \bar{s}^2) \sum \mu. \]  

(2)

From (1) and (2) and letting \( K = V_n/\bar{s}^2 \) we get the estimating equations

\[ \sum \mu = (1 + K) \frac{M^2}{V} \]  

(3)

\[ \bar{s} = \left( \frac{1}{1+K} \right) \frac{V}{M} \]  

(4)

\[ V_n = \left( \frac{V}{2M} \right) ^2 \left[ 1 - \left( \frac{1-K}{1+K} \right) ^2 \right]. \]  

(5)

Since there are three quantities to be estimated and only two observed quantities, \( M \) and \( V \), no explicit solution is possible. However, \( K \geq 0 \), so we can write three inequalities that set limits on \( \sum \mu \), \( \bar{s} \), and \( V_n \),

\[ \sum \mu \geq M^2/V \]  

(7)

\[ \bar{s} \leq V/M \]  

(8)

\[ 0 \leq V_n \leq (V/2M)^2 \]  

(9)

Mutation rates

The results of the calculations are shown in Table 1. The minimum estimate of the mutation rate is about 0.16 per chromosome, or about 0.4 per gamete per generation. It is of course unrealistic to assume that \( V_n = 0 \). If the distribution of \( s \) is exponential, \( K = 1 \) and the mutation rate is twice as high; the mean value of \( s \) is then half as large. There seems no escape from the conclusion that the rate of polygenic, viability-reducing mutations is of the order of one per generation per zygote. The rate is at least 25 times as high as the lethal rate.

How interested Muller would have been in these experiments! Unfortunately,
Table 1.  Mutation rate and mean homozygous effect of minor deleterious mutations; lethals and severe detrimental effects are excluded. Estimates are given for two assumptions about the standard deviation of $s$ for the data of Mukai (1964) and Mukai et al. (1972).

<table>
<thead>
<tr>
<th></th>
<th>$V_s=0$</th>
<th></th>
<th>$V_s=s^2$</th>
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<tbody>
<tr>
<td></td>
<td>1964</td>
<td>1972</td>
<td>1964</td>
<td>1972</td>
</tr>
<tr>
<td>$\sum \mu s$</td>
<td>0.0038</td>
<td>0.0040</td>
<td>0.0040</td>
<td>0.0040</td>
</tr>
<tr>
<td>$\sigma_s$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.013</td>
<td>0.012</td>
</tr>
<tr>
<td>$\sum \mu$</td>
<td>0.141</td>
<td>0.172</td>
<td>0.282</td>
<td>0.344</td>
</tr>
<tr>
<td>$\bar{s}$</td>
<td>0.027</td>
<td>0.023</td>
<td>0.013</td>
<td>0.012</td>
</tr>
<tr>
<td>Lethal Mut. rate</td>
<td>0.0063</td>
<td>0.0060</td>
<td></td>
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</table>

his death in 1967 prevented his commenting on them. Actually, Mukai's first experiments were published in Japan (Mukai and Chigusa, 1962) and in the United States (Mukai, 1964) while he was still living, but Muller's health was very poor during the later years of his life and I believe he never knew of Mukai's work.

Mukai's estimates of the mutation rate, at least the minimum estimates in Table 1, are much more likely to be too low than too high, for the following reasons.

1. The calculations assuming $V_s=0$ are minimum, and it is unrealistic to assume that $s$ has zero variance.

2. The experiments were done with minimum selection during the accumulation period, but it is impossible to eliminate selection entirely. If there was selection during the accumulation period, the mutation rate is to that extent underestimated.

3. The calculations assume that the heterozygous mutations in $Cy$ are completely recessive, whereas there is good evidence for partial dominance, as I shall discuss later. The bias is not large, however (Mukai, 1980).

4. There may be a large class of very mildly deleterious mutations that are so nearly neutral as to be undetected.

There are two reasons why the mutation-rate estimates may be too high. One is simply unavoidable errors in the experiments. These may be large, for $V$ in particular is estimated with considerable uncertainty. However the four replicates agree in showing a high mutation rate. The minimum estimate of the three is 0.104 per chromosome per generation (1972, Table 5).

Another possibility is that Mukai had an actively transposing element in his system, such as the P factor. The repeated backcrosses of a chromosome from nature to laboratory females is the type known to generate hybrid dysgenesis. This, however, can be ruled out by the lethal rate, 0.006, which is the normal value. Unless Mukai was unlucky enough to have a transposing element that caused polygenic mutations, but not lethals, this hypothesis is very unlikely. Later, in other experiments when there was evidence for actively transposing elements, the rate was much higher (Mukai et al., 1985).
Mukai’s experiments were greeted with skepticism by many geneticists. A mutation rate of the order of one mutation per zygote per generation seemed to be much too high. Geneticists were accustomed to think of a value more like 10 percent. I believe, however, that Mukai’s values are correct, at least of the right order of magnitude.

Mukai’s data have forced geneticists to consider the consequences of a high rate of deleterious mutations. How can the population survive such a high mutation load? I shall return to this topic.

PARTIAL DOMINANCE OF VIABILITY MUTATIONS

Another result of Mukai’s work was further evidence for the partial dominance of viability polygenes. A number of studies had been done earlier on the partial dominance of lethals, showing this to be about 4–5 percent for new lethals and about 2 percent for those in a natural population. The reason for the lesser value in natural populations is that the mutations with greatest heterozygous expression are most quickly eliminated and the average is weighted more heavily by the longer persisting mutations.

Mukai did similar experiments for genes with minor effects, using the covariance of viability for homozygous and heterozygous chromosomes. Several experiments were done, using data from different populations, with consistent results. The dominance of viability polygenes from natural populations is about 0.2 (Mukai et al., 1972). This is in sharp contrast with the dominance of lethals from natural populations, which is about 0.02, only one tenth as large.

Although many hypotheses have been suggested, the explanation is not yet known. Mukai and Cockerham (1977) suggested that, because of the high mutation rate of viability polygenes in comparison to isozyme genes, the viability effects are due to changes outside the coding regions. It is reasonable that mutations affecting regulatory processes have greater dominance than those causing a drastic effect.

HETEROZYGOUS EFFECTS ON FITNESS

These experiments all dealt with viability only, but of course the greatest evolutionary interest is in fitness. Although measuring fitness directly is very difficult, we can use measurements of viability to provide an indirect estimate of the heterozygous effects of mutations on fitness.

The persistence, p, is average number of individuals affected by a mutation before it is eliminated from the population by natural selection. In an infinite population this is the mean number of generations that new mutations persist in the population before they are eliminated. For a specific mutation the persistence is the reciprocal of the selective disadvantage of the mutant heterozygote
(assuming that elimination is through heterozygous rather than homozygous effects). Averaged over loci, the mean persistence is the reciprocal of the harmonic mean of the heterozygous fitness effects.

The mean persistence is

$$\bar{\rho} = \frac{\sum \mu sp}{\sum \mu s}$$  

(10)

The mean persistence, $\bar{\rho}$, is weighted by the product of the mutation rate, $\mu$, and viability effect, $s$, or the load, which is the proper weight since we want to emphasize those mutations with the greatest effect on the load (Mukai et al., 1972).

The numerator of (10) can be obtained from the homozygous effects of chromosomes extracted from natural populations. The denominator is the regression of viability on generation number in Mukai’s experiments. A representative set of results is given in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Viability reduction</th>
<th>Persistence</th>
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<tr>
<td></td>
<td>$\sum \mu s$</td>
<td>$\sum \mu s p$</td>
</tr>
<tr>
<td>Lethals</td>
<td>0.006</td>
<td>0.25</td>
</tr>
<tr>
<td>Minor mutations</td>
<td>0.004</td>
<td>0.10–0.15</td>
</tr>
</tbody>
</table>

The value 0.10 for the homozygous load of mild mutations is measured against random heterozygotes as a standard. If there is partial dominance, this is an underestimate and the value 0.15 is probably closer.

The striking result is that heterozygous mild mutations cause about as much reduction in fitness as heterozygous lethals do. The effect on the population of a mild mutation is about the same as that of a lethal, not only ultimately (by the Haldane principle), but also immediately through its equally large heterozygous effect.

**VIABILITY VARIANCE COMPONENTS**

Mukai also did an analysis of variance for viability polygenes (Mukai et al. 1974). The study involved chromosomes from natural populations, and again lethal and strongly deleterious chromosomes were eliminated from consideration.

In a representative experiment (Mukai et al., 1974) the additive component of the variance was 0.009 whereas the dominance component was 0.0012. Later experiments also showed a much larger additive than dominance variance. Mukai’s data offer little evidence for overdominance as a major cause of variability.
in viability. Mutation-selection balance is more likely. In this, and especially in subsequent experiments the additive variance is higher than would be expected from simple calculations based on mutation-selection balance. It also varies between populations and seems to be associated with environmental differences, suggesting genotype-environment interactions (Tachida and Mukai, 1985; Takano et al., 1987). There is much that remains to be understood.

IMPlications OF A HIGH MUTATION RATE

I should now like to consider the implications of Mukai’s finding of a high mutation rate. One would expect a high mutation rate to produce a very large genetic load. Such a high load in many organisms, especially those with a low reproductive rate, such as most mammals, could well lead to extinction.

The genetic load is defined as \( L = (W - \bar{W})/W \), where \( W \) is the mean fitness and \( \bar{W} \) is the fitness of a (perhaps hypothetical) mutation-free individual. From Haldane (1937) we learn that the load for a recessive locus is simply the mutation rate, \( \mu \), and for a partially dominant one 2 \( \mu \). For small loads in the absence of epistasis this is simply summed over loci. This suggests the generalization that the load is equal to \( 2\Sigma \mu/n \) where \( n \) is the number of mutant genes eliminated with each “genetic death”. This is approximately correct; an exact formulation is given by Kondrashov and Crow (1988).

This tells us that the mutation load can be reduced if selection can remove mutations in bunches, thus picking off several mutant genes with one genetic death. In other words, with strong epistasis it might be possible to lower the mutation load. However, epistasis such as that shown in Figure 3 is not enough to offset the mutation rate that Mukai observed. We must search farther.

It has long been known that truncation, or rank order, selection is very efficient and can eliminate many deleterious genes at once. But strict truncation is not likely to happen in nature, although in density regulated populations something approximating it might happen. Following a lead by Milkman (1978) we showed (Crow and Kimura, 1979) that even a rough approximation to truncation selection has almost the same mutation-eliminating efficiency. I suspect that many organisms are density-regulated, so that quasi-truncation occurs. If so, this can permit a high deleterious mutation rate with a moderate load. Although Mukai himself was skeptical of the truncation selection explanation (Mukai, Schaeffer and Cockerham, 1972), I believe the hypothesis is still worth serious consideration.

I note, however, that such a reduction in the mutation load requires Mendelian segregation and recombination. It is not possible in an asexual population (Kimura and Maruyama, 1966). I suggest therefore, as has Kondrashov (1984), that one reason for the prevalence of sexual reproduction is the ability to eliminate deleterious mutations efficiently. To give a single numerical example: Suppose \( \Sigma \mu = 0.5 \) and that 10 mutations are eliminated per generation by quasi-truncation
selection. In a sexual population the mutation load is \(2\sum \mu/10 = 0.1\). In an asexual population with independently occurring mutations the load is \(1 - \exp (2\sum \mu) = 0.63\), much higher than the sexually reproducing population.

Such a difference becomes important only if the mutation rate is high. Thus, another consequence of Mukai’s work is to give an additional evolutionary advantage to sexual reproduction.

**MUKAI’S LASTING INFLUENCE**

As I have emphasized, Mukai’s major influence on genetics has been the evidence for a high mutation rate. He did a large number of other experiments, with a variety of results. He was one of the first to show that there is very little linkage disequilibrium in Drosophila except with very tight linkage or inversions (Mukai and Voelker, 1977). Some of his findings, such as the difference in additive variance in different regions, call for additional studies to determine the causes. Other results are very hard to explain in terms of conventional genetics. Mukai and Yamazaki (1968) reported a coupling-repulsion effect that seems contrary to existing knowledge of genetics, as do his experiments suggesting an optimum level of heterozygosity (Mukai, 1969a). A satisfactory explanation of these results remains to be found.

One evidence of Mukai’s lasting influence is that Mukai-type mutation experiments are being planned in several laboratories. David Houle, at the University of Chicago, is accumulating mutations as Mukai did and testing these for effects on fecundity, mating success, and adult viability. Austin Burt, at the University of California at Santa Cruz, is planning to accumulate experiments on both second and third chromosomes affecting male fertility. John Willis is starting experiments with Michael Lynch at the University of Oregon, using the plant *Arabidopsis*. The small size, short generation time, and self-fertilizing habit of this species make it possible to plan large-scale experiments.

More than anyone else, Mukai has shown that a high mutation rate is a problem to be taken seriously. Whether the rates he observed in Drosophila also occur in other organisms, and man in particular, remains to be determined. I hope the molecular comparison of different DNA regions between man and his primate relatives will give some evidence on this question.

In both Japan and United States a great deal of time, money, and effort is spent discovering and assessing environmental mutagens. Mukai’s work argues that we should devote much more effort to understanding the evolutionary importance and the social impact of spontaneous mutation. It could be a serious environmental and public health problem. I believe that future work will show the wisdom of Mukai’s being awarded the Duke of Edinburgh Prize.

I thank Dr. Hidenori Tachida and Fumio Tazima for reading the manuscript and making very useful comments.
REFERENCES


