[Slide 1]

We've been studying the biological effects of the radioactive materials released following the Fukushima nuclear disaster, by monitoring the pale grass blue butterfly from the first generation after the accident. And I'm here today to share with you some of our significant findings that were much more serious than what we initially expected.

[Slide 2]

To begin with, the pale grass blue butterfly, *Zizeeria maha*, is a small butterfly of just over a centimeter in size, and it belongs to the family Lycaenidae, in the order Lepidoptera. As you can see, it develops through the stages of egg, larva and pupa, before becoming adult.

[Slide 3]

There are mainly two reasons why we chose the pale grass blue butterfly to study.

The first one is that, it is an ideal model organism to study wing colour patterns. As you can see, its wing colour patterns are simple, and therefore easy to determine whether the patterns are normal. It's also very small in size, so it's easy to take care of, in our tiny lab of limited means. It has a short life cycle, which makes it easy to monitor the changes from generation to generation. Also, this species is very abundant in Japan, and easy to collect in the field. But above all, because our lab had been studying its wing colour patterns since before the accident, we had the accumulation of experiences in rearing this butterfly under standard conditions. And that allowed us to give an elaborate analysis of their abnormalities.

Secondly, the pale grass blue butterfly is also an ideal environmental indicator species. Because it lives just above the soil level, it's directly affected by the radiation dose in the air just above the ground. It also shares the same living environment as humans, whether in cities or in the countryside, and therefore serves as an indicator of what might be the effects on the environment where people live. Furthermore, this species is distributed throughout Japan with the exception of the very North part of Japan, Hokkaido. It only feeds on the leaves of *Oxalis corniculata*, which makes it suitable for internal exposure experiments.

These conditions led us to think that our existing experimental system might be utilized, when the accident occurred on March 11th in 2011.

[Slide 4]

We published our first paper on this research on August 9th in 2012, the day when the atomic bomb was dropped in Nagasaki around 70 years ago.

[Slide 5]

And immediately, we received strong responses from countries overseas, including Germany and France.

[Slide 6]

This is a copy of the article in Le Monde. In Japan, however, we received not only very poor public reaction, but also many criticisms that were emotional and irrelevant to the scientific content of the paper. But despite all this, some journals and academic societies gave fair evaluations on the research.

[Slide 7]

This study is different, and therefore superior to other studies, in that we've been monitoring since the very early phase of the nuclear accident. That is, we've been monitoring the *Z. maha* butterflies since their first generation to be exposed to radiation. Secondly, experiments to evaluate the possible effects were carried out in Okinawa, which is one of the areas least affected by the accident. Also, with our breeding experiments, we examined the effects on their progeny and grand progeny. Lastly, we designed artificial external and internal exposure experiments to reproduce the results observed in the field.

However, our conclusion that low-dose exposures do affect this species of butterfly was not accepted, based on the results from the existing studies, in which insects, especially moths, were shown to be resistant to high-dose radiation.
However, unlike the traditional short-term high-dose exposures, this study examined the effects of long-term low-dose exposures: the experimental conditions are not the same, and therefore it would not be appropriate to compare the two directly.

[Slide 8]

We've done four sets of experiments so far, and the first is the field sampling in the Fukushima area in early May of 2011. Here, we collected the first generation to be exposed to radiation, and obtained the F1 and the F2 offspring in Okinawa to observe possible effects.
Next, we did another field sampling in September of 2011, to collect the fourth to fifth generations in the field, and also obtained their F1 offspring.
Third, we carried out an artificial external irradiation experiment using radioactive cesium 137, with butterflies from Okinawa.
Lastly, we did an artificial internal exposure experiment in Okinawa, in which contaminated leaves collected in the Fukushima area were fed to butterflies from Okinawa.

[Slide 9]

Shown on this map are the ten sampling sites for the field sampling of butterflies in May

2011. The Fukushima Dai-ichi nuclear power plant is located here. We used sampling sites from four prefectures, Fukushima, Tokyo, Ibaraki, and Miyagi.

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In total, approximately 6,000 individuals of this butterfly were used for these sets of experiments.

Au total, 6000 de ces papillons ont été utilisés pour ces expériences.

[Slide 11]

And here are the results.

Et voici les résultats.

[Slide 12]

First, the results of the field sampling of the parental generation, and the subsequent breeding experiments of the F1 and the F2 offspring.

Tout d'abord, les résultats des papillons collectés sur le terrain, et leurs descendant F1 et F2

[Slide 13]

For butterflies, the state of their development can be evaluated by looking at their wing sizes, just like heights and weights for humans.
As you can see, we observed a significant reduction in the forewing size in the population collected in Fukushima, compared to the populations from Shiroishi, located directly north of Fukushima, Tsukuba, located south of Fukushima, and Tokyo.
In addition, the forewing size seems to decrease in response to the increase of the ground radiation dose. This does not follow the standard rule in which butterflies in colder regions tend to have bigger wing sizes.

[Slide 14]

Next is the correlation between collection periods and abnormality rates. The green bars indicate samples collected in May, and the red bars indicate samples collected in September of 2011. The graph on the left is for the parental generation, and on the right is for the F1 generation.
The abnormality rates were higher in September populations for both the parental and the F1 generation in all areas except for Mito.
Also, among the criticisms seen on the internet, there was a comment suggesting that abnormality rates in butterflies would increase with latitude. Our conclusion is that no correlation was seen between latitude and abnormality rates. This yellow dot indicates

the latitude of the nuclear power plant. In terms of correlation, our results possibly suggest higher abnormality rates in areas closer to the power plant.

[Slide 15]
(a)

We then looked at the growth and development of the butterflies. The y-axis indicates the percentage of individuals who had undergone eclosion. The x-axis indicates the number of days it took for them to undergo eclosion. The population from Tsukuba, located farthest from the nuclear power plant, grew up faster than populations from other areas. The population from Hirono, located 20 km south of the power plant, which is the closest of all areas sampled, showed the slowest growth.

(b)

This plot shows the number of days it took for the half of each population to undergo eclosion, against the distance from the power plant. As you can see, it takes longer to undergo eclosion for the populations closer to the power plant, thus displaying growth retardation.

[Slide 16]

For morphological abnormalities seen in the F1 generation, we observed individuals with truncated leg segments, dented compound eyes, smaller wing on one side, and broken or curled wings.

[Slide 17]

We also looked at the F2 generation for their abnormalities. We chose females that displayed abnormalities from each locality and mated them with normal healthy males from Tsukuba, thus creating a total of eight breeding pairs. Not very many eggs were produced, and many individuals didn't survive in the first place, for us to look at their abnormalities.
The population from Motomiya of Fukushima prefecture, which experienced the highest radiation dose, showed the highest abnormality rate, and the F2 generation from Iwaki showed an abnormality rate of over 50 %.
Also, the abnormal traits seen in the F1 generation appeared to be inherited by the F2 generation.

[Slide 18]

Morphological abnormalities seen in the F2 generation included an individual with an antenna that was split into two. This is an individual with normal antennae. We also observed individuals with a short leg or with abnormal colour spots.
However, these experimental results are not sufficient as evidences to prove that these aberrations are the result of genetic damage. Therefore, we've started last year a new

project to address this by mutation analysis at the genetic level.

[Slide 19]

Among the criticisms we received for our paper, there was an argument that since the frequency of colour spot modifications usually increase with latitude, abnormal colour spots seen in the individuals from Fukushima, which is located in the relatively northern part of Japan, is to be expected. This is a false claim.
Shown at the top are the wild type normal wings, and the bottom are the wings with abnormal colour spots.
Abnormalities seen in the colour spots of the F1 and the F2 generations of Fukushima population are very characteristic, in that the modifications are random with no recognizable pattern.
Modification of colour spots under cold-shock do not involve other morphological aberrations, infertility or behavioral abnormalities seen in the Fukushima population.

[Slide 20]

If you look at the difference in colour spots between those seen in the Fukushima individuals and individuals under cold-shock treatment, colour spots under cold-shock treatment have distinct patterns, and there are only these three types recognized.

[Slide 21]

We’ve been looking at the results from observations made in the field sampling and the breeding experiments of the F1 and the F2 generations.
Next, let me show you the results from the artificial external irradiation experiment using butterflies in Okinawa, the part of Japan located farthest from the nuclear power plant.

[Slide 22]

We did two sets of experiments by exposing the butterflies to doses of radiation at 55 mSv and 125 mSv, for a long period of time throughout their larval to pupal stage.
The morphological abnormalities seen as a result were similar to what was seen in the Fukushima individuals and their F1 and F2 offspring. Those are, abnormalities of legs, antennae, colour spots and compound eyes.

[Slide 23]

Wing sizes, which are the indication of their growth, were significantly smaller both in males and females for the individuals exposed to external radiation.
We also looked at the trend in the survival rates as these butterflies develop from larva through pre-pupa, pupa, eclosion, and to adult.
While the control populations showed survival rates around 95%, the population exposed to 55 mSv of radiation showed a survival rate in the 70 % range, and the population exposed to 125 mSv showed a survival rate in the 50 % range. It is apparent that the

survival rate decreases in a dose-dependent manner.

[Slide 24]

Lastly, I'd like to show you the results from the internal exposure experiment. Here, we exposed butterflies in Okinawa to internal radiation by feeding leaves collected in the Fukushima area.
I'd like to emphasize the importance of this experiment, because the results from the fieldwork, which I've talked about earlier, were shown to be reproduced in this experiment. In other words, internal radiation is responsible for what was observed in the field, at least partly.

[Slide 25]

In this internal exposure experiment, leaves collected at various locations in Fukushima and also at a control site located approximately 1,000 km from Fukushima were fed to butterflies born in Okinawa, where the butterflies were subsequently reared.
We quantified the radioactivity in the leaves such as the amount of radioactive cesium, and identified abnormalities of adult butterflies, measured their wing sizes and scored the survival rates.

[Slide 26]

For the experiments performed in the year 2011, leaves were collected in Ube as a control, which is located approximately 1,000 km from the power plant, in Fukushima city, about 60 km from the power plant, in the Iitate flatland, 40km from the power plant, in the Iitate montane region, about 33 km from the power plant, and in Hirono, located 20 km south of the power plant. All butterflies were reared in Okinawa, located approximately 1,800 km from the power plant.

[Slide 27]

The amounts of radioactive cesium 137 in the leaves were measured to be 0.5 Becquerel per kilogram in Ube, 770 Bq in Hirono, 4,170 Bq in Fukushima city, 5,420 Bq in the Iitate flatland, and 23,100 Bq in the Iitate montane region.

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When these values are expressed relative to the control values measured for the leaves from Ube, where the values for the Ube leaves are set to 1, you can see that while the ground radiation dose, measured at the soil level, is only a 100 times as high as the control, the amount of radioactive cesium 137 are 10,000 times or even 40,000 times higher. These values are from 2011. Initially, as we wanted to know the effects of radiation, we intentionally chose those collection sites where we measured relatively high radioactivity.

[Slide 29]

As a result of internal exposure, we identified various morphological abnormalities in the butterflies.
As seen in these images, abnormalities of antennae and palpi, depressed compound eyes, eclosion failure, bent wings and abnormal wing colour patterns were seen. Abnormalities very similar to those observed in the field were reproduced here.

[Slide 30]

Next, shown in this figure is the transition of the survival rates. The x-axis indicates the developmental stages from larva through pupa, pre-eclosion to adult after eclosion. The y-axis indicates the survival rates.
As you can see, butterflies raised on the control Ube leaves showed a survival rate of 95 %, whereas butterflies raised on leaves from the Fukushima areas showed survival rates of 68 % in Hirono, 53 % in the Iitate motane region, 42 % in Fukushima city, and 37 % in the Iitate flatland.
Next, when we evaluated their growth by measuring their forewing sizes, in males for example, we observed a significant reduction in forewing size in the Fukushima city and the Iitate flatland populations, when compared to the Ube population, which suggests growth retardation.

[Slide 31]

When we looked at the individuals who did not survive the internal exposure experiment, those larvae that were raised on leaves from the Fukushima areas were often found to be dead in the process of molting, whereas a control Okinawa individual shown here is dead in a natural form. There were also cases of eclosion failure, which is the final molting from pupa to adult. Even if they survive eclosion, those adults were unable to stretch their wings after eclosion.
These observations suggest some kind of physiological change to have taken place inside the body.

[Slide 32]

To summarize these results, for individuals collected in the field and their F1 and F2 generations reared in the lab, we observed growth retardation, dwarfing, morphological abnormalities, increase in abnormality rates from May to September, and inheritance of morphological abnormalities.
In the external and internal exposure experiments, observations in the field such as decrease in survival rates, dwarfing, and morphological abnormalities were reproduced in the lab.

[Slide 33]

Now, I’d like to talk briefly about more recent findings from our internal exposure experiment.

[Slide 34]

We plotted the change in mortality rate and abnormality rate in response to the cesium dose ingested by a larva throughout its development.
The x-axis indicates the cesium dose ingested by a larva until its pupation, and the y-axes indicate the mortality rate and the abnormality rate.

As the graphs indicate, we observed a sharp increase in both mortality and abnormality rates at low doses of ingested cesium. Both mortality and abnormality rates then reached a plateau at approximately 3 Bq/larva, without further increase in response to increasing cesium dose.

[Slide 35]

We observed cesium activity retained inside the dead bodies of pupae for all populations except for the control Ube population. The pupae that consumed leaves from Hirono retained the highest amount of cesium activity inside their dead bodies, even though the contamination level and the mortality rate were not as high despite of the area being located only 20 km away from the power plant.

[Slide 36]

So far in our internal exposure experiments, we've been using leaves collected in the areas of high contamination in Fukushima prefecture that are located inside the evacuation zone. On the other hand, the results I'm about to show you are from the internal exposure experiments performed in 2012, in which we used leaves collected in areas such as Koriyama and Motomiya of Fukushima prefecture, where people live normal lives. Both areas are located approximately 60 km from the power plant.
I'm just going to present to you the effects observed on the F2 generation.

[Slide 37]

The y-axis indicates the survival rates and the normality rates, and the x-axis indicates the developmental stages from larva and pupa to adult.
As a result, the graphs were clearly divided into two groups.
Regardless of the origin of the leaves consumed by the F1 generation, individuals of the F2 generation that consumed Okinawa leaves showed survival rates over 70 %.
On the other hand, individuals raised on Koriyama leaves over two generations showed a normality rate of 16.7 %, and those raised on Motomiya leaves over two generations showed a normality rate of 0.8 %.
As you can see, the effects of radioactivity accumulated over the F1 and the F2 generations were significant in individuals raised on Motomiya leaves, which measured the highest radioactivity.
Concentrations of cesium 137 in the Koriyama leaves and the Motomiya leaves were 72

and 98 Bq/kg, respectively.

[Slide 38]

And that concludes my presentation. In closing, I'd like to thank those who live in Fukushima for their help with these experiments. Also, I'd like to send my heartfelt support to those who still live with hardships, especially children. Thank you so much for your attention.