Fukushima and the pale grass blue butterfly

Joji M. Otaki
The BCPH Unit of Molecular Physiology, University of the Ryukyus, Okinawa, Japan

IUR Consensus Symposium, Miami, November 18, 2015
Introducing myself:

Joji M. Otaki, Ph.D.
The BCPH Unit of Molecular Physiology
Department of Chemistry, Biology and Marine Science
University of the Ryukyus, Okinawa, Japan

【Research Topics】
Long-range signaling (developmental biology)
Real-time evolution (evolutionary biology)
Protein decoding (protein science, bioinformatics)
Radiation biology?

【Organisms】
Butterflies (the pale grass blue, the painted lady, the peacock pansy, and the blue pansy, etc.),
Fish (mandarin fish, etc.), E. coli (for protein science)

2006 and after: University of the Ryukyu, Okinawa, Japan【Butterfly studies】
Earlier: Kanagawa University, Hiratsuka, Japan【Butterfly studies resumed】
Earlier: University of Cambridge, UK, Wellcome Trust Immunology Unit (Post Doc)
Earlier: Columbia University, New York, Department of Biological Sciences (Ph.D.)
Earlier: University of Massachusetts, Amherst, Department of Chemistry (B.S.)
Earlier: University of Tsukuba, Department of Biology (B.S.)
Earlier: Nagasaki Minami High School
The pale grass blue
*Zizeeria maha* (Kollar, 1844)

Butterflies are the most studied group of animals in terms of life histories.
Habitat for the pale grass blue

Creeping wood sorrel (*Oxalis corniculata*)
Why the pale grass blue?

As a model organism to study color pattern development
1. Easily distinguishable color pattern (black dots)
2. Small size
3. Short life cycle (About 1 month)
4. Easy to collect in the field in Japan
5. Rearing system established (Entomol. Sci. 13, 293-302, 2010)

As an indicator species for environmental changes
1. Living on the surface of the ground.
2. Sharing living space with humans.
3. Found in most places throughout Japan.
4. Eats Katabami (monophagous).

Fukushima Nuclear Accident (March 11, 2011)

Our experimental system may be useful

Katabami = Oxalis corniculata
Sampling points

7 major localities
Collecting samples (Butterflies & Leaves)

Staying in Aizu-Wakamatsu, organizing collected samples

Fukushima City, collecting leaves
Abnormality rates in May and September 2011

Normal abnormality rate in Japan is less than 10%.
Field data were reproduced in laboratory. Wing size reduction (growth retardation) and dose-dependent decrease of survival rates.
Field data were reproduced. We observed (1) a dose-dependent decrease of survival rate and (2) reduction of the forewing size.
### Cs γ ray and β ray from *Oxalis* leaves

<table>
<thead>
<tr>
<th>Distance from the NPP (km)</th>
<th>Ground (μSv/h)</th>
<th>β ray (cpm/kg dry)</th>
<th>$^{137}$Cs (Bq/kg dry)</th>
<th>$^{134}$Cs (Bq/kg dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ube</td>
<td>966.0</td>
<td>0.18</td>
<td>$6.33\times10^2$</td>
<td>$5.3\times10^{-1}$</td>
</tr>
<tr>
<td>Hirono</td>
<td>20.7</td>
<td>1.06</td>
<td>$4.35\times10^3$</td>
<td>$7.71\times10^2$</td>
</tr>
<tr>
<td>Fukushima</td>
<td>61.1</td>
<td>3.57</td>
<td>$2.13\times10^4$</td>
<td>$4.17\times10^3$</td>
</tr>
<tr>
<td>Iitate (Flatland)</td>
<td>40.0</td>
<td>6.22</td>
<td>$2.73\times10^4$</td>
<td>$5.42\times10^3$</td>
</tr>
<tr>
<td>Iitate (Montane)</td>
<td>33.7</td>
<td>18.86</td>
<td>$7.61\times10^4$</td>
<td>$2.31\times10^4$</td>
</tr>
</tbody>
</table>

Dry specimen measured using Ge semiconductor radiation detector.
Summary 1

Field and reared samples
• Growth retardation and small forewing size
• Morphological abnormalities
• Increase of abnormality rates (from May [P, F1] to September [P, F1])
• Inheritance of adverse effects

External and internal irradiation
• Dose-dependent decrease of survival rate
• Small forewing size
• Morphological abnormalities
Further internal irradiation
Next generation
First-Second generations
(Contaminated/Non-contaminated Food)

<table>
<thead>
<tr>
<th>F1-F2</th>
<th>O-O</th>
<th>K-O</th>
<th>M-O</th>
<th>O-K</th>
<th>K-K</th>
<th>O-M</th>
<th>M-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate [%]</td>
<td>83.5</td>
<td>88.3</td>
<td>73.7</td>
<td>20.9</td>
<td>16.7</td>
<td>10.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

O: Okinawa
K: Koriyama
M: Motomiya

Non-contaminated
Contaminated
Cumulative dose and forewing size are correlated

\[ r = -0.93 \]
\[ p = 0.0028 \]
Summary 2

• Survival rate is internal dose-dependent.
• Survival of the next generation (F2) does not dependent on the parents’ ingestion (F1).
• However, cumulative dose in two generations and the forewing size are correlated: Parents’ effect exists.
aAR(P) = adult Abnormality Rate of the Parent generation (field-caught)

(Fall 2011 + Spring 2012) + (Others)
tAR(F1) = total Abnormality Rate of the F1 generation

High in Fall 2011 and Spring 2012.
Summary 3

- Occurrence and accumulation of adverse physiological and genetic effects in early generations (5\textsuperscript{th} and 7\textsuperscript{th} generations).
- Decrease and leveling off to a normal level in 3 years (17\textsuperscript{th} generations).
- This study is probably the most comprehensive record of biological dynamics after a nuclear accident available today.
Robert Koch (1843-1910)

- A founder of modern bacteriology
- Identified specific causative agents of tuberculosis and cholera.
- Proposed “Koch’s Postulates”, and demonstrated infectious disease.
- Nobel Prize in Physiology or Medicine (1905)
Koch’s Postulates of Infectious Disease

Can be used as a determination of the cause of most infectious diseases

• 1) The organism must always be present, in every case of the disease. **CORRELATION**
• 2) The organism must be isolated from a host containing the disease and grown in pure culture. **REDUCTION**
• 3) Samples of the organism taken from pure culture must cause the same disease when inoculated into a healthy, susceptible animal in the laboratory. **REPRODUCTION**
• 4) The organism must be isolated from the inoculated animal and must be identified as the same original organism first isolated from the originally diseased host. **REPRODUCTION**
Postulates of pollutant-induced biological impacts

• 1) Spatial relationship **CORRELATION**
• 2) Temporal relationship **CORRELATION**
• 3) Direct exposure **REDUCTION**
• 4) Phenotypic variability or spectrum **CORRELATION**
• 5) External exposure **REPRODUCTION**
• 6) Internal exposure **REPRODUCTION**

Our butterfly studies mostly meet these postulates.
“No effect” statement

• In scientific (experimental) research, it is difficult to demonstrate “no effect” without thorough investigation of a restricted system. (no effect = failure of detection?)

• Dosimetric studies can show a theoretical possibility of “no effect” based on many assumptions.

• Assumptions on the system are often wrong without experimental evidence, because any biological system is a large black box.

• “Unknown” should not be confused with “no effect”.

• “No effect” should not be confused with “not detected”.

“Effects could exist” is a starting point of scientific (experimental) research. Degrees and detectability of the possible effects are to be studied.
Thank you for your attention

Otaki Lab (The BCPHUnit) at U of the Ryukyus
Atsuki Hiyama
Chiyo Nohara
Seira Kinjo
Wataru Taira
Masaki Iwata
Mayo Iwasaki

Instrumental Research Center at U of the Ryukyus
Akira Tanahara
Shinichi Gima

And many others inside and outside the University.
Thank you!!

Please visit us at w3.u-ryukyu.ac.jp/bcphunit/index.html
The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly

Atsuki Hiyama¹*, Chiyo Nohara¹*, Seira Kinjo¹, Wataru Taira¹, Sinichi Gima², Akira Tanahara² & Joji M. Otaki¹

Subject Areas:
Environmental Sciences
Ecology
Biodiversity

Published on August 9, 2012
The Fukushima nuclear accident and the pale grass blue butterfly: evaluating biological effects of long-term low-dose exposures

Atsuki Hiyama, Chiyo Nohara, Wataru Taira, Seira Kinjo, Masaki Iwata and Joji M Otaki

Abstract

Background: On August 9th 2012, we published an original research article in *Scientific Reports*, concluding that artificial radionuclides released from the Fukushima Dai-ichi Nuclear Power Plant exerted genetically and physiologically adverse effects on the pale grass blue butterfly *Zizeeria maha* in the Fukushima area. Immediately following publication, many questions and comments were generated from all over the world. Here, we have clarified points made in the original paper and answered questions posed by the readers.

Results: The following points were clarified. (1) There are many advantages to using the pale grass blue butterfly as an indicator species. (2) The forewings of the individuals collected in Fukushima were significantly smaller than in the northern and southern localities. (3) We observed growth retardation in the butterflies from the Fukushima area. (4) The aberrant colour patterns in the butterflies obtained in the Fukushima area were different from the colour patterns induced by temperature and sibling crosses but similar to those induced by external and internal exposures to the artificial radionuclides and by a chemical mutagen, suggesting that genetic mutations caused the
The biological impacts of ingested radioactive materials on the pale grass blue butterfly

Chiyo Nohara¹, Atsuki Hiyama¹, Wataru Taira¹, Akira Tanahara² & Joji M. Otaki¹

¹The BCPH Unit of Molecular Physiology, Department of Chemistry, Biology and Marine Science, Faculty of Science, ²Instrumental Research Center, University of the Ryukyus, Okinawa 903-0213, Japan.

A massive amount of radioactive materials has been released into the environment by the Fukushima Dai-ichi Nuclear Power Plant accident, but its biological impacts have rarely been examined. Here, we have
Ingestion of radioactively contaminated diets for two generations in the pale grass blue butterfly

Chiyo Nohara¹, Wataru Taira¹, Atsuki Hiyama¹, Akira Tanahara², Toshihiro Takatsuji³ and Joji M Otaki¹*

Abstract

Background: The release of radioactive materials due to the Fukushima nuclear accident has raised concern regarding the biological impacts of ingesting radioactively contaminated diets on organisms. We previously performed an internal exposure experiment in which contaminated leaves collected from polluted areas were fed to larvae of the pale grass blue butterfly, Zizeeria maha, from Okinawa, which is one of the least polluted localities in Japan. Using the same experimental system, in the present study, we further examined the effects of low-level-contaminated diets on this butterfly. Leaves were collected from two localities in Tohoku (Motomiya (161 Bq/kg) and Koriyama (117 Bq/kg)); two in Kanto (Kashiwa (47.6 Bq/kg) and Musashino (6.4 Bq/kg)); one in Tokai (Atami (2.5 Bq/kg)); and from Okinawa (0.2 Bq/kg). In addition to the effects on the first generation, we examined the possible transgenerational effects of the diets on the next generation.

Results: In the first generation, the Tohoku groups showed higher rates of mortality and abnormalities and a smaller forewing size than the Okinawa group. The mortality rates were largely dependent on the ingested dose of caesium.
Spatiotemporal abnormality dynamics of the pale grass blue butterfly: three years of monitoring (2011–2013) after the Fukushima nuclear accident

Atsuki Hiyama, Wataru Taira, Chiyo Nohara, Mayo Iwasaki, Seira Kinjo, Masaki Iwata and Joji M Otaki

Abstract

Background: Long-term monitoring of the biological impacts of the radioactive pollution caused by the Fukushima nuclear accident in March 2011 is required to understand what has occurred in organisms living in the polluted

Cumulative results of 3 years

• Hiyama et al. (2015) BMC Evolutionary Biology 15: 15.
Fukushima’s Biological Impacts: The Case of the Pale Grass Blue Butterfly

Wataru Taira, Chiyo Nohara, Atsuki Hiyama, and Joji M. Otaki

From the BCPH Unit of Molecular Physiology, Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan (Taira, Nohara, Hiyama, and Otaki).

Address correspondence to Joji M. Otaki at the address above, or e-mail: otaki@sci.u-ryukyu.ac.jp.

Abstract

To evaluate the effects of the Fukushima nuclear accident on the surrounding area, we studied the pale grass blue butterfly Zizeeria maha, the most common butterfly in Japan. We here review our important findings and their implications. We found forewing size reduction, growth retardation, high mortality rates, and high abnormality rates in the field and reared samples. The abnormality rates observed in September 2011 were higher than those observed in May 2011 in almost all localities, imply-