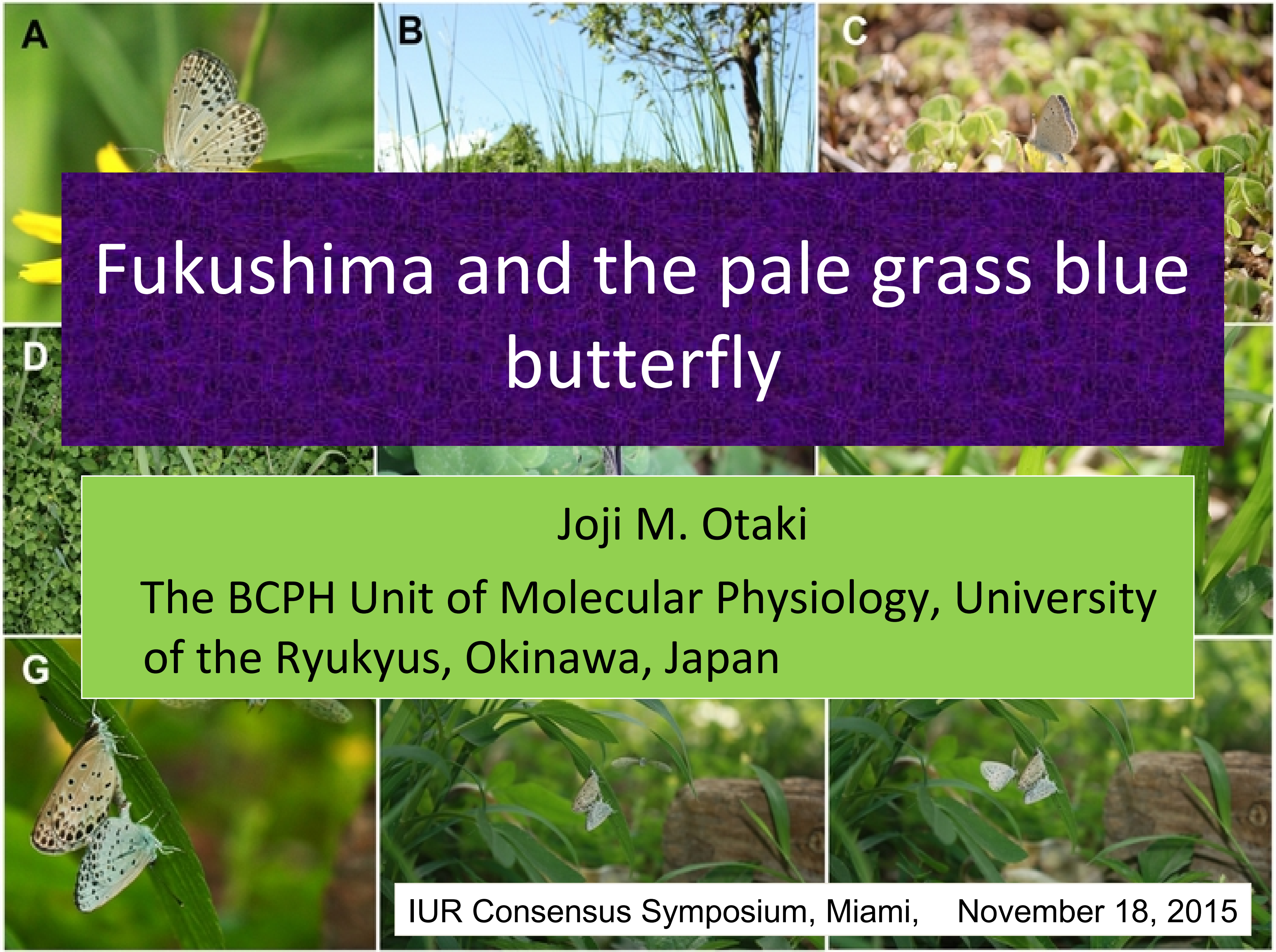


Fukushima and the pale grass blue butterfly

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 Ryukyus, 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

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

The pale grass blue

As an indicator species for environmental changes

①Living on the surface of the ground.
②Sharing living space with humans.
③Found in most places throughout Japan.
④Eats Katabami (monophagous).

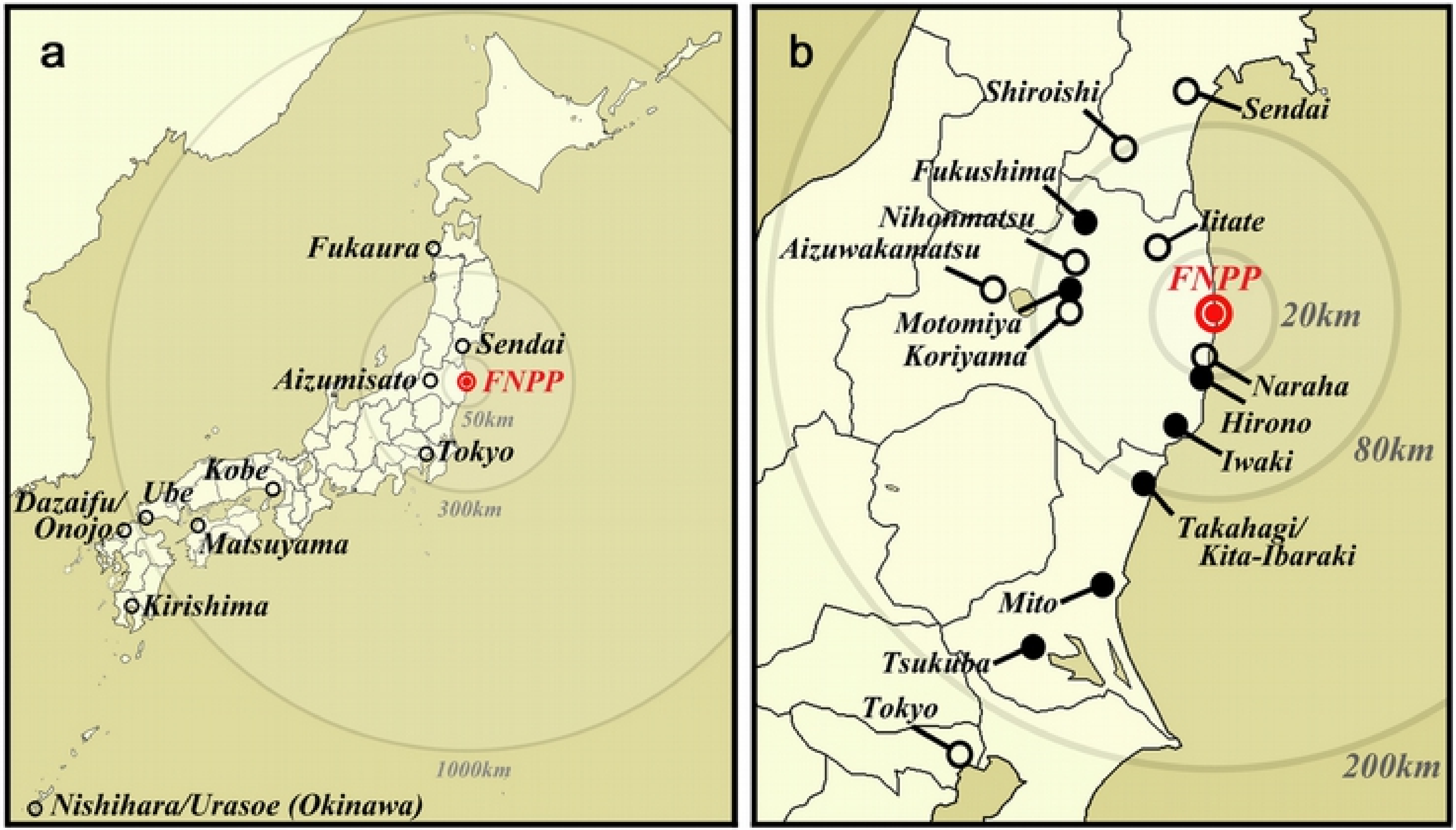
Katabami = *Oxalis corniculata*

Fukushima Nuclear Accident（March 11, 2011）

→

Our experimental system may be useful

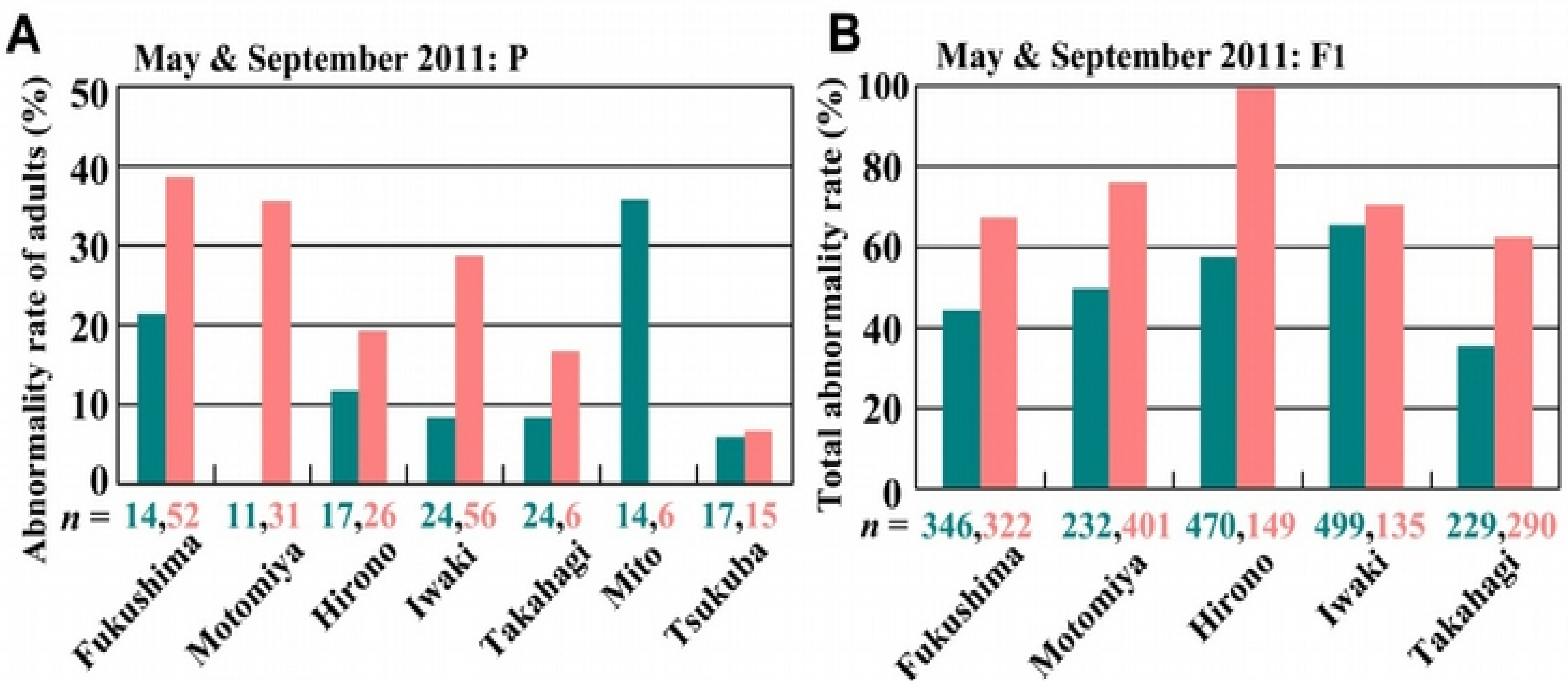
Sampling points



7 major localities

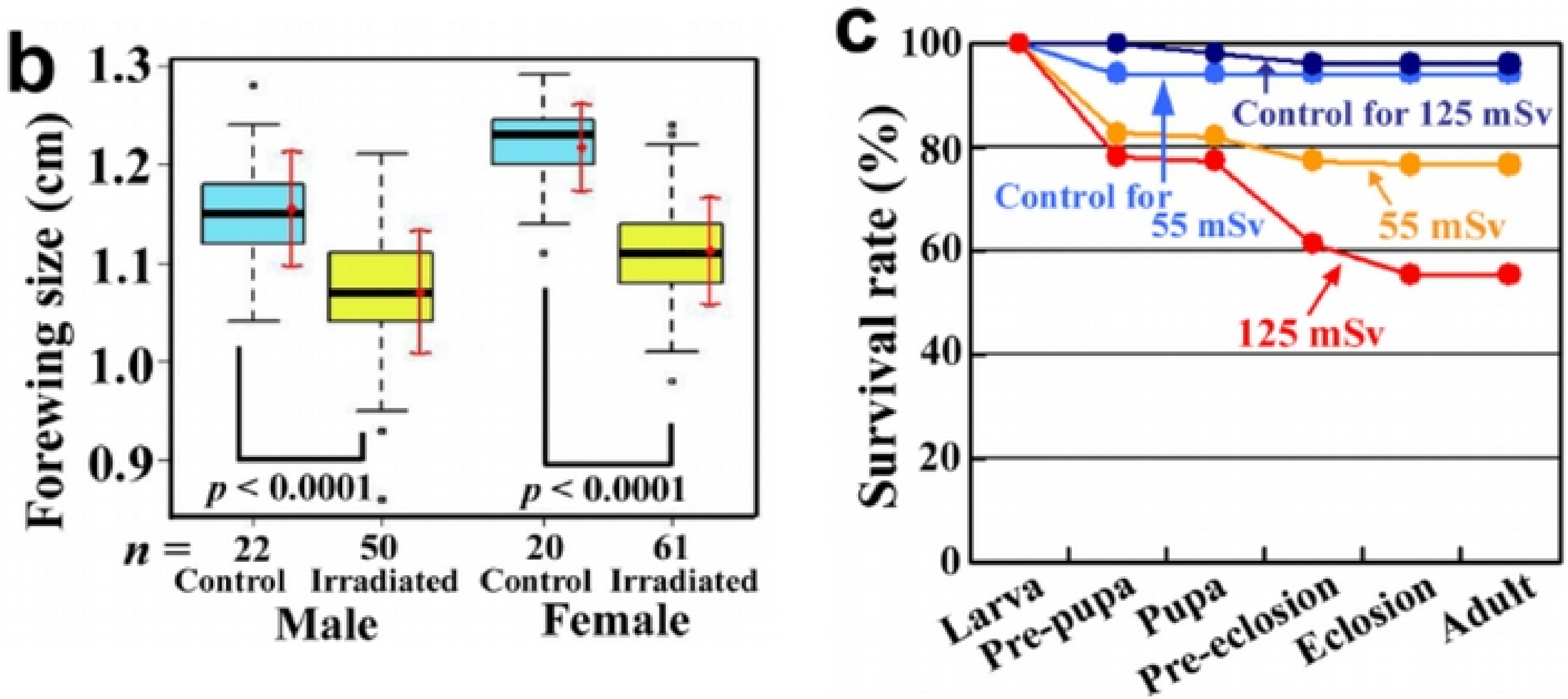
Collecting samples（ Butterflies ＆ Leaves ）

Abnormality rates in May and September 2011



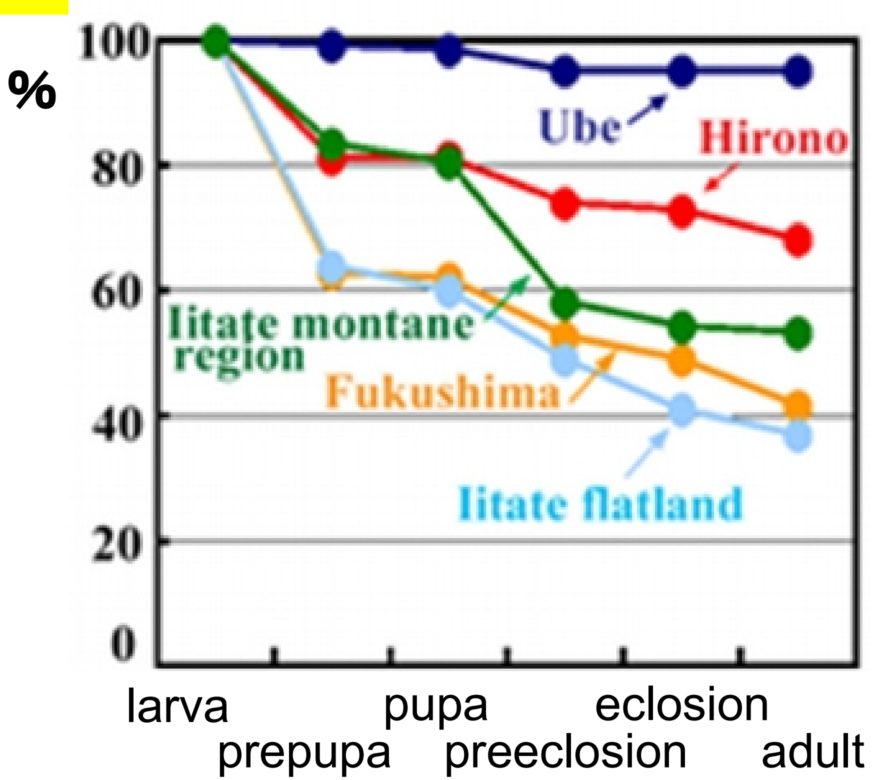
Normal abnormality rate in Japan is less than 10%.

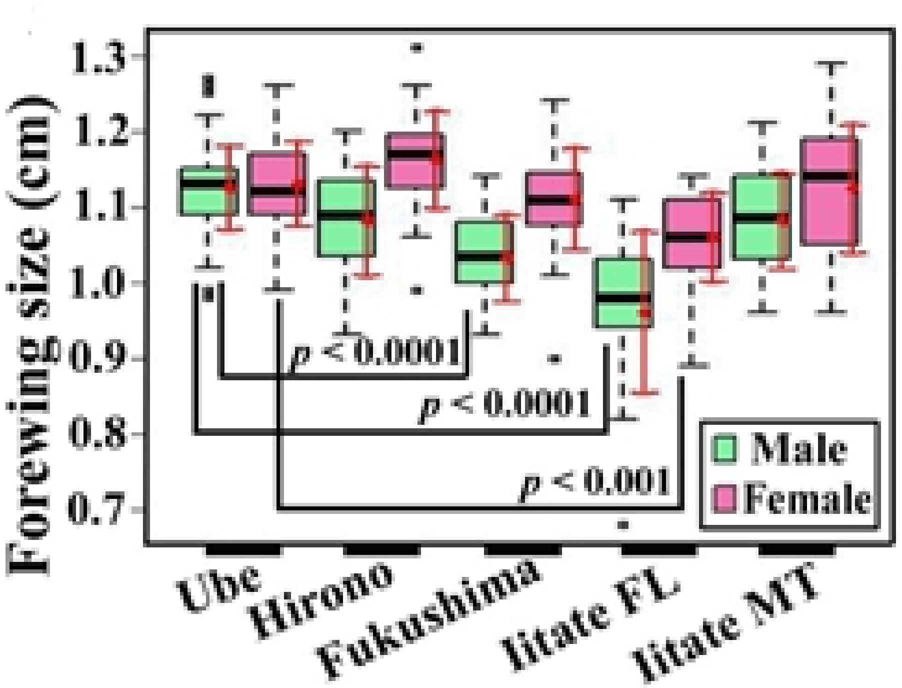
Forewing size and Survival curve
【 External irradiation 】



Field data were reproduced in laboratory.
Wing size reduction （ growth retardation ） and dose-dependent decrease of survival rates

Survival curve and Forewing size 【 Internal irradiation 】





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

	Distance from the NPP (km)	Ground (μSv/h)	β ray (cpm/kg dry)	^{137}Cs (Bq/kg dry)	^{134}Cs (Bq/kg dry)
Ube	966.0	0.18	6.33×10^{2}	5.3×10^{-1}	3.9×10^{-1}
Hirono	20.7	1.06	4.35×10^{3}	7.71×10^{2}	5.83×10^{2}
Fukushima	61.1	3.57	2.13×10^{4}	4.17×10^{3}	3.16×10^{3}
Iitate (Flatland)	40.0	6.22	2.73×10^{4}	5.42×10^{3}	4.07×10^{3}
Iitate (Montane)	33.7	18.86	7.61×10^{4}	2.31×10^{4}	1.75×10^{4}

Dry specimen measured using Ge semiconductor radiation detector

Summary 1

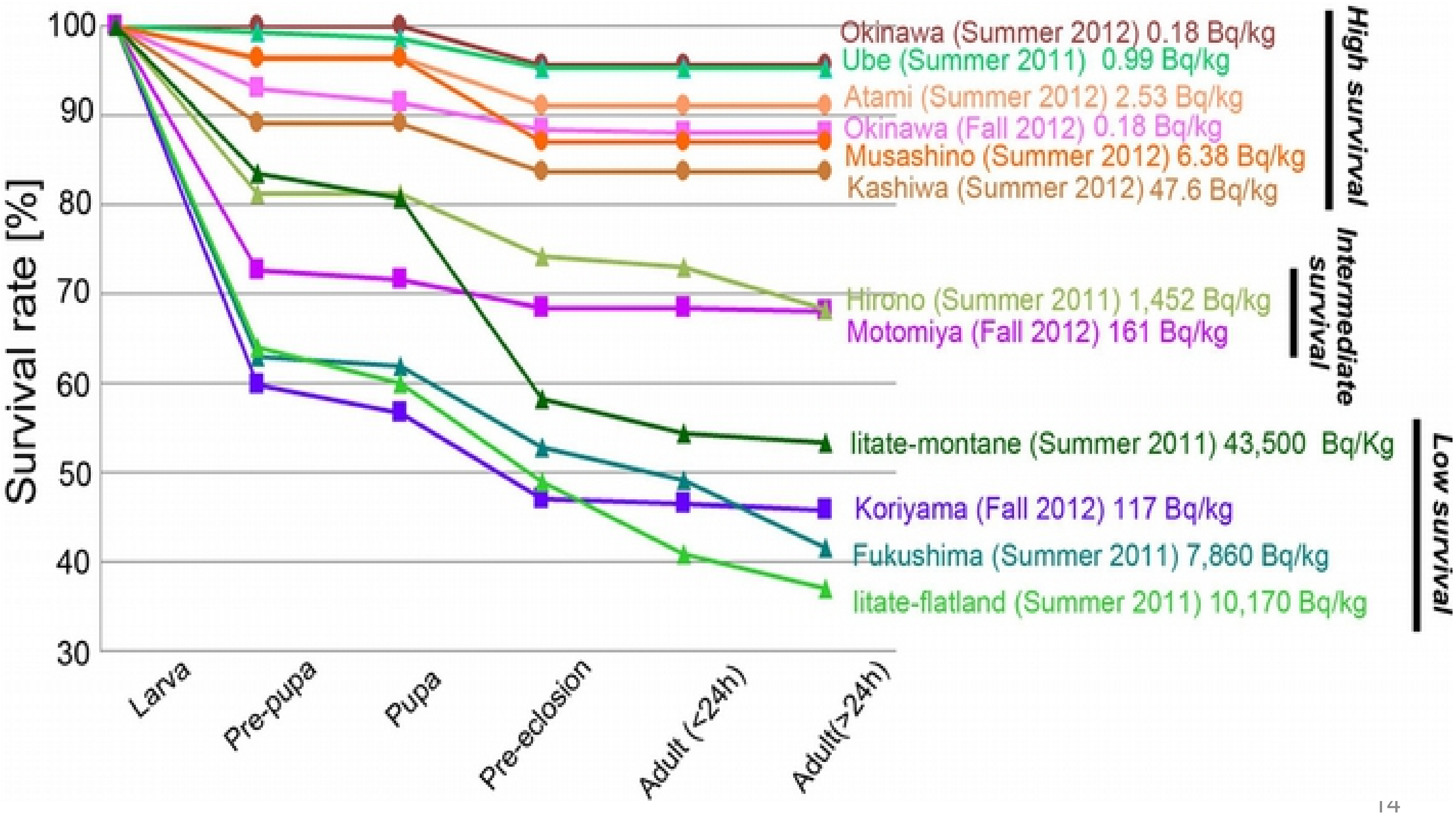
【 Field and reared samples 】

- Growth retardation and small forewing size
- Morphological abnormalities
- Increase of abrnormality rates（ from May [P, F1] to September [P, F1] ）
- Inheritance of adverse effects

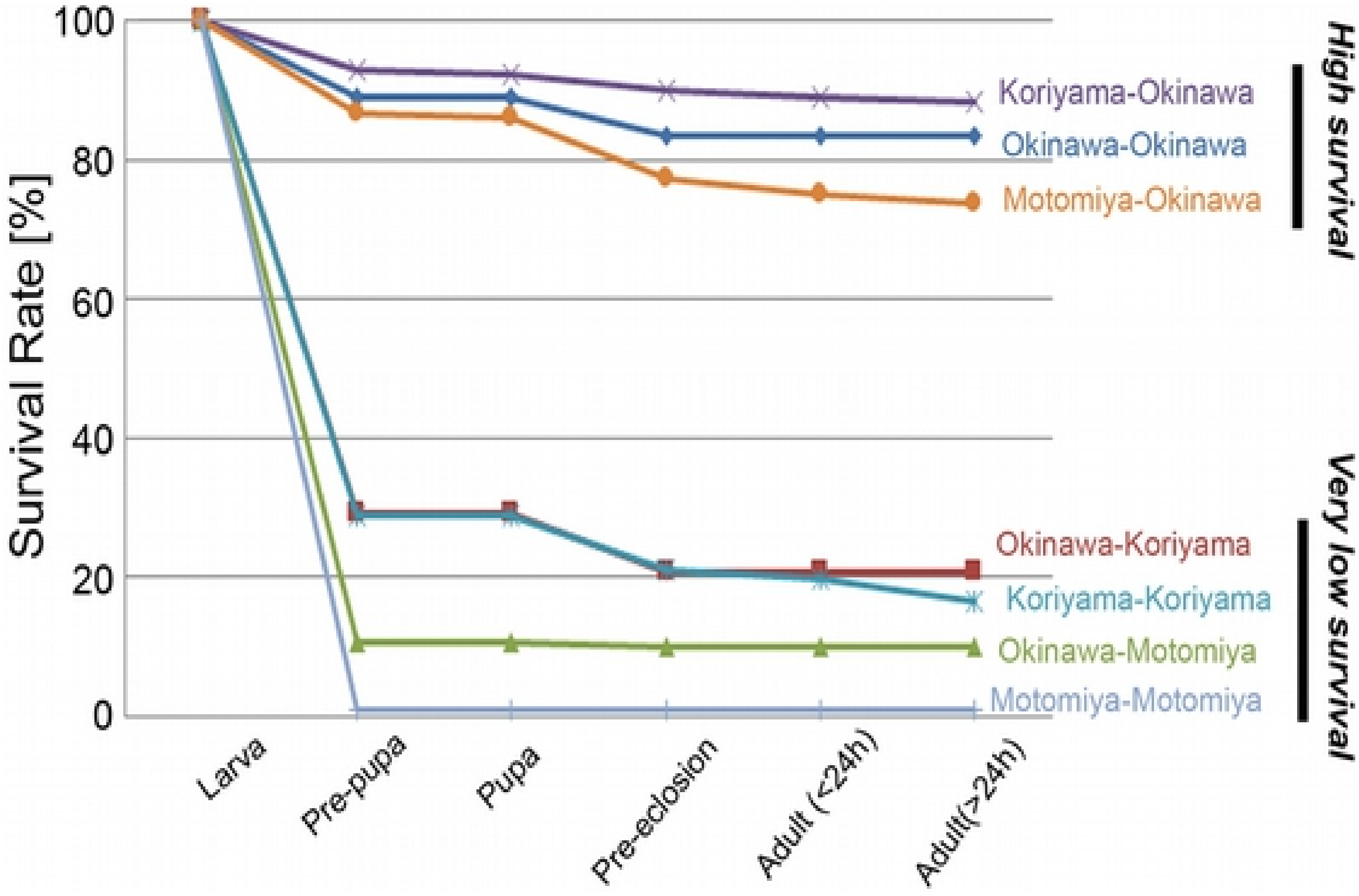
【 External and internal irradiation 】

- Dose-dependent decrease of survival rate
- Small forewing size
- Morphological abnormalities

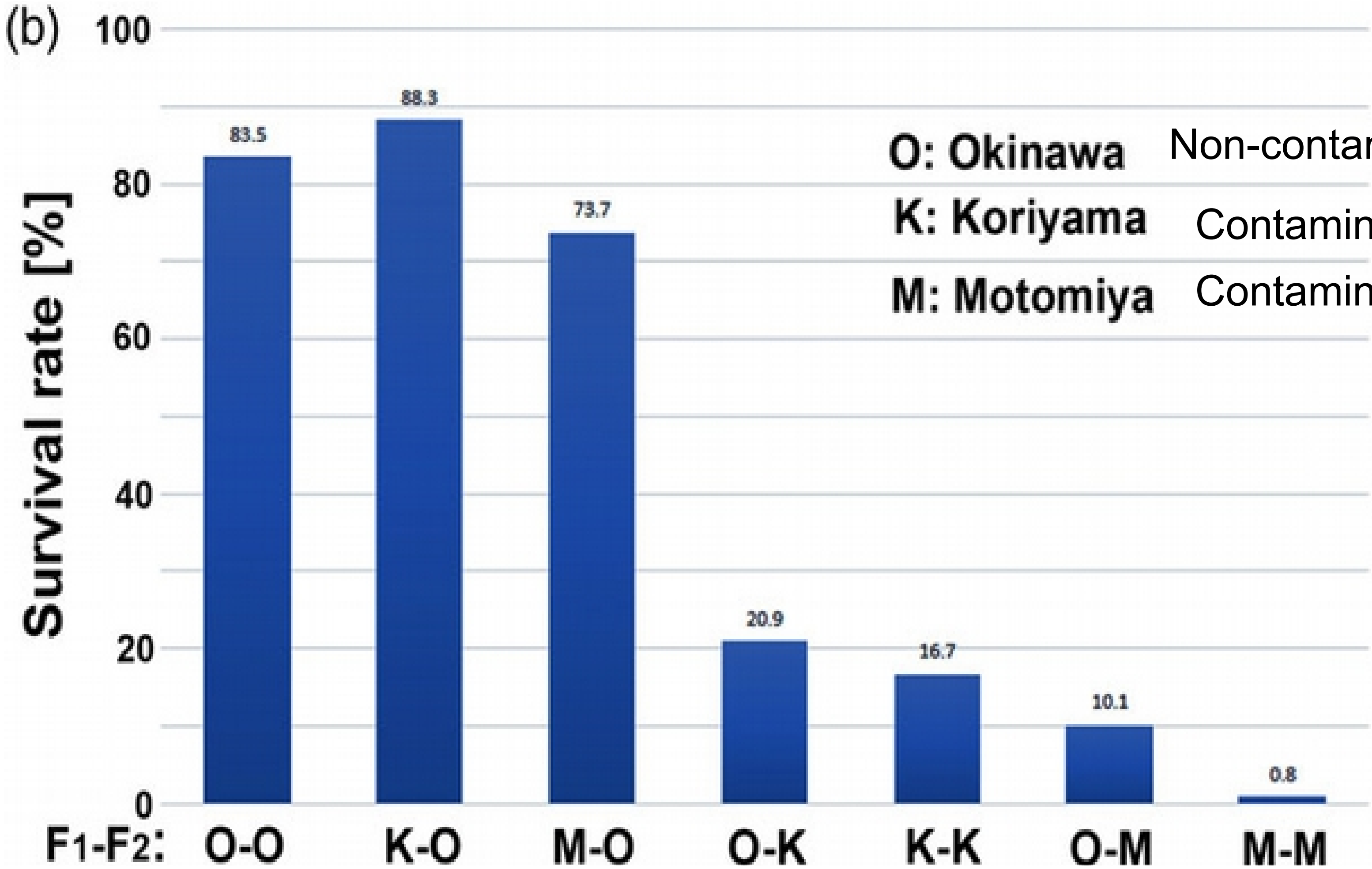
Further internal irradiation



Next generation



First-Second generations (Contaminated/Non-contaminated Food)



O: Okinawa Non-contamined
K: Koriyama Contaminated
M: Motomiya Contaminated

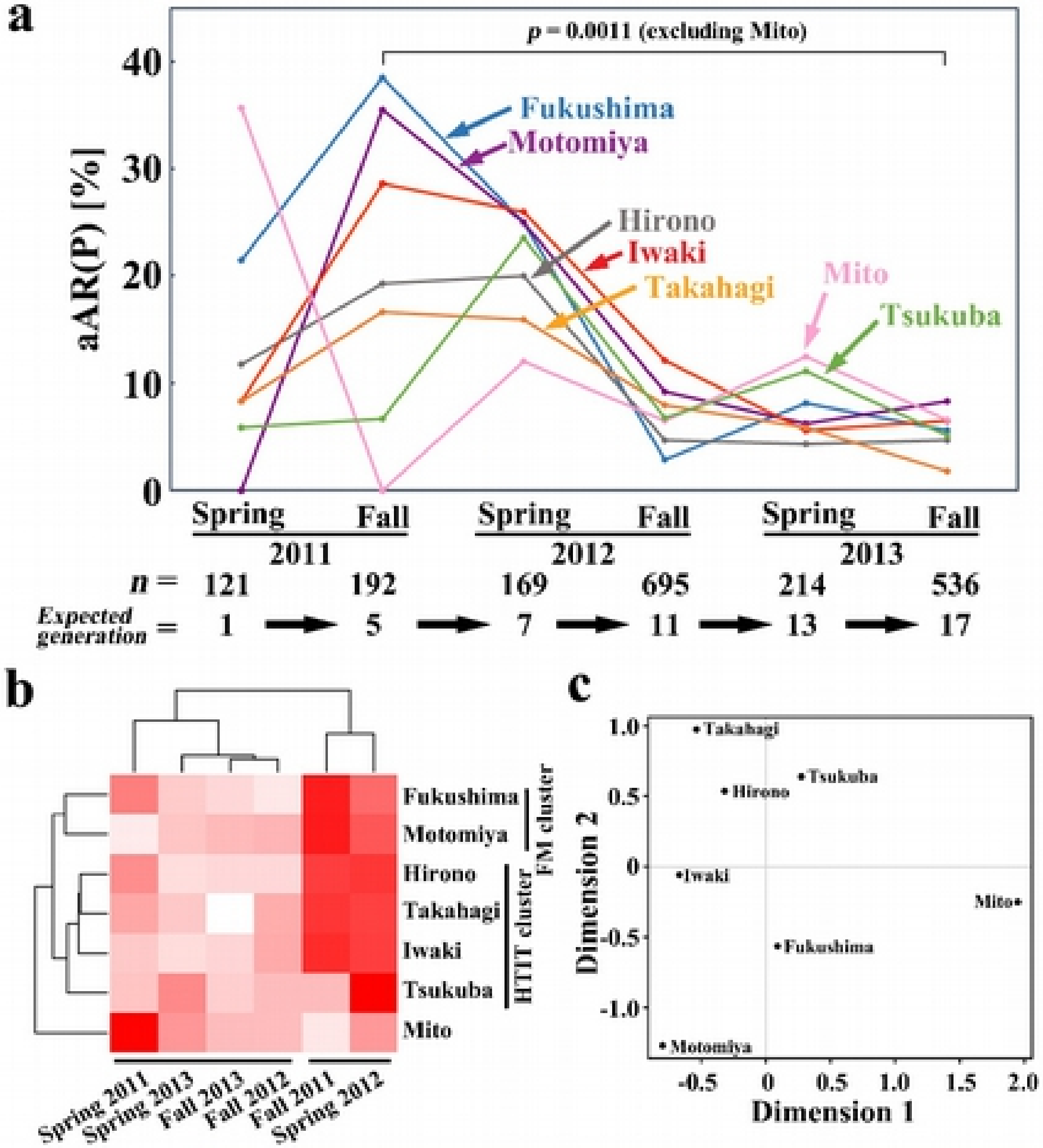
Cumulative dose and forewing size are correlated

r = -0.93
p = 0.0028

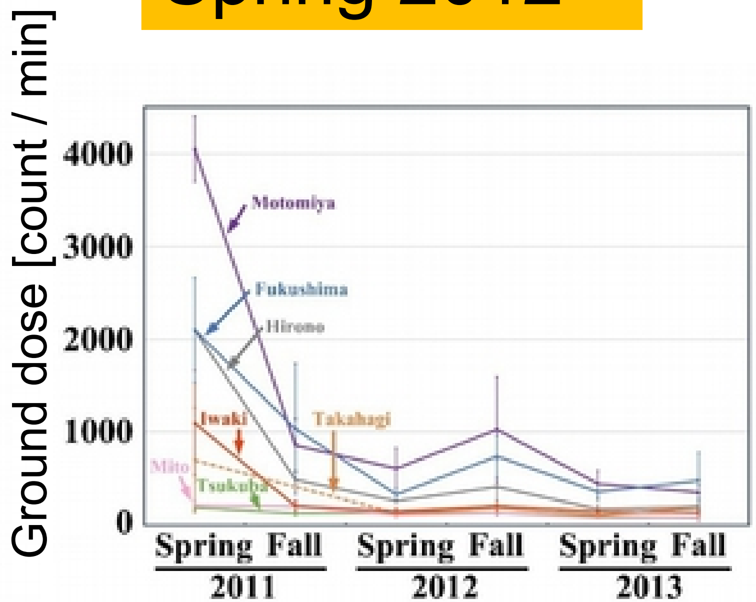
Summary 2

- 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)

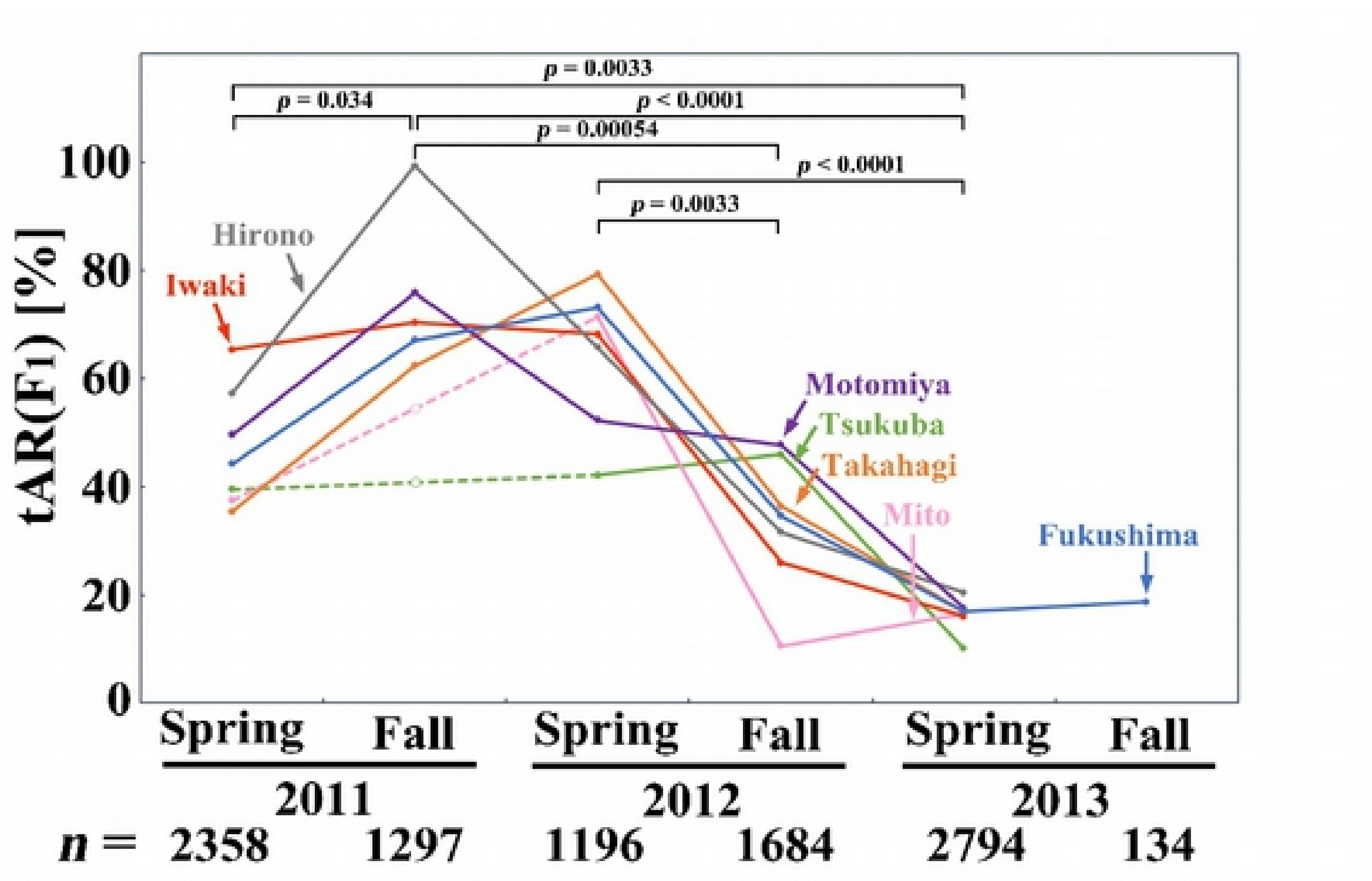


Fall 2011 and Spring 2012



(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 (5th and 7th generations).
- Decrease and leveling off to a normal level in 3 years (17th generations).
- This study is probably the most comprehensive record of biological dynamics after a nuclear accident available today.

21

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

SCIENTIFIC REPORTS

OPEN

The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly

SUBJECT AREAS:
ENVIRONMENTAL SCIENCES
ECOLOGY
BIODIVERSITY

Atsuki Hiyama[1]*, Chiyo Nohara[1]*, Seira Kinjo[1], Wataru Taira[1], Sinichi Gima[2], Akira Tanahara[2] & Joji M. Otaki[1]

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Hiyama *et al. BMC Evolutionary Biology* 2013, **13**:168
http://www.biomedcentral.com/1471-2148/13/168

BMC Evolutionary Biology

CORRESPONDENCE Open Access

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

SCIENTIFIC REPORTS

OPEN

The biological impacts of ingested radioactive materials on the pale grass blue butterfly

SUBJECT AREAS:
ENVIRONMENTAL SCIENCES
RISK FACTORS
ENVIRONMENTAL HEALTH

Chiyo Nohara[1], Atsuki Hiyama[1], Wataru Taira[1], Akira Tanahara[2] & Joji M. Otaki[1]

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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

Nohara *et al. BMC Evolutionary Biology* 2014, **14**:193
http//www.biomedcentral.com/1471-2148/14/193

BMC
Evolutionary Biology

RESEARCH ARTICLE Open Access

Ingestion of radioactively contaminated diets for two generations in the pale grass blue butterfly

Chiyo Nohara[1], Wataru Taira[1], Atsuki Hiyama[1], Akira Tanahara[2], Toshihiro Takatsuji[3] and Joji M Otaki[1*]

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.

Hiyama *et al. BMC Evolutionary Biology* (2015) 15:15
DOI 10.1186/s12862-015-0297-1

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

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.

Journal of Heredity 2014:105(5):710–722
doi:10.1093/jhered/esu013

Fukushima's Biological Impacts: The Case of the Pale Grass Blue Butterfly

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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-