

One World, One Health

「One World, One Health」の概念に基づき、生態系の健康に着目して、人の健康は全ての生き物や環境の健全性によって支えているのだということを、改めて認識し、今後の生き方（ライフスタイル）を模索する。

《主催》日本野生動物医学学会

《司会／座長》

「保全医学と生態系の健康」

村田浩一氏（日本大学生物資源科学部教授／よこはま動物園スーラシア園長）／日本野生動物医学学会会長

《演者》

「霊長類の自己治療行動―予防と治療」

ハフマン・マイケル氏（京都大学 霊長類研究所 准教授）

「蚊が運ぶ病気と生態系の構造」

津田良夫氏（国立感染症研究所 昆虫医科学研究室 室長）

「タニが語る生物多様性／寄生生物の進化的重要単位の意義」

五箇公一氏（独立行政法人 国立環境研究所）

Workshop V

ワークショップ V

“One World, One Health”

Based on the concept 'One World, One Health', we will focus on the health of the ecosystem, reconfirm that our health is supported by the health of all living creatures and the environment of this planet, and identify the ideal 'lifestyle' for the future.

Organizer: Japanese Society of Zoo and Wildlife Medicine

MC / Chairperson:

“Conservation Medicine and Ecological Health”

Koichi MURATA, PhD, Professor, College of Bioresource Sciences, Nihon University,
Director, Zoorasia Yokohama Zoological Gardens
President, Japanese Society of Zoo and Wildlife Medicine

Speakers:

“Self-Medication in Primates- Prevention and Cure”

Michael A. HUFFMAN, PhD, Associate Professor, Primate Research Institute, Kyoto University

“Structure of Animal Communities and Transmission Dynamics of Mosquito Borne Diseases”

Yoshio TSUDA, Chief, Laboratory of Taxonomy and Ecology, Department of Medical Entomology,
National Institute of Infectious Diseases (NIID)

“Mites Talk about Biodiversity – Ecological Significance of Evolutionarily Significant Units in Parasites.”

Koichi GOKA, PhD, Invasive Alien Species Research Team, National Institute for Environmental Studies

保全医学と生態系の健康

Conservation Medicine and Ecological Health

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多くの人は、保全医学（Conservation Medicine）という言葉が初めて耳にするかもしれない。それも当然のことで、本学問領域が産声を上げたのは1990年代後半であり、初の教科書とも言える会議報告書（“Conservation Medicine: Ecological Health in Practice”）が出版されたのは2000年代に入ってからである（Alonso et al., 2002）。しかし、本書の出版が大きな契機となり、保全医学に関する研究が先進諸国で急速に展開されている。

保全医学は、「ヒトの健康、動物の健康および生態系の健康に関わる研究分野を統合する学問領域」と定義されている（Tabor, 2002）。また、保全医学が目指しているのは、人間や家畜などの動物を縦割りのように区別した健康概念ではなく、それらの相互関係の中で維持される単一かつ横断的な健康、すなわち“One Health”であり、そ

のために必須となる生物多様性（Biodiversity）の保全である。本学問領域は、健康や医療に関する学問領域を連携させ、生態学的健康（Ecological Health もしくは Ecohealth）を維持するための学際的で実践的な研究分野と言える。

保全医学における感染症対策は、病原体と宿主を取り巻く有機的および無機的環境の相互関係を理解し、それらの微妙なバランスで成り立っている生態系を考慮して図られる。とくに新興感染症（Emerging Infectious Diseases: EID）については、その原因が野生動物の生息域への人間の侵入であり、そのことから生物多様性の維持が根源的な発生源予防につながることを強く認識している。

Many of you may never have heard the term “Conservation Medicine.” That is only natural because this field of study was only born in the late 1990's and the first conference report, which is referred to as the textbook for this new discipline (“Conservation Medicine: Ecological Health in Practice”) was only published in this decade (Alonso et al. 2002). However, inspired by the publication of the textbook, studies related to Conservation Medicine have been evolving rapidly in developed countries.

Conservation Medicine is defined as “a discipline that unites the fields of human health, animal health and ecosystem health” (Tabor, 2002). Conservation Medicine is not based on a health concept that posits human and animal in vertical divisions. It is aimed at horizontal health that can be sustained in interrelationship between humans, animals and the ecosystem, that is to say, “One Health” and conserving Biodiversity that is indispensable. This new field of study can be considered as very pragmatic and interdisciplinary in the way that it links related health and medical study fields and helps sustain the Ecological or Eco-Health.

In Conservation Medicine, countermeasures to control infectious diseases are designed based on an understanding of organic and inorganic host-pathogen interactions and the ecosystem built on their subtle balance.

Emerging Infectious Diseases (EID) are, in particular, caused by human intrusion into the habitats of wild animals and all the more, for this very reason, conservation of Biodiversity is strongly recognized as fundamental to the prevention of those diseases.

霊長類の自己治療行動—予防と治療

Self-Medication in Primates- Prevention and Cure

京都大学霊長類研究所 准教授・ハフマン A. マイケル

Michael A. Huffman, PhD, Associate Professor

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チンパンジーを初めとする野生霊長類は日常、栄養価に富んだ果実や葉、若い芽などを食べるが、それ以外に、特殊な二次代謝産物を含む多くの植物の部位を食べる。栄養的には乏しいと考えられるこれらの種あるいは部位の非栄養的採食意義に、ここ数年、興味をもたれ、その一つとして薬理的効果が指摘されている。更に、非栄養的なある種の植物を食べると、寄生虫感染症の制御や、その二次的病徴である腹痛の治療などに有効であるとする仮説が、アフリカの大型類人猿(チンパンジーとゴリラ)研究により実証されてきている。

すなわち、東アフリカのチンパンジーにおいて、強烈な苦味をもつ髄部液を摂取し、又は葉をそのまま呑み込むといった行動が生態学的・寄生虫学的解析から寄生虫感染症の軽減に役立っていることが知られている。寄生虫は多くの病気を誘発し、個体それ自身の行動や、繁殖能力にも影響を及ぼす。したがって、これらの悪影響を取り除くことは重要である。寄生虫感染症が宿主へ与える

Chimpanzees and other primates eat a variety of nutritious items including fruits, leaves, young buds on a daily basis. Besides these items, a number of plant parts containing peculiar secondary plant compounds are occasionally eaten. In the recent years, an interest has been shown in understanding the significance of ingesting these nutritionally deplete plant items. One explanation is that these items are used for their pharmacologically active properties. Strong evidence in support of this hypothesis has come from research on African great apes (chimpanzees and gorillas) demonstrating anti-parasitic properties and or relief from parasite related gastro-intestinal upset.

From ecological and parasitological analysis of the behavior of chimpanzees in East Africa it has been shown that the ingestion of extremely bitter juices from the pith of plants or the swallowing of the rough leaves of other species plays an important role in the suppression of parasite infections. Parasites are responsible for a variety of diseases that directly affect the behavior and reproductive capacity of animals. Therefore, it is extremely important that animals should be able to eliminate parasites. It is

影響や、感染した際の宿主への反応は、長い進化の過程で培われてきた産物であることは間違いない。アフリカの大型類人猿についての最近の研究は、偶然ではなく、薬効を期待してある種の植物を積極的に摂取していることが示唆されている。一方、マハレ山塊国立公園周辺では、ヒトとチンパンジーでよく似た病徴を示す疾病に対し、同じ植物を選択することが知られている。この事実から、両者が系統的にもっとも近縁であるため、又は人間が伝統的に動物の行動を観察することによって、新しい「薬」を得てきたという2つの説明ができる。

アフリカの大型類人猿やその他の野生霊長類の自己治療研究には、ヒト、家畜、飼育動物などの寄生虫感染症を効果的に治療することに対する天然物の有効利用や新しい治療方法の提供についての期待を抱かせるものである。

evident that the effects caused by parasites on the host, and the hosts' responses to infection are the results of a long evolutionary process. Research has also shown that African great apes do not accidentally ingest these items, but seek these plants out for the curative properties. Interestingly, humans and chimpanzees living in and around the Mahale Mountains National Park of Tanzania select many of the same medicinal plants for use when suffering from similar ailments. From this and other evidence, two explanations can be given. Firstly, they show similar behaviors because they are phylogenetically very close and secondly, humans have traditionally observed the habits of wild animals to obtain valuable new medicines for their own use.

There is hope that the study of self-medicative behaviors in the African great apes and other primates in the wild will provide new methods of healing and new medicinal plants effective in the treatment of the parasite disease in humans, livestock and other domestic animals.

蚊が運ぶ病気と生態系の構造

Structure of Animal Communities and Transmission Dynamics of Mosquito Borne Diseases



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蚊は生態系の分解者として、また天敵生物の食物源として生態系を支えているが、他の昆虫にはないユニークな役割を演じている。蚊は吸血習性を持つことで有名である。わが国には 100 種あまりの蚊が生息しているが、このうち 60 種ほどの蚊が人を吸血する性質を持っていると言われる。人を刺さない蚊の中には、大型の哺乳類を好んで吸う種類、野鳥を好んで吸う種類、カエルを吸う種類や、魚を吸う種類なども報告されている。蚊が持つユニークな役割は、この吸血習性に関係している。動物の病気の中には蚊によって伝播されるものがある。これは、病気に罹った動物の血を吸った蚊が、病原体を血液とともに体内に取り込み、別の健康な動物を吸血するときにその病原体を注入することによって起こる。

蚊が病気をうつす力は個体群の形質のひとつで、いくつかの生物学的、生態学的性質によって決定されている（例えば、病原体との親和性、吸血源動物嗜好性、成虫

密度、寿命など）。これらの性質は環境条件に影響されるため、蚊の病気伝播力も時間的に変動する。蚊がうつす病気の流行が、特に媒介蚊の吸血パターンに大きく依存しているという研究例が、いくつか報告されている。

蚊の吸血パターンと病気流行の関係を考えるとき、蚊が病原体の宿主になる動物から吸血することと、宿主ではない動物から吸血することを区別することが重要である。動物群集に病原体の宿主動物が多数含まれるとき、蚊と宿主動物の接触は頻繁になり、病原体の受け渡しが容易で病気の流行も盛んになる。逆に宿主動物が少ない動物群集では、蚊が宿主でない動物を吸血することが頻繁になり、病原体の受け渡しの機会が減って病気の流行が抑えられる可能性がある。このような考えから、生物多様性と病気流行の関係に関する研究も進められている。

Mosquito larvae work as scavengers and decompose organic matters in aquatic ecosystems. They are important food resources supporting predator populations in aquatic communities together with other aquatic insects, such as Chironomids, midges, etc. The adult mosquitoes also provide a food source for wild birds, bats, spiders, and ants, among others. Therefore, mosquitoes are an important member of aquatic ecosystems and help maintain the structure and species diversity of animal communities.

Mosquitoes are well known for their blood feeding habits and have unique roles within animal communities. More than 100 mosquito species live in Japan and about 60 of them bite human beings. There are mosquitoes that prefer to feed on wild birds, or on larger mammals, or frogs, even fishes. It is through their blood feeding habits that some mosquito species transmit infectious disease to humans as well as wild animals. Females that have fed on infected animals ingest the pathogen with the blood meal, and transmit it to uninfected animals. The capacity of mosquitoes to transmit pathogens is determined by several biological and ecological characters, such as vector competence, host preference, biting density, feeding pattern,

and longevity. The transmission dynamics of the pathogens in animal communities depends largely on the feeding pattern of vector mosquitoes in the communities.

It is important to distinguish the amplifying- and the diluting-vector-animal contacts in order to predict the epidemic of mosquito borne diseases in animal communities. When mosquito borne pathogens are introduced into communities composed of many pathogen hosting animals, vector mosquitoes have a high chance of taking blood meals from host-animals and thus, the amplifying-vector-animal contacts are frequent. Conversely, in animal communities where host animals are rare, a large proportion of vector mosquitoes feed on non pathogen hosting animals, the diluting-vector-animal contacts occurs frequently and thus, the pathogen transmission rate is low. Therefore, we can expect some relationships between community structure and transmission dynamics of mosquito borne diseases within animal communities. Theoretical as well as empirical ecological studies have been conducted to demonstrate the relationships between species diversity and transmission of vector borne diseases.

ダニが語る生物多様性～寄生生物の進化的重要単位の意義～

Mites Talk about Biodiversity - Ecological Significance of Evolutionarily Significant Units in Parasites.



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生物多様性の保全が唱われて久しいが、生物多様性を語る上で対象となる生物は、目で見て分かる、美しい、かわいらしい、あるいは格好がいい動物や植物が主流を占める。しかし、目に見えない、(一般には) 美しくない、微小な生物達も立派な生物多様性の構成員であり、重要な生態系機能を担っている。特に、寄生生物は宿主生物との間に軍拡競争的共進化をもたらし、進化や種分化の強力な駆動力として、生物多様性の創成において重要な役割を果たしている。しかし、現在の人間活動による生物の生息地の破壊や生物の人為的移送は、宿主-寄生生物間における共進化の歴史を崩壊させ、寄生生物の感染爆発というかたちで生態系や人間社会に脅威をもたらしている。

近年、問題とされる新興感染症や再興感染症も、それらの病原体生物の住処である野生生物を、人間がハビタットごと減少させたことにより、病原体微生物が生き残り

をかけて、人間への宿主転換を図った結果と考えられる。寄生生物の多様性および宿主との共進化の歴史を知るとは、寄生生物との共生関係を維持する上でも重要な知見となる。

ダニは Macro-Parasite (目で見える寄生生物) 群の中で代表的な種群であり、多くの人是不快なイメージしか抱かない生物であるが、その種数は分かっているだけで約 5 万種、未記載種を含めると 100 万種を超えともいわれ、その生活史も生息場所も多岐に渡る。自由生活者、寄生者、捕食者など、彼らもまた生態系システムの中で重要な役割を果たしており、立派な生物多様性の要因である。本講演では、ダニ学者でもある講演者自身のこれまでの研究成果を交えながら、寄生性ダニと宿主生物の間に繰り広げられる共進化の世界を紹介し、ミクロな生物多様性の重要性について考察をしたい。

Biodiversity conservation has recently become a boom field within environmental science. When we talk about biodiversity people usually only focus on the visible, beautiful, pretty or stylish animals and plants. But obviously the less beautiful microorganisms also have membership within biodiversity and play important roles in ecological systems. Notably, parasitic organisms have served as powerful causative agents behind evolution and wildlife speciation through a co-evolution arms race between parasites and host species. However, habitat destruction and biological invasion by large-scale modern day human activity has caused the historical co-evolution of host and parasite to collapse resulting in parasite pandemics. Furthermore, some of the recent emerging and reemerging disease problems are considered to have resulted from host switching by infectious viruses. These viruses once only co-habited, in symbiosis, with certain specific wildlife hosts but they have since lost their usual habitat due to human activity. Maintenance of the symbiotic relationship between human and parasites must be an important process

for understanding the biodiversity of parasites and co-evolutionary history between hosts and parasites.

The Acarian is a representative species group within macro-parasites (visible parasites). While people almost always have an unpleasant image of this species group, the varieties already recorded within the group approximate to a total of 50,000, and the actual total number, including non-recorded species, may exceed 1,000,000. Furthermore the life history and habitats of all these species ranges widely over free living, parasite, predator and so on. So, we can say that acarians are really important within biodiversity. In this workshop I will introduce, as an acarologist, the world of co-evolution produced by parasitic mites and their host species, and discuss about the significance of miniature organism biodiversity.



WS5-Record

WS5- 記録集

保全医学と生態系の健康

Conservation Medicine and Ecological Health

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Hello everyone. I am Koichi Murata, President of the Japanese Society of Zoo and Wildlife Medicine, and I will be acting as the coordinator of today's symposium.

Let me begin by saying a big "thank you" to all of you for coming. It is wonderful to see so many people here despite it being a cold Sunday morning.

First of all, I would like to speak to you about the purpose and intent of this symposium project. The theme of the present symposium is "One World, One Health." In other words, conservation medicine is an academic and research discipline aimed at the concept that the entire earth has its own health.

Now, what does conservation medicine actually consist of? In all probability, most of you here are hearing the term "conservation medicine" for the first time. That is reasonable and totally understandable because the discipline of conservation medicine was not pioneered here in Japan but in North America, where it was first established just 15 years ago in 1997.

This slide shows how conservation medicine is aimed at achieving human health, animal health and ecosystem health in a comprehensive manner that addresses the relationship between all three. Put simply, the concept of health does not exist individually and separately in people, animals or ecologically, but stems from their mutual relationships. This is a quite radical and essential definition of health.

Conservation medicine, which focuses on addressing these issues, is an idea that at heart pursues ecological

health. It is an interdisciplinary approach that reaches into a variety of research fields including political and social issues. It involves NPOs and ordinary citizens rather than scholars, per se.

The core aim and target of conservation medicine is the protection and preservation of biodiversity because maintaining biodiversity is extremely important. I'm sure you have all had many opportunities to hear the word "biodiversity". The mass media takes up the subject frequently and in 2010 an international conference called the 'COP 10 (The 10th Conference of the Parties to the Convention on Biological Diversity)' was held at Nagoya. But what exactly is biodiversity? And why is it so important? When we ask these questions again, we see that there are some areas that remain unclear.

As this figure indicates, the global environment is facing many threats at present. The red marks show the causes of this critical situation. Among the critical factors harming the Earth that we hear about often are climate change (or global warming), the eutrophication of lakes, and the problem of acid rain. But in fact it is the disappearance of biodiversity itself which is considered to be the single biggest risk to the Earth at present. The entire global ecosystem itself may be endangered if that risk continues to increase. Actually, biodiversity is considered to have a greater effect on the global environment than is usually stated in the mass media.

So what is the significance or meaning of protecting biodiversity? Up until now, various researchers and

academics have tried to explain why it is so important. A great many theories have been postulated and, among them, the idea put forward by Stanford University researcher Dr. Gretchen C. Daily of “ecosystem services” is considered particularly important. The idea behind ecosystem services is that protecting and preserving biodiversity can yield benefits for people. Accordingly, we must preserve biodiversity sustainably so that people can continue to enjoy the benefits of biodiversity. Personally I don't like this idea very much because it is rather anthropocentric but, as a theory, it has the merit of being very easy to understand and is useful for providing a rationale for why biodiversity should be preserved.

These ecosystem services can be grouped into several categories. For example, there are so-called “cultural services,” so-called “conservation services,” and what can be termed “supply” services. Ecosystems are considered to provide various services that are beneficial to people, and these services are related to each other. Among them are so-called “regulating services”. These include such things as flood regulation, weather regulation, and disease regulation. Or in other words, by conserving biodiversity we can enjoy the benefit of controlling diseases. So from this point of view, biodiversity is very important.

Now I would like to speak a little more on the subject of disease regulation, which is one of the points we will be discussing during this symposium. In the context of disease regulation there is a phenomenon called the ‘dilution effect’. What this means is that biodiversity has the effect of diluting the incidence of disease. First proposed by the ecological researchers Ostfeld and Keesing, this is now a fairly well understood and accepted theory that mainly deals with the relationship between the amount of biodiversity and the prevalence of vector-borne diseases carried by arthropods such as ticks and mosquitoes.

I would like at this point to explain the dilution effect in some detail. The main idea is that certain kinds of diseases are kept under control where there is sufficient biodiversity, such as in environments where many

species coexist. A specialized book has been written on the subject, as is shown on the slide. In illustrating the dilution effect, Keesing took up the subject of Lyme disease, an infectious disease transmitted by arthropods. Lyme disease is a zoonosis, meaning that it is a disease transmitted between animals and humans. It is actually a bacterial infection commonly spread by ticks. The disease-causing agents are bacteria belonging to the genus *Borrelia*. Essentially, the disease is maintained through a relationship linking the disease-causing bacteria, ticks and wild animals. From time to time, people also become infected. The disease causes a distinctive circular skin rash (known as erythema migrans), as well as flu-like symptoms such as fever. Lyme disease is not life threatening although, if treatment is delayed, symptoms can become severe. However it is not something we need to fear very much.

These days however, the incidence of people infected by Lyme disease is spreading worldwide. In particular, many cases of infections are reported among urban dwellers who go camping or pursue other outdoor activities in the countryside.

In a short while I would like to talk in a little more detail about the connection between Lyme disease and the preservation of biodiversity. But before that, I will try to give a simple description of the life cycle of ticks. Near the end of this symposium, I believe Dr. Goka of the National Institute for Environmental Studies will be talking about ticks. But in simple terms, ticks grow through the larval and nymph stages by sucking the blood of many kinds of vertebrate animals, and then, when they reach the adult stage they lay eggs. That's their life cycle in a nutshell.

To sustain their life, ticks require nutrition in the form of blood from mammals or birds. However, they don't draw this blood from pre-selected hosts, but rather, they encounter a host animal at random and then begin sucking its blood. They may end up drinking the blood of an animal whose blood is unpalatable to them and if the blood is unpalatable they may die, (or at least their growth may be retarded).

In the case of Lyme disease, it turns out that ticks harboring the bacteria that cause Lyme disease are particularly fond of the blood of mice. It is well understood that if they get the opportunity to drink mouse blood, they grow very well and tend to proliferate. This is a key point. For instance, let's take a look at an environment model. There are lots of animals here. This environment has lots of different kinds of animals that ticks feed on, and within it there are some mice. But there are also animals not favored by the ticks that transmit Lyme disease, such as birds and skunks. For some reason the blood of skunks appears to be particularly unpalatable. Ticks feed on the blood of individual animals at random so in this environment their chances of encountering a mouse are extremely low. As a result, their rates of growth and proliferation are not very high. But on the other hand, within a different environment where biodiversity is extremely low, (such as, for example, an environment in which most animals are mice, with only the occasional raccoon - a foreign exotic species), then most ticks will encounter mice, their favorite food source, and so their rates of growth and proliferation will be high.

Keesing and his fellow researchers conducted field studies to investigate how Lyme disease is actually propagated in a species-rich environment. In this figure, the horizontal axis shows the species diversity and the ratio of ticks infected with Lyme disease is plotted along the vertical axis. The environment on the left is extremely simple with impoverished biodiversity and with hardly any mammals apart from mice. When ticks obtained from this environment were examined, a large number of *Borrelia* bacteria, the pathogen causing Lyme disease, were found on the ticks' bodies. But when the same kind of survey was carried out on ticks from within a biodiversity-rich environment, only a very few were found to be carrying *Borrelia*.

Naturally, there are counterarguments. But on the other hand, there are reports that a similar dilution effect can be observed in the case of West Nile virus and other viral diseases such as the Sin Nombre virus. I'm sure you have all heard of the term "emerging infectious diseases." These are infectious diseases that have

recently begun to spread in the human world, and in most cases emerging infectious diseases are zoonoses. A zoonosis occurs when a disease-causing agent that is present in one or more species of animals newly crosses over to infect and cause disease in humans. As this map shows, most zoonoses originate in tropical regions such as Africa, South America and Southeast Asia.

The reason is that originally in such areas, wild animals, pathogens and vectors had been living quietly together in a delicate balance, but that balance became destroyed because of developments such as agriculture. The collapse of the relationships between wild animals, pathogens and vectors allows new infections to spread to the people and livestock that colonized the areas. This map suggests that perhaps airplanes and ships carry diseases that occur in such places to industrialized countries such as the nations of North America, Europe and Japan where they can spread further.

On the other hand, there are some areas that are known as biodiversity hotspots. This slide shows the distribution of rare animals, endangered species and species indigenous to only a small area. Actually, many rare species of wildlife are found where biodiversity is abundant and the environment is rich. The places marked in red on the map are such hotspots and they actually overlap quite well with the places I showed earlier where new infectious diseases emerge.

As you are such an intelligent audience, I am sure you have understood what I've said up to now. Although the wild animals and pathogens living in these biodiversity hotspots affect each other and the balance between them is delicate, under natural conditions things tend to work out somehow and diseases do not spread very much. But when people go into such places and exert an influence on the environment or damage it with development, this balance can collapse. And when the balance collapses, the pathogens can exert an influence on livestock or people. In other words, we can say that by damaging biodiversity, people unleash a process of destruction that comes back to people again.

I've been talking about ecosystem services so far, but more fundamentally, unless people establish a better relationship with pathogens, vectors and wild animals, in my opinion we will not be able to maintain the earth's ecological health. This is one of the major reasons why we planned this symposium at this time.

So, in order to spread understanding of this purpose more widely, I have requested three lecturers to give talks on the subject.

The first lecturer is Prof. Michael Huffman, an associate professor at the Primate Research Institute, Kyoto University. Prof. Huffman has studied self-medication by primates, and specifically by the great apes. He has observed that apes control their own bodies, which means they can maintain their own health, heal themselves when they become ill, and even prevent illnesses from arising in the first place. From before, I have read Prof. Huffman's papers and specialized books with interest. I've invited him to talk to us today because I thought it would be a good opportunity for us to learn about the intelligence of apes and about their lifestyles, which are adapted successfully to the environment.

Our second lecturer today is Prof. Yoshio Tsuda, a researcher at the National Institute of Infectious Diseases. Prof. Tsuda is proceeding with his research from a stance of looking at disease from the perspective of mosquito biology rather than that of the relationship between mosquitoes and infectious disease. When you think of mosquitoes, you may only be thinking about how to squash them, but I think that if there is an environment in which mosquitoes can't live, then perhaps it is an environment where people can't live either. In this sense, I would like us all to learn how skillfully mosquitoes are engaged in their environment and how important an element of the environment they are.

Dr. Koichi Goka, who is also a researcher at the National Institute of Infectious Diseases, will give today's last lecture. Dr. Goka is now working mainly on the control of invasive alien species. However, originally he was

researching tick damage to agriculture. Ticks are also very important organisms in the environment, and probably the soil cannot be kept in a healthy condition without their help. This means that environments without ticks are environments where people cannot live either. I would like everyone to realize this, and that is why I have invited Dr. Goka to speak to us.

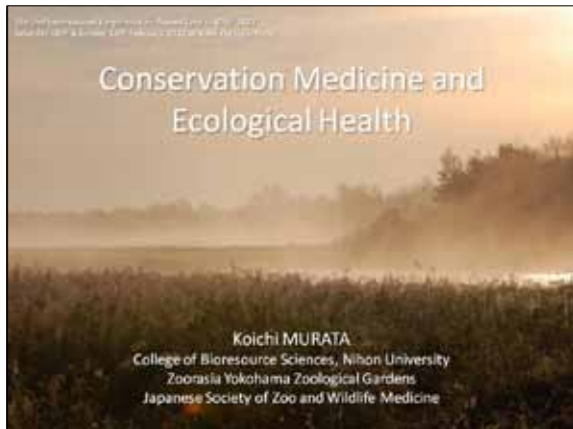
Now, I'd like to tell you about the relationships I have with these three lecturers.

As for Prof. Huffman, although I have known his name for a long time, as I mentioned I first became acquainted with him in a research context at an international conference held several years ago on the non-human primate malaria parasite *Plasmodium*. At that time, Prof. Huffman was also working as a coordinator of the International Primatological Society (IPS). In spite of his busy schedule, he participated in the primate malaria conference, where he delivered a lecture. Perhaps because he was tired after that, I remember he slept deeply during the conference.

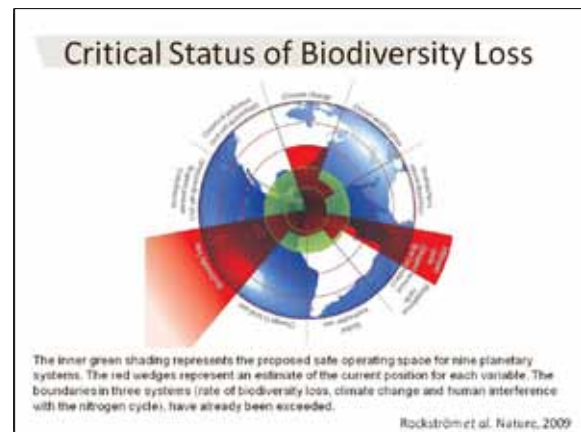
I met Prof. Tsuda for the first time five or six years ago. Although my specialty was avian malaria, I was almost totally ignorant about mosquitoes. I wanted to develop avian malaria research in some way by obtaining Prof. Tsuda's cooperation, so I have accompanied him on fieldwork across Japan and carried out research with him as his co-researcher. Even today I receive guidance from him about mosquito biology, behavior and classification.

I have also been acquainted with Dr. Goka's name for many years. Perhaps you too are aware of his activities through the mass media including TV. Although he is extremely busy with his research, he actively participates in drinking sessions outside of research hours and he has developed a habit of taking photos with his students. These are posted on Facebook. I introduced one of these photos earlier showing him and me together. At this symposium too, he has been taking photos with many people, and these will also be posted on Facebook.

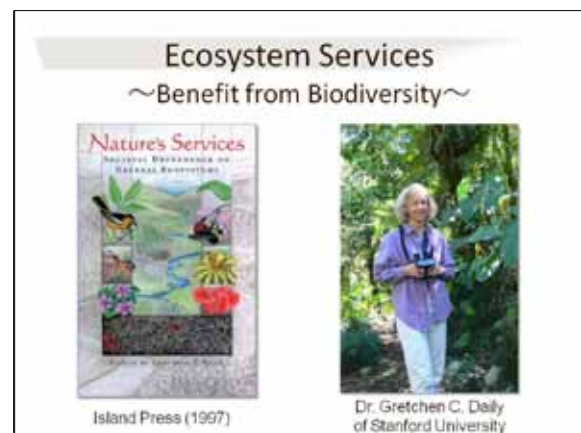
Now it is time to hear the lectures from these three academics, which is the original purpose of this symposium. First, Prof. Michael Huffman, please start your talk now. I invite you all to enjoy his lecture.



【Slide 1】



【Slide 4】



【Slide 5】

What is Conservation Medicine?

History

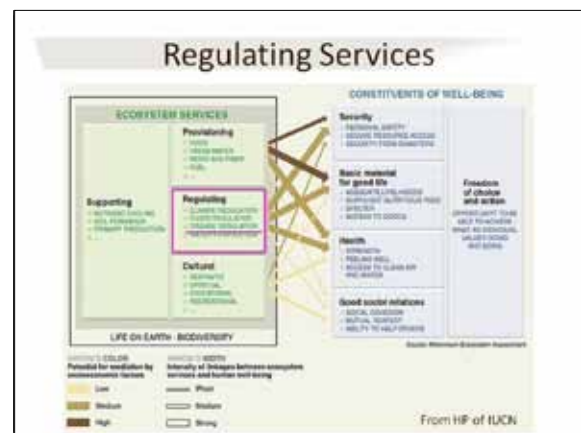
- The term of "Conservation Medicine" primarily appeared in 1997.
 - Petras, M., Tabac, G., Pearl, M., Sherman, D., Epstein, E. Conservation Medicine: An Emerging Field. In Raven, PH, (ed.), Nature and Human Society: The Quest for a Sustainable World, pp. 553-556. National Academy Press, Washington, D.C., 1997.
 - "Conservation Medicine: Ecological Health in Practice (2002)" the proceeding of the first meeting

Definition

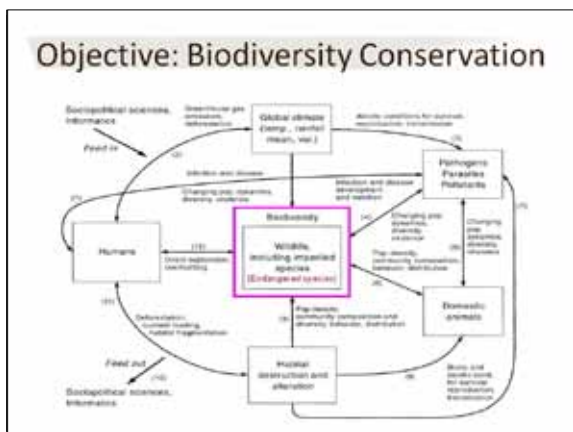
- "Conceptually, conservation medicine is at the **nexus of the fields of human health, animal health, and ecosystem health.**" (Tabac, 2002)

☞ nexus: relationship

【Slide 2】



【Slide 6】



【Slide 3】

Disease Regulation

Dilution Effect by Biodiversity

- Relationship between Vector-borne diseases and Biodiversity
- Species richness will reduce infectious diseases caused by arthropod-transmitted pathogens
- Ostfeld & Keesing. Biodiversity and Disease Risk: the Case of Lyme Disease. Conservation Biology 2001.

【Slide 7】

Dilution Effect of Lyme Disease

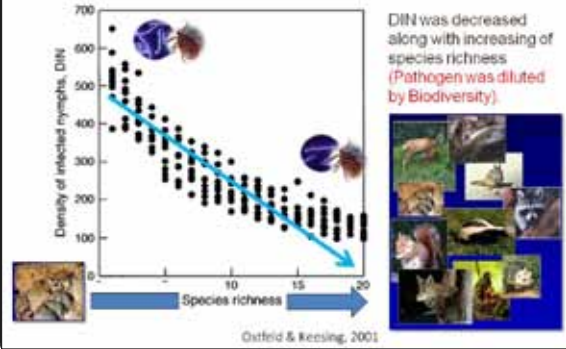
Lyme Disease as Zoonosis

- ✓ Infectious tick-borne disease caused by bacteria belonging to the genus *Borrelia*
- ✓ Fever, headache, depression, and a characteristic circular skin rash called erythema migrans (EM)
- ✓ Delayed or inadequate treatment can lead to the serious symptoms



【Slide 8】

Species Richness & DIN



【Slide 12】

Tick Life Cycle & Hosts



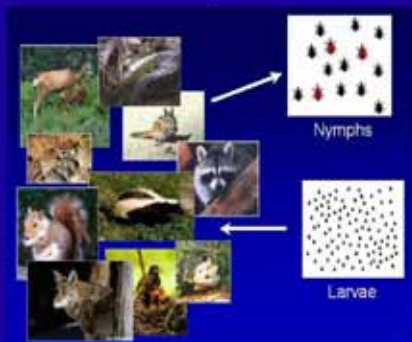
【Slide 9】

Global Distribution of EID



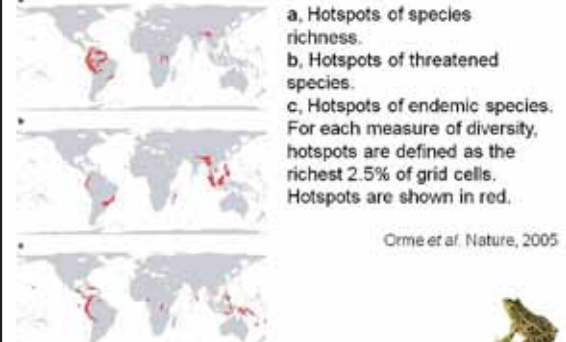
【Slide 13】

Habitat of Species-Rich



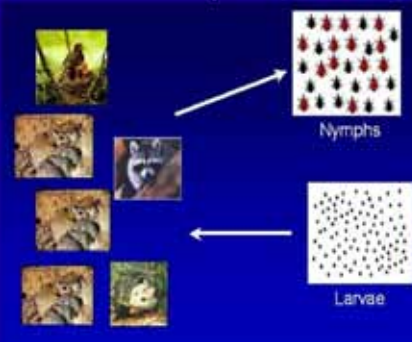
【Slide 10】

Biodiversity Hotspots



【Slide 14】

Habitat of Species-Poor



【Slide 11】



【Slide 15】

Speakers and Titles



Dr. Michael A HUFFMAN: Self-medication in primates- prevention and cure



Dr. Yoshio TSUDA: Structure of Animal Communities and Transmission Dynamics of Mosquito Borne Diseases



Dr. Koichi GOKA: Mites talk about biodiversity – Ecological significance of Evolutionarily Significant Units in parasites.

【Slide 16】



【Slide 20】



【Slide 17】



【Slide 18】



【Slide 19】

霊長類の自己治療行動—予防と治療

Self-Medication in Primates- Prevention and Cure

京都大学霊長類研究所 准教授・ハフマン A. マイケル
Michael A. Huffman, PhD, Associate Professor
Primate Research Institute, Kyoto University



Good morning everybody. First of all, I would like to express my thanks to all the people who have been involved in planning and hosting this highly significant international conference, beginning with Prof. Murata. Yesterday, I heard a lot of talks, and although they addressed fields somewhat different to my own, I still found them very interesting and informative. I enjoyed them a great deal.

I would like to talk to you now about my own research. My talk will overlap to some extent with what Prof. Murata was talking about earlier so let me introduce that part from now.

Today I am going to talk on the subject of “Self-Medication in Primates - Prevention and Cure”. To begin with, there is one point on which I must insist. Many people may think that primates are capable of self-medication because they are clever. But I personally don't think this reasoning is correct. As Prof. Murata stated earlier, we humans too are animals. We shouldn't forget that just like us, plants and other animals are living things, and that we all live together in one world. With regard to self-medication, I don't believe that primates lead a particularly special kind of existence. On the contrary, I believe that all living things must perform self-medication in some way or another. Because unless organisms - whether they be primates, birds, fish, or even insects - have some means of maintaining their own health when facing disease or stress, then they would not be able to continue to exist on Earth.

Probably, the first living things to begin practicing self-medication were the plants, because predators eat plants if they can't protect themselves. Plants rely on their leaves to collect energy yet the parts of plants

that have a lot of nutritional value, such as the leaves, are also the parts that get eaten by animals. Plants are considered to employ two strategies in order to defend themselves against being eaten. Their first defense strategy is to produce various chemical substances. Due to the presence of toxic secondary metabolites, important parts of many plants are rendered distasteful to animals. Many of these substances are bitter to the taste. For instance, if a predator overeats a certain kind of leaf and the animal develops a disorder (such as bad digestion, etc.) it will, as a result, tend to avoid feeding on the species of plant in question. The second strategy is a physical defense. Some plants grow large burs or thorns, while others have almost invisible small, hard projections that are closely packed on the surface of their leaves. These small projections, which make the rough textured surface of the leaves, are made of a glassy substance called silica which is very difficult to digest. So if an animal eats such leaves, it will develop indigestion.

This is a photograph of the surface of such a leaf taken though an electron microscope. It gives a good idea of how many tiny burs cover the leaf surface.

In short, third parties have taken over these two methods of defense, which originally evolved in order to protect plants, for use in protecting their own bodies. There are reports that many kinds of animals use these two plant defense methods as “medicines”. Insects are considered to have been the first animals to begin self-medication as an evolutionary process. Around the time the first flowers and fruit began to grow, there was also an explosive increase in the number of insect species. In the course of that process, insects began using the plants for purposes other than nutrition. Plants were also co-evolving with insects, and relationships of deep

mutual dependence developed between the two.

This figure illustrates such close relationships between specific plants and insects. Some species of plants and insects have co-evolved into relationships of mutual dependence such that neither would be able to continue existing without the other. Ecosystems in which a single species of plant and a single species of insect function as a single unit have formed.

Within these relationships, insects play a role in making plant reproduction possible by carrying pollen. Of course, from the insects' standpoint, they are using the plants as a source of nutrition. And they also use the plants as "natural medical foods", which means they literally ingest medicinal plants. While on the one hand the insects help the plants, after ingesting the nutrition of the plant, they also use them to stimulate various functions by accumulating the plants' secondary metabolites within their own bodies. These secondary metabolites serve a variety of functions. For example, insects use them to stimulate various interactions between individuals of the same species, such as their function as precursors in the formation of pheromones. These help the sexes come together for mating. Some insects also use secondary metabolites as social communication tools or for defense against natural enemies. They make themselves unpalatable to predators by accumulating large amounts of these substances in their bodies. So if a predator puts an insect (such as a butterfly, which has adopted the defense strategy) into its mouth, the presence of the substance will cause the predator to spit out the insect. In that way, the insect can avoid being eaten. In addition, some insects use secondary metabolites for their anti-parasitic effects. Poisonous substances created in plants and ingested by the insect can function to destroy parasitic infections.

From insects to primates, many animals have been observed to practice self-medication. Animals try to protect themselves and maintain their health on several levels when they come into contact with diseases. Now I am going to change the subject to the self-meditative behavior of primates, and in particular, of chimpanzees.

I'd like to introduce you to how chimpanzees maintain their own health using the two kinds of plant defense systems I mentioned earlier, as well as to various other kinds of behavior they engage in to maintain health. Roughly speaking, these behaviors can be considered as operating on three levels.

Level 1 is behavior aimed at avoiding becoming infected with disease-causing agents and at reducing contact with such agents.

Two examples of this behavior are shown in these photographs. The one on the left shows olive baboons that live in the savanna on the Serengeti Plain.

There are four individuals and at dawn they go out foraging. Overnight, they stay in different trees, moving from one to another every few days. At times when food is plentiful they may remain on the same tree for a number of consecutive days. When they do this, a parasitic infection begins to break out from the excrement they leave beneath the tree. Sometimes it takes a few days for the infection to begin, but if the baboons climb the same tree everyday or carry out activities on the ground beneath the tree, the probability that they will contract the infection becomes very high. It is said that the baboons change the place where they stay overnight periodically in order to minimize that risk.

The other example I want to show you is the method illustrated in the photograph on the right. These monkeys are hamadryas baboons that live on the Arabian Peninsula. Their habitat is a desert area that doesn't have an abundant supply of water all year round. When water levels become very low, all the animals in the area gather close to the limited water sources. Due to the small volume of the water, the temperature of the water rises, creating a perfect environment for disease-causing agents (carried by the various animals in the area) to propagate. By drinking this water, animals have a very high probability of coming into contact with a wide range of pathogens. These baboons do a very clever thing in order to avoid the problem. In this photograph, you can see green

water flowing slowly at the bottom of the picture. The baboons are digging a hole in the sand at a place some distance from this stream. The groundwater emerges at the place where the baboons dig their hole. By making something like a well they are able to obtain cleaner water.

Through these two examples, we can ascertain that monkeys know some ways of avoiding being infected by disease and of reducing the risk of coming into contact with pathogens.

At level 2, animals eat foods that are nutritious and produce medicinal effects. In terms of the way humans look at the world, these foods fall into what we call the 'supplements' and 'spices' categories.

As you can see from this photograph, chimpanzees follow a largely frugivorous diet and they can quickly gain energy this way. The sugars contained in fruits can be used as an immediate energy source. Many fruits are also rich in protein and provide the animals with necessary fiber and minerals too.

On the other hand, chimpanzees also eat plants that contain various other ingredients in addition to nutrients at certain times of the year. These plants are classified as medicinal foods. Some plants contain substances that have bactericidal effects or which inhibit parasitic infections.

For example, for many years I have been conducting research on chimpanzees in the Mahale Mountains National Park in Western Tanzania. In that park, I have focused on a group called the M Group and researched how much of these medicinal foods are included in the food they actually eat. In the course of this research, I have found that approx. 22% of the 172 kinds of food they usually eat coincide with natural medicines that humans use to ward off parasitic infections. More generally, it has been established that throughout Africa both chimpanzees and humans use the same parts of the same plant species for easing specific symptoms of specific diseases such as parasitic infections or gastrointestinal diseases. Interestingly, apart from fruit,

people in Africa use many other plant parts such as leaves and leafy stems, trunks, seeds, etc., as medicinal plants.

Now, I will show the situation with another primate species, the gorillas. In the same way as I did with chimpanzees, I surveyed how much overlap there was between the plant parts that gorillas eat and the plants that local people use for medicines, with reference to a list compiled of all the foods known to be eaten by the three sub-species of gorillas. Surprisingly, I found that the plant parts taken by both gorillas and humans contain substances that have a variety of effects. For example, some of them contain factors that inhibit the growth of cancers, promote cardiac function, work as stimulants, boost the immune response, etc., in addition to working as antiseptic, anti-parasitic and antiviral agents. Unfortunately, it has not yet been observed how much, in what way, or in what situations gorillas use these plant parts.

The next level up is Level 3. At this level, primates are using medicinal plants precisely as humans use medicines. This is self-medicative behavior in which primates are observed to use medicinal plants when they are sick or when disease symptoms appear. In cases where large amounts of the plants are poisonous, they use limited amounts and employ special methods to treat disease. Even if their health condition deteriorates, they only use limited small amounts of these plants.

Now, let me explain how this works in the case of chimpanzees. They have two ways of self-medicating that I would like to introduce.

In the photograph on the left side of the slide, you can see a chimpanzee named Linda, who is about to put a leaf into her mouth. The plant is of the genus *Aspilia*, which has leaves with a roughly textured surface similar to the one I showed you in the electron microscope photograph earlier.

Chimpanzees put the leaves into their mouth one by one, rolling each leaf around in their mouth and then

swallowing it without chewing it. Sometimes they may swallow up to a hundred leaves in this way at a single sitting. As I mentioned earlier, such leaves are difficult to digest. Chimpanzees eat things that are hard to digest in order to promote the body's rejection responses. When the leaves pass through the digestive system, they act as a de-worming agent, helping to remove the parasitic nematode worms that infest the chimpanzees' intestines. This self-medication method involves a physical effect, and the chimpanzees are engaging in what is known as "leaf swallowing behavior".

Another method is to use *Vernonia amygdalina*, a plant in the family Asteraceae. The chimpanzees chew on the bitter pith of the plant aggressively and suck out its juice. This is known as "bitter pith chewing behavior". This pith juice doesn't contain anything of nutritional value, but it is effective for treating oesophagostomiasis, an illness resulting from a parasitic nodular worm infection. This infection is quite a serious disease, but based on my own observations, by drinking the bitter juice of the pith of this plant, visually confirmable symptoms such as fatigue, decreased appetite and abnormally colored urine disappeared within 24 hours, and the parasites' egg laying activities also ceased. I have reported this observational data in scientific journals.

In the Mahale Mountains National Park, there are clear differences in how the nodular worm infection rates wax and wane between the rainy and dry seasons. Within the approximately six months when the region receives heavy rain, these parasites actively and repeatedly infect chimpanzees.

This figure shows the life cycle of the nodular worms. When the chimpanzees excrete the eggs of these worms, they remain in the soil or on the surface of low plant leaves until they reach the L3 stage. At this stage they become larvae capable of re-infecting chimpanzees. About the time when the rains start, the environment becomes favorable for the development of the eggs and young larvae excreted in chimpanzee feces. When chimpanzees unknowingly take in the infectious L3 stage larvae, these parasites burrow into

the chimpanzee intestinal wall and produce nodules. After an incubation period of about one month, the negative effects of the infection appear in the chimpanzees. If the degree of infection (the number of adult worms infecting the host) becomes very high this leads to symptoms such as intestinal inflammation, diarrhea, stomach pain, etc. If the infection is particularly heavy, it may even kill the host chimpanzee. In the period from the beginning to the middle of the rainy season, infected chimpanzees frequently engage in both bitter pith juice ingestion behavior and leaf swallowing behavior.

Sick individuals carry out these two activities frequently and interchangeably. Though as I mentioned above, because *Veronia* is toxic, they do not practice this behavior as much as leaf swallowing. Now, I will introduce the behavior in more detail.

This photograph shows a chimpanzee putting a leaf into its mouth, rolling it around and trying to swallow it in a gulp.

I once rolled up such a leaf and attempted to swallow it in the same way as the chimpanzees do, but it was extremely difficult. The burs grow downward, so the leaves tend to stick in the throat and will not travel down smoothly. It was a thoroughly unpleasant experience for me. However, chimpanzees casually swallow up to a hundred of these leaves on occasion.

The chimpanzee in this photograph is again Linda. She is about to swallow the leaf she is holding in her hand. The other photograph shows a leaf on which you can see an adult modular worm of the species *Oesophagostomum stephanostomum*, which is about 3cm in length. Fighting infestations by this parasite is one of the aims of this particular form of chimpanzee self-medication.

On average, two leaves is effective in expelling one worm. Sometimes, 20 to 30 adult worms are forced out from a single 'dose' of 40-60 leaves. The de-worming effect is not due to the plant's secondary metabolites but the result of transient diarrhea simply increasing

movements of the intestines, which rapidly expel the leaves and worms with them. Even if the chimpanzees ingest the leaves every day, it doesn't seem to cause them any problems. During the rainy season, there is a high possibility that individual chimpanzees will be infected by parasitic worms many times, but this method seems to be sufficient for controlling the number of worms. Looking at the infection rates data, the treatment seems to have this effect.

Chimpanzees engage in this behavior frequently at the beginning of the rainy season when each individual's modular worm infection burden increases. Not every individual chimpanzee becomes infected, and this behavior is not observed everyday in the rainy season. But in the dry season it is hardly observed at all. However, from about one month into the rainy season, a marked increase is observed in bitter pith chewing behavior.

As research into the African great apes - chimpanzees, bonobos and gorillas - has progressed over the past 20 years, this leaf swallowing behavior has been observed at 16 research sites, not only at Mahale. The same behavior has also been noted at many sites being used to counter infection by the nodular worm *Oesophagostomum stephanostomum*. Yet another target of the same behavior is the tapeworm *Bertiella studeri*. Leaf swallowing behavior has a similar deworming effect on both infections. The adult stage worms and the tapeworm proglottids are excreted together with the leaves. In the case of the nodular worm, the number of adult-stage worms is clearly reduced, so there seems to be a definite effect. As for the tapeworm nodule excretion effect, there has not been much discussion on the subject. Up to now, it has been established that chimpanzees engage in leaf swallowing behavior while making use of the leaves of about 40 different species of plants. All of these species have similarly rough textured leaves.

Next, let's take a closer look at the chimpanzees' bitter pith juice ingestion behavior. The plant they use at Mahale is *Vernonia amygdalina*. This plant is very well known in both folk medicine and in pharmacognosy.

It has been demonstrated to lower malarial fever, blood cholesterol and blood sugar levels, and is very effective against parasitic infections. People are widely reported to use it for their own livestock. However, although this plant has been studied for a century, the fact that primates use it as a medicine for treating parasitic infections was not discovered until 1987. It was in fact myself who made the first observation and reported about this behavior. Interestingly, I found that both humans and chimpanzees exhibit the same symptoms after ingesting *Vernonia amygdalina*. The plant is very bitter. When people use it, they first beat it and then soak it in water until the water becomes deep brown, which doesn't take very long. Then they drink the water down all at once. This is said to result in an improvement in their condition within 24 hours.

In the case of chimpanzees also, within about 24 hours of drinking the bitter juice from the pith of the plant, their body condition shows clear signs of improvement. By observing their behavior we can clearly see improvements in their appetite and physical strength. The fact that humans and chimpanzees have this response in common is very interesting.

People have been researching *Vernonia amygdalina* as a natural compound for over a hundred years. A long list of compounds have been extracted from the plant, with many kinds of sesquiterpene lactones among them. Every report without exception mentions the powerful activity and poisonous ingredients of the plant.

However, until 1987, nobody had paid attention to the plant pith, the part that is used by chimpanzees, so the chemical composition of the pith was unknown. To remedy this situation, I carried out joint research with Prof. Koshimizu and Prof. Ohigashi of the Faculty of Agriculture at Kyoto University regarding the chemical analysis of *Vernonia amygdalina* and its bioactivity. One of the results of the research was that we discovered 13 new chemical compounds. These were identified to be steroid glucosides and were named Vernonioside A1, 2, 3, 4, B1, 2, 3, etc.

In summary, chimpanzees engage in leaf swallowing

and bitter pith juice ingestion behaviors as self-medication during the rainy season when infection rates of the nodular worm *Oesophagostomum stephanostomum* and the tapeworm *Bertiella studeri* are high. Other medicinal plants are also used intensively throughout the rainy season when parasite infection rates peak. The chimpanzees appear to use these plants from time to time up until the end of the rainy season.

In the dry season, leaf swallowing and bitter pith juice ingestion behavior are rarely observed. And the chimpanzees reduce their intake of natural medicinal foods too. When the season changes, the animals change their food menu and employ more direct treatment methods.

Lastly, I would like to talk about ethnopharmacognosy, which is another of my research themes. In this connection I would like to introduce you to Mohamedi Seifu Kalunde, with whom I cooperate on chimpanzee research in Tanzania. Mohamedi is very knowledgeable about medicinal plants and he works actively as a traditional healer treating the diseases that affect the local people. He was born and raised in the forests of the Mahale Mountains, and has a wealth of knowledge about local medicinal plants that has been handed down for generations in his family. I've walked around the forest together with Mohamedi for almost 20 years and he has taught me a great deal about chimpanzees and plants. For example, he has told me that among his own relatives there are people who tell how they obtained many new medicines by observing how sick animals act. Even while we were working together, he discovered a new anti-diarrhetic medication. Having seen a sick chimpanzee with diarrhea practicing self-medication he correctly surmised that the plant being used might be effective for people too. So he tried it out on himself and found that it did work well. It is widely used nowadays.

I have another interesting story to relate, this time about Babu Kalunde, Mohamedi's grandfather, who was a traditional healer. He discovered a new drug just by watching the strange behavior of a porcupine. He

noticed a weak porcupine digging up and eating the root of a plant his tribe called *mulengelele* which he recognized as being highly poisonous. Babu Kalunde knew there must be a reason behind the behavior so he brought a few roots back to his village and used them to treat some seriously ill patients. Mohamedi and other healers still use this root today to treat infections for which western doctors would use antibiotics.

The next speaker, Prof. Tsuda, will be talking about malaria, so I'm going to end my talk by giving a brief introduction to primate malaria. There is no doubt that malaria has had a huge effect on primates as well as on humans. It is an established fact that all four malaria species infecting humans are derived from strains that infect non-human primates. Recently, in Southeast Asia, the simian malaria parasite *Plasmodium knowlesi* (which usually only infects macaques) has begun infecting humans too. Since this is an infectious disease that the local monkeys have been living with for a long time, it does not represent a major problem for the monkeys. But as an emerging disease in humans, it causes serious symptoms in people who contract it.

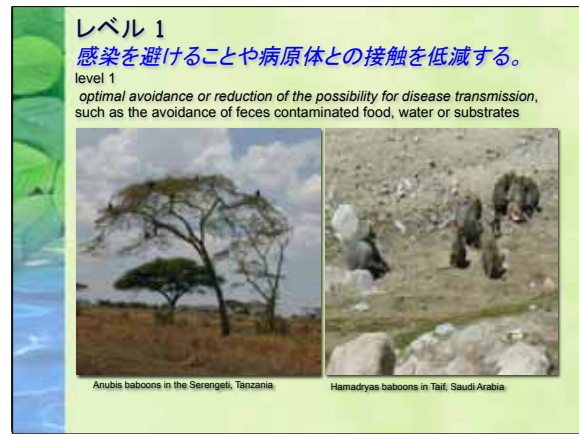
At present, there are no effective medicines for treating the disease but in the course of recent research it has been found that chimpanzees are also infected by malaria. As part of our 1990s research into the ingredients within the medicinal plant *Vernonia amygdalina* - as used by chimpanzees - we have discovered that chemical compounds have a strong anti-malarial action. More recently, another chemical compound has been reported which also has anti-malarial parasitic action. It was isolated from a species of plant called *Trichilia rubescens* and it is a plant that chimpanzees in Uganda use. So there is great anticipation that this discovery can be turned into a new medicine for people.

Several years ago I checked out the latest reports about *Vernonia amygdalina* (using Google) and found that the University of Missouri had obtained a patent to use a substance obtained from this plant as a medicine for treating breast cancer. Animals and humans have been living together for a long time in one world but since

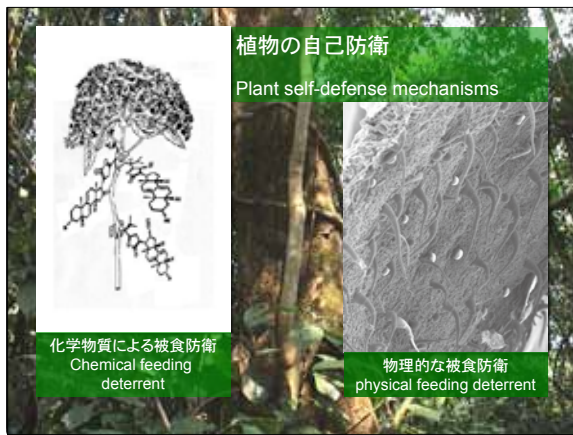
we humans have come to live in modern societies we have gradually lost touch with nature. As a result we have started to lose the natural knowledge that we had previously gathered. I believe there is a link between preserving the natural environment and biodiversity and protecting our own health. We need to reconsider the importance of the global environment and our position in nature. In closing I would like to thank you very much for listening.



【Slide 1】



【Slide 5】



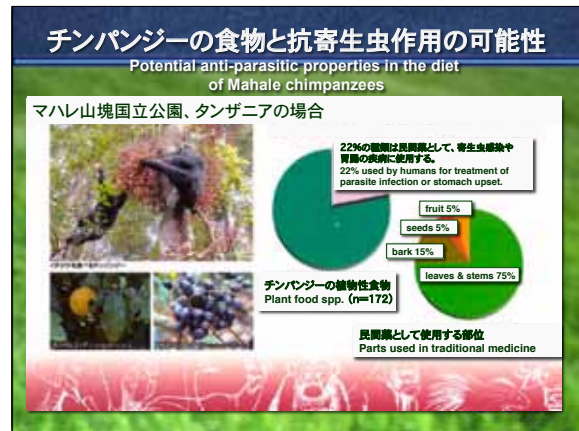
【Slide 2】



【Slide 6】



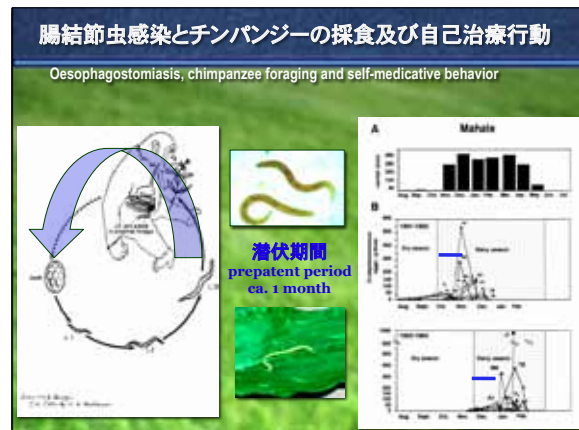
【Slide 3】



【Slide 7】




【Slide 4】



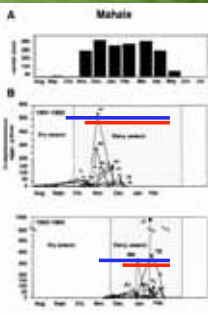
【Slide 8】

腸結節虫感染とチンパンジーの採食及び自己治療行動

Oesophagostomiasis, chimpanzee foraging and self-medicative behavior




MEDICINAL FOODS MEDICINE



【Slide 9】

ザラザラ葉の呑み込み行動

Rough, whole leaf swallowing



【Slide 13】

ゴリラの食物メニューに含まれる不思議な生理活性

Pharmacological properties in the gorilla diet



認められた作用:
強心、幻覚、免疫賦活
腫瘍成長抑制、殺菌
駆虫、抗ウイルス...


Recognized pharmacological properties: stimulant, cardiotoxic, hallucinogenic, immuno-stimulant, tumor growth inhibitor, bactericide, fungicide, anti-parasitic, antiviral...

Analysis based on published feeding lists of all long-term gorilla study field sites in Africa and pharmacological literature search. N = 110 spp. 59 plant families Cousins & Huffman 2002 African Study Monographs

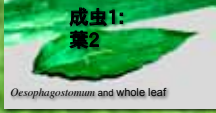
【Slide 10】

葉呑み込み行動による寄生虫感染に及ぼす影響

The effect of leaf swallowing on parasite infections



成虫1:
葉2



1. 早朝や空腹時に葉を呑み込むと、腸のぜん動運動が活発になり、成虫体が駆逐される。
Swallowed on an empty stomach, intestinal activity increases and worms are expelled.
2. 呑み込む葉2枚あたり成虫一匹が排泄される。
Average of 1 worm expelled / 2 leaves (mean 19.8, range 1 – 55 leaves per bout)
3. 雨期に葉呑み込み行動を度々行うことによって、腸結節内の寄生虫感染を抑制する効果が期待できる。
Frequently performed during the rainy season, leaf swallowing effectively suppresses level of Oesophagostomum infection.
Huffman et al., 1996; 1997; Huffman & Caton, 2001

【Slide 14】

レベル3

病気やその症状を治療するため、生理活性のある植物部位の少量を限定した回数で利用する。栄養価値が低い。

level 3
ingestion of a substance for the curative treatment of a disease or the symptoms thereof: use of toxic or otherwise biologically active items at low frequency or in small amounts, having little or no nutritional value



【Slide 11】

アフリカ類人猿におけるザラザラ葉飲み込み行動が見られる場所

Sites where whole leaf swallowing is seen in the African Great Apes



1. Dvobanu
2. Nimba
3. Tai
4. Puhi Luango
5. Ndoko
6. Lomako
7. Lyama
8. Warma
9. Kahari-Binga
10. Mahale
11. Gashaka
12. Mt. Itabi
13. Rutundu
14. Kalimbu
15. Fozzoli
16. Kiroko
17. Bulindi

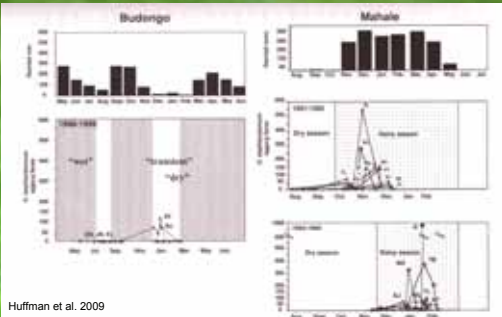


Over 40 plant species known to be swallowed and all are rough & hispid

【Slide 15】

チンパンジーの腸結節虫感染症-感染度と雨量との関わり

Oesophagostomiasis- infection intensity and rainfall



Huffman et al. 2009

【Slide 12】

チンパンジーの苦汁摂取行動



【Slide 16】

ベルノニアから単離された新成分

New compounds isolated from *Vernonia amygdalina*

STERIOD GLUCOSIDES
vernonoside

SESQUITERPENE LACOTONES
vernodaline, hydroxyvernolide, vernolide, vernolopin, vernodalol, vernomenin

vernonoside:
発見された新成分の分類群
Newly discovered class of
compounds

sesquiterpene lactones:
ベルノニアの多くの種に存在が確認されている。
Widely reported in *Vernonia* spp.

【Slide 17】

<p>ヒトマラリア Human Malaria Ape</p> <p><i>Plasmodium falciparum</i> (ゴリラ由来 gorilla origin)</p> <p><i>P. vivax</i> (アジアのマカク由来 Indian - Asian primate origin)</p> <p><i>P. ovale</i> (チンパンジー由来 chimpanzees origin)</p> <p><i>P. malariae</i> (マカク由来 Macaque origin)</p> <p><i>P. knowlesi</i> 新人獣共同感染症 Newly emerging zoonoses</p>	<p>サルマラリア Macaque Malaria</p> <p><i>P. reichenowi</i>, <i>P. gaboni</i></p> <p><i>P. simium</i> Indian - Asian primate origin)</p> <p><i>P. ovale</i> variant type chimpanzees origin)</p> <p><i>P. knowlesi</i> Macaque origin)</p>
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【Slide 21】

ベルノニアに含まれている成分の抗マラリア採用

Anti-malarial properties of *Vernonia*

TOWARD THE CHEMICAL ECOLOGY OF MEDICINAL PLANT USE IN CHIMPANZEES: THE CASE OF *Vernonia amygdalina*, A PLANT USED BY WILD CHIMPANZEES POSSIBLY FOR PARASITE-RELATED DISEASES

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DAISUKE IZUTSU,¹ KOICHI KOSHIMIZU,^{1,*}
MASANORI KAWANAKA,³ HIROMU SUGIYAMA,³
GEOFFREY C. KIRBY,⁴ DAVID C. WARHURST,⁴
DAVID ALLEN,⁴ COLIN W. WRIGHT,⁵ J. DAVID PHILLIPSON,⁵
PIERRE TIMON-DAVID,⁶ FLORENCE DELMAS,⁶
RIAD ELIAS,⁷ and GUY BALANSARD⁷

Journal of Chemical Ecology, Vol. 20, No. 3, 1994

【Slide 18】

サルまね?

Human see, human do?

【Slide 22】

トリキリアの抗マラリア活性

Anti-malarial properties of *Trichilia*

Novel Antimalarial Compounds Isolated in a Survey of Self-Medication Behavior of Wild Chimpanzees in Uganda

Sabrina Eral,¹ Math Thimmann Marud,² Philipp Grollman,² Janis Koenen,² and Thierry Schuster^{1*}

Journal of Chemical Ecology, Vol. 20, No. 3, 1994

【Slide 19】

タンザニアの伝統医が野生動物の行動観察から新薬の発見をする。

Traditional healers in Tanzania acquire new medicines from the observation of wild animals

Babu Kalunde and the crested porcupine & elephant

Mohamed Seifu Kalunde and the chimpanzee

Joha Kasente and the snake

'mulengele' *Aeschynomene nyassana*

'mhefu' *Trema orientalis*

【Slide 23】

サルマラリア ヒトマラリア

【Slide 20】

【Slide 24】

ベルノニアから乳がん治療の研究が行われている。

In 2004, a patent was registered in the US by researchers at Jackson State University, Missouri for the phytochemotherapeutic applications of *Vernonia amygdalina*. Plant extracts demonstrated inhibitive activity against growth of neoplastic cells, including those of human breast cancer.

【Slide 25】



蚊が運ぶ病気と生態系の構造

Structure of Animal Communities and Transmission Dynamics of Mosquito Borne Diseases

国立感染症研究所 昆虫医科学部第一室 室長・津田 良夫

Yoshio TSUDA, Chief, Laboratory of Taxonomy and Ecology, Department of Medical Entomology, National Institute of Infectious Diseases (NIID)



Hello, I am Yoshio Tsuda of the National Institute of Infectious Diseases.

As Prof. Murata mentioned during his introduction, I am first and foremost engaged in research on mosquitoes that transmit diseases to humans. These days however, just like Prof. Huffman who is working on simian malaria, and Prof. Murata who is working on avian malaria, I have also been working on animal malaria and particularly on the vectors that spread malaria.

I expect most of you have an image of mosquitoes as disagreeable insects that suck blood and spread diseases. But today, let's try to improve our understanding a little more about mosquitoes and the mechanisms by which they spread diseases, including the fact that not all species of mosquitoes actually spread diseases. And on that basis, let's explore how mosquitoes spread infectious diseases in animal communities including to humans. To begin with, I would like to talk more about what Prof. Murata mentioned in his introductory talk because, although mosquitoes do carry diseases, this need not be such an enormous problem. We should also be thinking about what the ecosystems that include mosquitoes can achieve.

In terms of content, I will be talking about the dilution effect that Prof. Murata introduced at the start.

Among the questions I am often asked about mosquitoes are "what good do mosquitoes do, and wouldn't it be better all round if they didn't exist?" Certainly, we tend to see only the bad side of

mosquitoes. But let's take a closer look. This is a copy of a page from a textbook about mosquitoes, and it shows a mosquito larva, which is commonly known as a wriggler. This schematic diagram shows an image of a rice paddy. Perhaps most of you have not had this experience, but if you go to a rice paddy not long after the rice seedlings have been planted and look closely, you will see that there are a lot of wrigglers in the water. There are lots of rice paddies, particularly in Southeast and East Asia including Japan. And these places where wet-paddy rice cultivation is carried out are among the most important sources of wrigglers and mosquitoes.

At the same time, there are many different organisms also living in rice paddies. In particular, during the Japanese rainy season in June, when wrigglers are common, the fry of fish such as killifish, crucian carp and loach are emerging from their eggs. These young fish rely on wrigglers as a major part of their food intake. Water fleas also feed on the newly hatched mosquito larvae and other insects also use them as a food source. The number of wrigglers that hatch each year is enormous, and they can be considered an important food resource for many other creatures.

While we are focused on the image of the adult mosquitoes that come around to suck our blood, these creatures are in fact eaten by a host of natural predators such as birds, bats, frogs, spiders and dragonflies that prey on insects. So they are an extremely important food source for natural predators. Additionally, the wrigglers feed on the straw from the previous year's rice crop found in the waters of rice paddies. In so doing they help break this material down.

Given this other role as scavengers they are a vital component of the ecosystem and their absence would cause problems.

Of course, mosquitoes do not only live in rice paddies and, worldwide, there are about 3,000 known species. In Japan alone, about 112 species exist in the wild. These various species of mosquito have different breeding habits and their wrigglers hatch in different places. I'd like to talk about that next.

Mosquitoes live in a wide range of habitats, but for today's talk I will show you some data from the rice paddies of Izumo in Shimane Prefecture. I'm sure everybody here today has heard of Izumo, which is around here on the map. Here is Lake Shinji, and here is the rice paddy zone where the data was gathered.

The reason why this particular area was chosen for a survey is that it is famous for the enormous number of migratory waterfowl that visit, as you can see from this photograph taken in October of the survey year. These birds overwinter in Izumo but there are other species of birds that are summer visitors. Because this small area attracts so many migratory birds, if individual birds carrying infectious diseases arrive, it is easy for the infections to spread. For instance, a mosquito biting an infected bird and sucking its blood also takes in disease-causing agents, or pathogens, present in the bird's blood. Later, this same mosquito may go on to bite another bird or even a human being, thereby infecting them with the pathogen too. My main work involves investigating these kinds of infection routes and the amount of risk they entail. I survey mosquitoes in various places where migratory birds congregate, and Izumo is one of these places.

This figure is small so it may be difficult to make out. It is a relief map, and just here, where you can see a grid pattern, is the rice paddy zone. And naturally, because rice paddies need irrigation, there is a river. Here you can see the characteristics of Izumo. This is the hilly area behind the rice paddies, and you may be able to see some small ponds dotted about, which are marked in blue. These are irrigation ponds. Even today some of

them are still in use but in the old days water from all of these reservoirs was used to provide irrigation water for rice growing. These days a different water supply system is used so most of the irrigation ponds are no longer used. For the most part nobody takes care of the unused ponds so they have become places where the wrigglers, the mosquito larvae, hatch and grow. This is a photo showing one of these ponds. The water here is an ideal breeding ground for wrigglers and so I select places like this for catching mosquitoes.

The surveys are carried out in this kind of hilly area and also along riverbeds in locations where there are a few trees growing up from the riverbed.

Within the rice paddy areas, capturing mosquitoes inside the paddies themselves is difficult. So, because in most rice-growing communities there is a Shinto shrine surrounded by thick growing vegetation, I try to capture mosquitoes there using traps.

Here in Izumo, there is another interesting survey spot known as the "Sagi Yama" which translates as "heron mountain" in English. In summer, it attracts a great many herons, but various other kinds of birds also congregate there and build their nests, so there are a lot of nests concentrated in the same location. Such places are commonly called "sagi yama" in Japan, so when I was informed that there was a heron mountain in Izumo, I decided to collect mosquitoes there. Today, looking hard at my own slides, I've noticed that although the subject of today's talk is "mosquitoes", there is not a single mosquito image among them. Although I said I was going to talk about mosquitoes this talk is actually less concerned with the mosquitoes themselves.

This is the mosquito species *Culex tritaeniorhynchus* which is a vector in transmitting Japanese encephalitis. Japanese encephalitis has been the biggest single infectious disease problem in Japan. These days there are almost no patients with the disease, but there is still a problem in that a number of people do become sick every year who appear to be infected with it on first glance. These mosquitoes were captured in the vicinity

of a pond. You can see that there are mosquitoes of different colors, which means that they belong to different species. So from this you can see that there are various species present. About one quarter of the individuals collected were of *C. tritaeniorhynchus*. At the shrine however, the mosquitoes collected were of only three colors so the total number of species was very small. Again, *C. tritaeniorhynchus* accounted for about one quarter of the individuals. In riverbed areas we come across individuals of a few other species, but in these places too *C. tritaeniorhynchus* is extremely common. This also shows that we capture different kinds of mosquitoes in different places.

Perhaps, what we can catch depends on what kinds of mosquitoes live in what kinds of places. But, more than that, because movement and behavior varies between different species of mosquito, each species tends to live in the places it finds convenient and easy to live.

To find out what a heron mountain is like, let us take a look at the data obtained from the heron mountain in Izumo. This particular heron mountain is not such a big one. When I talked to some locals at the start of the survey they told me that it was certainly big in the old days. When I went to the site, the first thing I noticed was a strong smell. There were lots of bird droppings lying around and the smell was overpowering. It was also very noisy. These birds tend to gather in places where it is difficult for people to walk. While the number of birds at this site was not particularly large it was a proper heron mountain in which night herons, as well as little herons and cattle egrets, had all built their nests en masse. Beneath this heron mountain, among the vegetation and the whitish bird droppings, if you dip into the soil where you can see these little channels, you can capture the mosquitoes that suck the blood of various animals.

Here is a different heron mountain that I visited in Tokushima Prefecture during the following year. This is a huge heron mountain that is literally covered in nests. The birds make their nest at heights from between two meters to five or six meters. This nest is positioned approximately two meters above the ground, which

is why I was able to take a photograph of it. Here and there in the heron mountain there are fallen trees, and lots of mosquitoes rest on the tree branches.

This species of mosquito was captured from a heron mountain but I am not going to talk about this particular species today. In total I have captured about 16 species of mosquito. Now I would like you to take a look at this red area. This is a mosquito that has sucked blood. It has a partially full belly but, as I'm sure you know, mosquitoes can keep sucking blood until their belly becomes really huge. That one there is a full-fed mosquito. Those with only partially full bellies have only sucked a little blood. Perhaps the host bird moved and caused them to stop while they were feeding. This one has a belly half full with blood while the other half has already been digested. When the blood has been half digested, the mosquito produces eggs while continuing to digest the remaining blood. In this case, the eggs are filling up about half of the belly space. The condition of this next one is that all the blood has been digested and the belly is bulging with eggs. This one has a flat belly because it has not yet sucked any blood at all. We catch different mosquitoes exhibiting a variety of physiological conditions.

My main subject today is the analysis of the blood that these mosquitoes hold. After bringing back the mosquitoes, I ask someone to assist me with the things I can't do myself such as removing the animal blood from the mosquitoes and extracting DNA from the blood. By obtaining the DNA base sequence from the blood and checking this against similar base sequences, we are able to identify the species of animal from which an individual mosquito has sucked blood.

Here, from the data obtained in Izumo you can see a lineup of the animal species we found to have been the sources of the blood taken from the mosquitoes analyzed. Here are the species of mosquitoes. Here, surrounded by a thin green line are the birds which include great egrets, cattle egrets and intermediate egrets. These mosquitoes were taken from a heron mountain so there were a lot of heron and egret samples but we also found some sparrow blood. Here

we have some mammal blood samples including blood from raccoon dogs and Japanese sika deer, which doubtless live in the area, as well as from mice, etc. Then there were frogs, such as tree frogs. And here at the bottom we have grass lizards.

As you can see, mosquitoes suck the blood of many kinds of animals ranging from birds to reptiles. Here, I have also split the mosquitoes into several groups. The red group is the group that feeds on frogs. This kind of mosquito is the most familiar one to most of us here today. It's the kind that buzzes in your ear during the night and wakes you up with an itchy mosquito bite. The mosquitoes that have this red part here mostly feed on birds. This group has a strong preference for bird blood. The next group is known as the striped mosquitoes. In Japan, when we go out in the summertime and sit on a shady bench in the park, we are likely to be bitten by a mosquito in no time. The mosquitoes lying in wait for us are the striped mosquitoes. As a group, they limit their blood-sucking activities to mammals in almost all cases. This next one is of the species *Uranotaenia novobscura*, known in Japan as "chibi ka" meaning "small mosquito". And indeed they are about twice as small as most other mosquitoes. They are particularly fond of feeding on frogs. The reason I was able to capture quite a lot of this particular mosquito species at this heron mountain was, as you might expect, because they came there to feed on the frogs. This next type is vary rare, but there are also mosquitoes that suck the blood of snakes, lizards, etc.

Many mosquitoes of the genus *Culex*, known as house mosquitoes, are extremely fond of birds and also feed partially on humans and other mammals. Moreover, there are species that feed almost exclusively on mammals, and, similar to *Uranotaenia novobscura*, there are species that like feeding on frogs, although they also drink the blood of mammals occasionally. And then, there are more adventurous eaters that like to feast on the blood of reptiles and amphibians. So all in all, there are different mosquitoes that follow a wide range of blood sucking patterns depending on the species or group to which they belong.

Despite all this variety, basically all the different mosquito species have a lot in common. First of all, if we consider how their so-called blood sucking patterns are decided we find that these patterns are determined by genetic factors. It turns out that house mosquitoes have the strongest disposition to suck the blood of birds while striped mosquitoes have a strong disposition to suck the blood of mammals. In this sense, their behavior is genetically determined.

However, that isn't the end of the story. For example, on a heron mountain, where there are lots of herons and egrets, it is clear that the mosquitoes will tend to suck the blood of these birds. Where there are herons, the mosquitoes nearby will try to drink their blood and where there are no herons, the local mosquitoes will try to drink the blood of other animals. If there are no birds, they will feed on mammals such as raccoon dogs or on other animals, depending on which species are present and in what ratio to each other. Also, some mosquito species prefer to feed at night and others prefer to feed during the daytime. Generally, in my understanding, the overall balance of biological, behavioral and genetic factors determines the blood-sucking pattern. In particular, whether or not it is easy to suck the blood of a particular kind of host is determined by ecological factors. For instance, it is easier to suck the blood of animals that do not move around at night.

Looked at in this way, the heron mountain results I outlined earlier can be much better understood. This slide is very difficult to understand, but let's take a quick look at it. Up to now I've been talking about mosquitoes, but now I'd like to talk a little about the diseases that are transmitted by mosquitoes. And then, to finish up, I will talk about how mosquitoes spread these diseases among groups of animals.

Mosquitoes act as vectors in transmitting a variety of pathogens. Among the diseases spread by mosquitoes that infect humans are malaria, dengue fever and Japanese encephalitis. As for animal diseases, mosquitoes carry avian malaria, simian malaria,

canine filariasis, etc. The mosquitoes' capacity to spread infectious diseases is determined by a number of properties. The first of these is their affinity with the pathogen in question, although not all species of mosquitoes can transmit disease. For instance, *Culex tritaeniorhynchus* is the most proficient mosquito at transmitting Japanese encephalitis. This is because the Japanese encephalitis virus does not propagate in the bodies of other mosquito species. So the capacity to spread infection is partially dependant on the compatibility between the host mosquito and the pathogen it carries.

And then, in the first place, there is the question of what kinds of animals a given species of mosquito prefers to feed on. Another important property is the lifespan of individuals of a given species. And lastly, there is the question of how many intervals of the species are produced. Ultimately, the capacity of a group of mosquitoes to spread disease depends on the cumulative effect all of these properties.

Today, I am particularly interested in two things, namely, 'affinity for pathogens' and 'blood-sucking preference'. These two things determine the combination of vectors - the mosquitoes that carry infections - and the animals that become the hosts for the particular pathogens carried. So this is the most interesting part for me.

This slide may also be a little difficult to understand. The uppermost part of the slide shows a mosquito's body cut in half longitudinally. Here is the head, this is the thorax, or chest, and here is the abdomen. The wings would be about here and the legs would be here, but these have been removed.

First, if we talk about malaria, for instance, whether it is simian malaria or human malaria or whatever, when a mosquito ingests the blood of an animal infected with malaria, the blood goes into a part of their abdomen known as the midgut. After that, the malaria pathogens in the blood form lump-like swellings called oocysts on the outside of the midgut. The pathogens develop into a form known as sporozoites inside the oocysts

until they eventually burst out in the form of worm-like vermicules. In this form, the sporozoites enter the mosquitoes' salivary glands. Then, when an infected mosquito sucks the blood of another animal, the sporozoites are transferred inside the animal along with the saliva. This is how malaria is spread.

As is written here, when a mosquito sucks the blood of an infected animal, it takes about 12 days for the sporozoites to enter the salivary glands. So until this amount of time has passed, the mosquito cannot propagate malaria. In this respect, the lifespan of the mosquito is very important.

Next, I would like to talk about how mosquitoes spread malaria among groups of animals, and my talk will cover basically the same subject as the dilution effect that Prof. Murata explained at the beginning.

For infectious diseases, groups of animals usually include those who become hosts for the pathogens and those that do not become hosts. Or in other words those that do not become infected with the disease in question. Mosquitoes pick up pathogens from infected host animals and transmit them to other animals when they bite with the result that the disease is spread or "amplified". This is known as the 'amplifier concept'. In short, a relationship exists between the mosquitoes and their hosts moderated by the activity of blood sucking.

As for animals that do not become hosts, for instance, if a mosquito carrying avian malaria bites an animal that cannot be infected with this pathogen, the disease will not be spread and so this will not aid the pathogen's propagation. The more often this happens, the more the transmission of the disease will be diluted.

Ultimately, this balance determines whether or not an epidemic will occur. If the mosquitoes come into contact with large numbers of animals that can serve as hosts, the amplification effect causes an epidemic to spread. On the other hand, if the dilution effect is larger, then the scale of epidemic will be limited. Unlike ticks, mosquitoes are good at choosing their hosts, and so the mosquito blood-sucking pattern is very important for

determining the balance of whether or not an epidemic will continue to spread.

Now, I would like to share with you some data taken from an article on simian malaria that was written over 50 years ago. As Prof. Huffman explained earlier, simian malaria has recently become a major problem in South East Asia, and half a century ago similar research was carried out on this disease. Here in a forest, on the branches of larger trees, platforms were set up in various locations with cages on them with monkeys placed inside. The mosquitoes that came to suck their blood were then captured. This research was conducted in 2007, but exactly the same kind of research was carried out 50 years ago. In some cases, humans sat in the cages to attract mosquitoes.

I really like this data. These mosquito species were captured in a coastal mangrove forest. Using the same method the researchers captured mosquitoes from the lowland swamp behind the mangrove forest, and from the hill country behind the swamp. Naturally, the mosquito species they found in these differing places were different. The problem was that there were spherical oocysts on the outside of the mosquito midgut and there were sporozolites in their salivary glands. This was what the data indicated.

We know that this part marked in red refers to simian malaria parasites. As the data shows, it means that the species of mosquitoes that were captured coming to bite the monkeys were carrying simian malaria. As I explained earlier, there is a connection between this and the “amplification” of malaria. What the green part shows is that, as the researchers worked hard to establish, these chevrotains, or mouse deer, become infected with this type of malaria. I don't really understand this blue part very well. But at the time, and even now, we are unable to classify all the things that emerge from the bodies of mosquitoes merely by their shape. Even so, from the shape of this one, I can tell that it is certainly not simian malaria. And looking at this one, I would guess it to be avian malaria. This means that, although these mosquitoes came to feed on monkeys, the malaria parasites they carried were

totally unrelated to monkeys. So this contact between mosquitoes and monkeys was an example of a full dilution effect.

With regard to simian malaria, we know the following things. We conduct research into avian malaria, and when avian malaria comes up as a subject of conversation, we can't avoid the feeling that this disease is happening in places where people have totally different lifestyles to Japan, such as South East Asia and Africa. But even so, when I was actually researching avian malaria, I found very similar stories. I would like to talk a little about this to close my talk.

This is a park in Tokyo where I conduct surveys. It is a rather large park. There are lots of trees growing, and I am always capturing mosquitoes in this area. In places where this sort of vegetation grows I can capture mosquitoes by sweeping a net about. The place may be in urban Tokyo but I am still able to find 11 species of mosquito here. This is a red house mosquito (*Culex pipiens pallens*) and this part surrounded in red is the blood sucking part. My analysis was centered on individual red house mosquitoes that had sucked blood.

My analysis found that between 3% and 5% of the mosquitoes that had not sucked blood were carriers of avian malaria. In the case of mosquitoes that had sucked blood, an analysis of the abdomen revealed that 18.6% were carrying avian malaria, while about 3% seemed to be carrying it in their chest or salivary glands. So out of every 100 of these mosquitoes, we can be sure that several are carriers of avian malaria.

This is a survey of the animals on which the mosquitoes I captured had been feeding. Those surrounded by the dotted lines are all birds. There was only one human blood sample, meaning that out of the 220 mosquitoes surveyed, only one had bitten a human. The above three species, namely jungle crow, sparrow, and great tit, accounted for 83.4% of the samples obtained. This part in red shows the numbers of avian malaria samples obtained. The ratios were 29%, 21% and 40%, respectively. So in all probability, these three species of birds can be considered as host species for avian

malaria in Tokyo. I think that under this situation, the fact that so many mosquitoes feed on these host birds creates a great many contact points for amplifying the spread of avian malaria and gives little opportunity for dilution effects to operate.

Actually, the species of mosquitoes that feed on birds change according to the season. For instance, the numbers of mosquitoes that feed on crows are low in May but increase gradually through June, July and August. And in line with this increase in numbers, the ratio of mosquitoes carrying avian malaria parasites also changes.

This chart shows data on various species of birds. We looked at three species - crows, sparrows and great tits. The vertical axis shows the risk of avian malaria infection for each species. For the sparrows, marked with a white circle, the risk of becoming infected with avian malaria from a mosquito bite is high at the start but becomes lower in the summertime. The great tits tend to be bitten little by little throughout the period but there is no particular time when lots of great tits are being bitten. So the risk of this species being infected with avian malaria, while present, is very low. In the case of the crows, which have the biggest problem, as the summer progresses through June, July and August, the mosquitoes come to feed almost exclusively on crows. Therefore the risk of these birds contracting avian malaria is very high. In the course of our research, we found out that another such high risk period occurs around October.

Put more simply, when we examined the red house mosquitoes captured at the park, we found evidence of blood from avian malaria host bird species such as crows, sparrows and great tits, and also of non-host bird species. In this case, a single mediator species and several host species are involved. When mosquitoes feed more on non-host species, we can expect the dilution effect to be favored, but when they feed more on host species we can expect to see the amplification effect happening. In this way, the overall propagation ratio of pathogens is influenced by how many host and non-host animals are available to the mosquitoes.

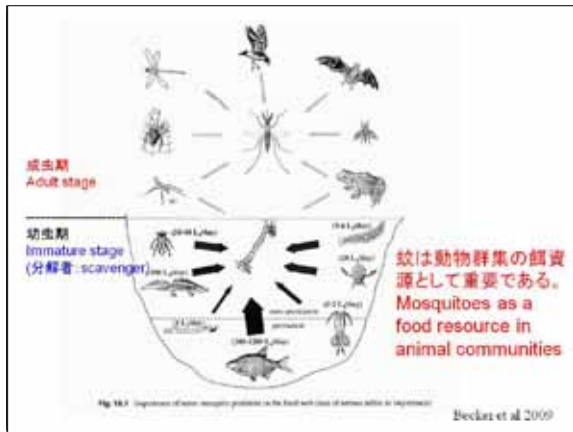
Moreover, in the survey area there were other animals besides birds and other mosquitoes than the species surveyed. We had to limit the species of mosquitoes in the survey, and so we chose those species that fed most often on birds. These included the red house mosquito that I have been discussing. On this occasion we also surveyed a member of the striped mosquito family, but we found that these mostly sucked the blood of mammals. Although on this occasion we targeted avian malaria, if we understand the conditions under which avian malaria is sustained in more detail, we will have a better idea about how this disease, which is transmitted to wild animals by mosquitoes, is able to exist.



【Slide 1】



【Slide 5】



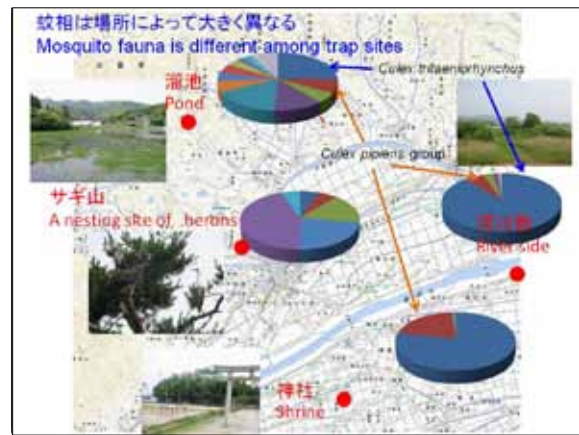
【Slide 2】



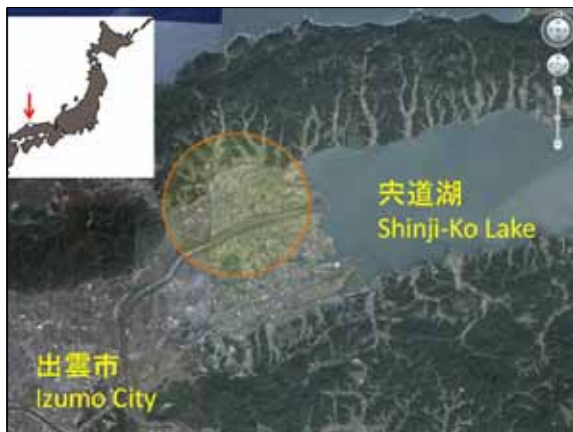
【Slide 6】



【Slide 3】



【Slide 7】



【Slide 4】

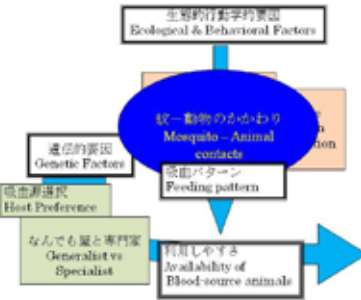


【Slide 8】



【Slide 9】

蚊の吸血パターンは変化しうる。
Feeding pattern of mosquitoes is flexible.



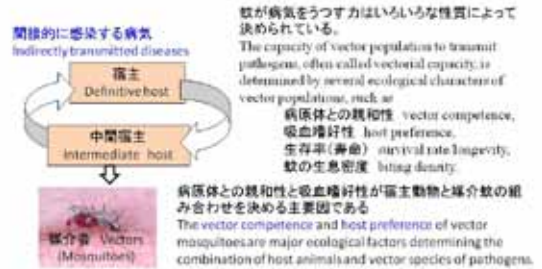
【Slide 13】

出雲のサギの巣で行った吸血蚊採集の結果
Results of sweeping collections of mosquitoes at a nesting site of herons at Izumo

Species	捕獲数 full	採集回 partial	変化 total	羽 David	本 吸血 total	小計 ♀TTL	小計 ♂TTL	合計 Total
1. <i>An. albopictus</i>	4			60	44	15	59	
2. <i>Cx. japonicus</i>		2		44	46	10	56	
3. <i>An. furciferus</i>	1	2		15	18	22	40	
4. <i>Cx. pyg. gr.</i>		11		6	26	2	28	
5. <i>Cs. nigropalpus</i>	1	1	2	1	11	15	9	24
6. <i>Cp. norobocera</i>		7	5	3	15	8	23	
7. <i>An. vassalli</i>	1			3	10	14	14	
8. <i>To. taeniosoma</i>				4	4	8	12	
9. <i>An. sulcatipes</i>				4	4	3	9	
10. <i>Cs. finlayianus</i>	2	1		2	5	1	6	
11. <i>An. sinensis</i>		1		1	2	3	5	
12. <i>Cs. (Chikungunya) sp.</i>				3	3	1	4	
13. <i>Cs. orientalis</i>	1				1	1	2	
14. <i>Cs. ruber</i>				2	2		2	
15. <i>Cs. fatigans</i>							1	1
16. <i>Cs. tritaeniorhynchus</i>				1	1		1	1
Total	12	9	24	9	146	200	86	286

【Slide 10】

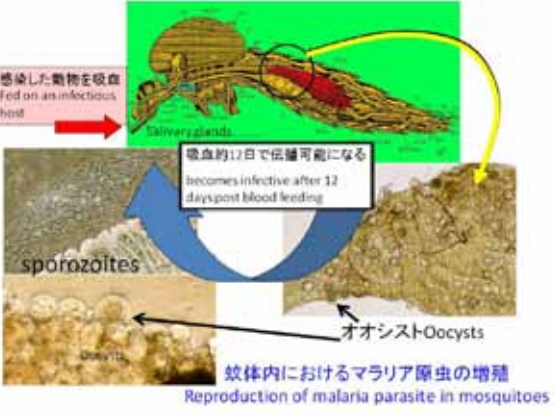
蚊は様々な病原体を媒介している。
Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as viruses, protozoan, nematodes, which cause serious diseases such as malaria, dengue, yellow and Chikungunya fever, encephalitis or filariasis.



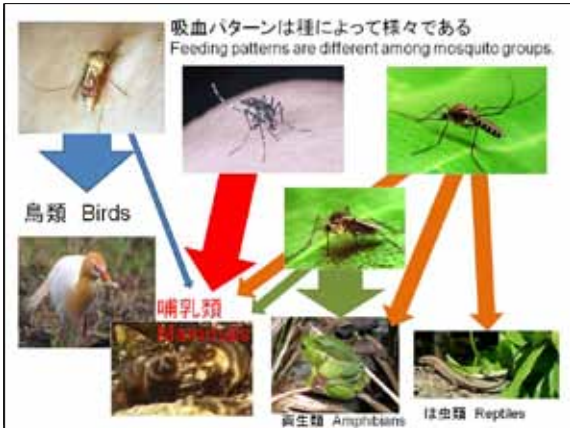
【Slide 14】

吸血動物の同定結果 Feeding pattern of mosquitoes collected at a nesting site of herons at Izumo: Results of blood-meal identifications

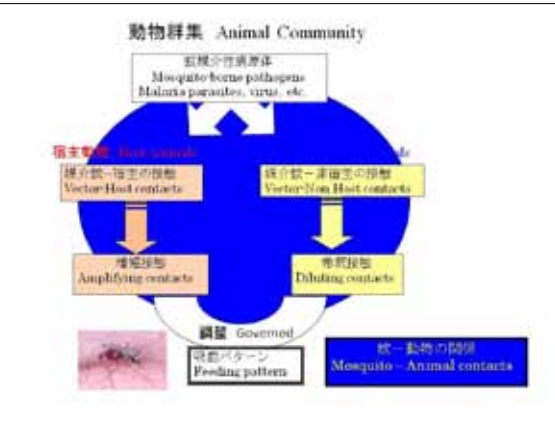
【Slide 11】



【Slide 15】



【Slide 12】



【Slide 16】



【Slide 17】



【Slide 21】



【Slide 18】



【Slide 22】

増幅接触と希釈接触の両方が起きている
Both amplifying or diluting contacts were recognized in primate malaria
(from Wharton et al 1964)

蚊の種類 Mosquito Species	サンゴ海岸 Coastal mangrove	低地 swamp forest Lowland swamp forest	山頂 森林 Summit hills
<i>Anopheles gr.</i>	14 sp. (22 sp. 0.01)	2 sp. 0.7 sp. (0.01)	1 sp. (0.01)
<i>An. punctipennis</i>	1 sp. (0.4)		
<i>An. dunnellii</i>			1 sp. (0.01)
<i>An. tritaeniorhynchus</i>			1 sp. (0.01)
<i>An. sinuatus</i>			1 sp. (0.01)
<i>An. walkeri</i>			1 sp. (0.01)
<i>An. nigri</i>			1 sp. (0.01)
<i>An. nigri</i>			1 sp. (0.01)
<i>An. walkeri</i>			1 sp. (0.01)
<i>An. walkeri</i>			1 sp. (0.01)
<i>Culex pipiens</i>			1 sp. (0.01)
<i>Culex pipiens</i>			1 sp. (0.01)
<i>Culex pipiens</i>			1 sp. (0.01)

増幅接触
Amplifying contacts

希釈接触
Diluting contacts

P. knowlesi, *P. cynomolgi*, *P. coatneyi*, *P. fieldi*, *P. ginsu* - Monkey malaria
Plasmodium fragile: *Plasmodium* of the mouse-least
Plasmodium other than primate malaria

【Slide 19】

2007年4月から12月に採集された蚊の種類と個体数
A list of mosquito species and the number of blood-fed and unfed females collected during April to December 2007

Species	half				unfed females	males	Total
	full	partial	gravid	Gravid			
1 <i>Cx. tritaeniorhynchus</i>	1	2	2	14	14071	14090	2802 16892
2 <i>Ae. albopictus</i>	71	133	236	9	4877	5326	1922 7248
3 <i>Cx. pipiens</i> sp.	91	25	132	75	605	928	1164 2092
4 <i>Cx. sasai</i>	9	1	8	4	54	76	421 497
5 <i>Lz. vorax</i>	2	5	17	6	81	111	155 266
6 <i>Or. anopheloides</i>	1		2	7	11	21	31 52
7 <i>Ar. subalbatus</i>					15	15	12 27
8 <i>Cx. orientalis</i>					14	14	2 16
9 <i>Cx. rubitorator</i>					12	12	1 13
10 <i>Cx. bitaeniorhynchus</i>					8	8	1 9
11 <i>Cx. inatomi</i>					4	4	4 4
Total	175	166	397	115	19752	20605	6511 27116

アカイエカの吸血蚊を分析した
Blood-fed samples of *Culex pipiens pallens* were analyzed.

【Slide 23】



【Slide 20】

アカイエカの鳥マラリア原虫陽性率
Positive rate of *Plasmodium* parasite in unfed and blood-fed *Cx. pipiens pallens*

Specimen	個体数 No. mosquito	陽性数 No. <i>Plasmodium</i> +	陽性率 Positive rate
未吸血蚊 Unfed	643 (71 pools)	33* (22 pools)	0.05(33/643) 0.03(22/643)
吸血蚊 Blood-fed	371	Abdomen 69 Thorax 11	0.186 0.03

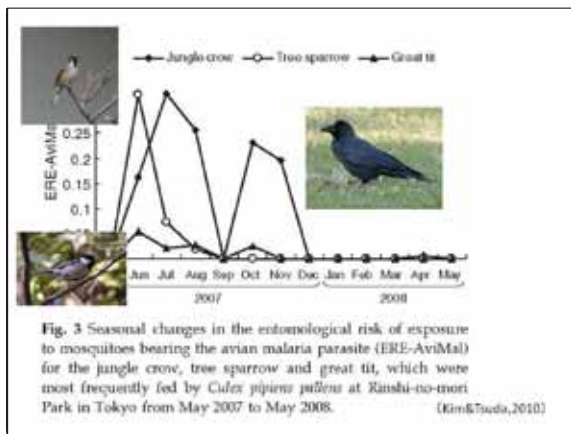
(Kim&Tsuda, 2010)

*This was calculated by 22 × 1.5
(1.5; a mean number of infected mosquito per positive-pool)

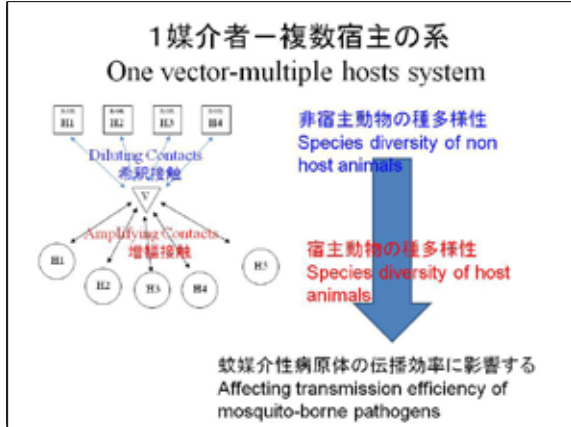
【Slide 24】



【Slide 25】



【Slide 26】



【Slide 27】



【Slide 28】

ダニが語る生物多様性～寄生生物の進化的重要単位の意義～

Mites Talk about Biodiversity - Ecological Significance of Evolutionarily Significant Units in Parasites.



独立行政法人 国立環境研究所 侵入生物研究チーム・五箇 公一
Koichi GOKA, PhD, Invasive Alien Species Research Team
National Institute for Environmental Studies

Hello everybody. I am Koichi Goka of the National Institute for Environmental Studies.

First of all, I must apologize in advance to those of you listening in English because I was late presenting my slides and the script of my speech is too long. This means I will have to speak so fast that the simultaneous interpreters may not be able keep up. So please read my English subtitles where they appear.

Also, as I have a great many slides please regard my presentation as if it was an animation.

At the request of Prof. Murata I am going to talk about mites and ticks which are my specialty subject. In particular, I intend to focus on biodiversity as seen through the study of mites. At the same time, I'd like to explain in an autobiographical manner about why I turned towards the study of mites and why I love them.

In today's workshop we have been hearing about various kinds of parasites, including mites. Parasites are organisms that have existed almost since the beginning of life on Earth. Ever since that time they have evolved together with the rest of the biosphere. Parasites attach themselves to other living things in order to live and, as you are probably aware, there is a theory that they played a role in the development of sexual reproduction. Many organisms have male and female individuals and reproduction between two sexes is the most common way in which reproduction takes place.

Many kinds of living creatures, from primitive organisms such as insects and plants to human beings, exist as male or female, as this slide illustrates.

As I mentioned just now, parasites played a major role in the evolution of sex according to an idea known as "the Red Queen" hypothesis". One of many current ideas concerning evolution, the hypothesis takes its name from the character in Lewis Carroll's second "Alice" book, *Through the Looking-Glass, and What Alice Found There*. In the story, the Red Queen says, "It takes all the running you can do, to keep in the same place." Here the Red Queen is used as a metaphor for the idea that species, individuals and genes have to keep evolving if they are to survive. In order for multicellular species to keep warding off continual attacks by viruses, the species needs to be equipped with sufficient variation at the genetic level. Clearly, there is a difference in the tempo at which evolution can take place in viruses and in multicellular organisms consisting of an enormous number of cells. So if multicellular organisms were to counter the attacks of viruses by means of ordinary mutation alone, they would never be able to win the evolutionary battle. Instead, they have evolved as a result of gene exchanges and the creation of new genotypes through sexual reproduction. This hypothesis is one of several that attempts to account for the evolution of sexual reproduction.

Parasites have various ways of making a living. For example, some kinds of organisms parasitize higher order organisms, while others practice kleptoparasitism. This is a photograph of a Great Reed Warbler (*Acrocephalus arundinaceus*) feeding a Common Cuckoo (*Cuculus canorus*) chick in an example of what is known as "brood parasitism". When we consider that parasitism itself is always associated with the evolution of organisms, and that some parasitic species exist inside almost all species of living things, parasites can

therefore be said to play a major role in promoting biodiversity.

When we think about it, we human beings are living as the biggest parasitic organisms on the Earth, and our style of parasitism creates numerous problems. But at the same time, parasitic organisms have very important functions within the ecosystem. As two other lecturers noted earlier, in a sense parasites play very important roles, and while they are universally disliked, we also have to recognize that their roles make them very precious organisms.

From this point on I am going to talk about mites.

My specialty is acarology (the study of mites and ticks), and although I am involved in a variety of work concerning the preservation of biodiversity, the things I actually like most are mites. Actually, there is an organization called the Acarological Society of Japan. And although the study of acarology is not very well known, there is also an International Congress of Acarology, which is held every four years. Two years ago, I attended the International Congress of Acarology held in Brazil where, for an entire week I took part in happy and harmonious conversations with acarologists from around the world about mites and nothing but mites.

Now, what kind of creatures are mites? Since many of you here today are specialists perhaps I don't need to explain, but many members of the general public are confused that mites are insects. In fact, mites are not insects. The mite body is very simple. It consists of only one section to which the limbs and mouth are directly attached.

The insect body is separated into three sections, the head, thorax and abdomen, with six legs and up to four wings attached to the thorax. That's the insect archetype. So insects have a far more complex structure than mites do. Like spiders, mites have eight legs. But the spider body is divided into two sections, cephalothorax and abdomen, so spiders also have a more complex structure than mites do.

However, even though mites have very simple bodies, these animals are distributed everywhere on Earth. Also, the life histories and food sources of different species of mites are amazingly varied, and the total number of mite species is enormous. There are around 50,000 known species recorded at present, and it has been estimated that there may be up to a million species of mites all told.

Mite habits also vary widely. For example, some species even live in the ocean. In this respect, mites have gone farther than insects as no insects live in the sea. Mites can inhabit both salt water and freshwater environments where they engage in eating plants and in sucking the blood of animals. Also, there are predatory mites that eat other mites and there are scavenger mites. There are many varieties of mites in the soil which break down organic matter and convert it to inorganic matter which helps create a rich soil. In these and other ways, mites are organisms that carry out a wide range of functions and help to support biodiversity.

Hearing such a story, you might very well be thinking that however important mites may be as an element in the web of life, as a human being you don't like them personally. You may wish also that mites were organisms that did not concern human life at all. However, mites of the species *Demodex folliculorum* are in all probability living happily on your face. As a matter of fact, almost 80% of Japanese people are hosts to this species. Of course, newborn babies don't have these mites to begin with but when a mother rubs her cheek against her baby's face, the mites are transferred. In this way, every human family has its own mites that are passed down the generations. So please bear in mind that mites are an excellent part of biodiversity as a whole.

Now I will proceed with my own story. I will explain about why I started to research the subject of mites in the first place. When I was a student in the Department of Agriculture at Kyoto University, I chose spider mites as my research subject. These are a family of herbivorous mites that parasitize plant leaves. From the

human standpoint spider mites are a terrible problem because they get onto various plants and suck the juices from the leaves. They eventually kill the plants and cause major problems as a worldwide agricultural pest.

Regarding their living environment spider mites begin life as eggs and go through several stages before reaching the adult stage. They exist as males and females and reproduce sexually. Mating between males and females is effective only one time. When a female engages in mating, her semen tank becomes full with the result that subsequent mating is ineffective. So for males, the obtaining of virgin females is a very important breeding strategy. In view of this, the kind of behavior spider mites have evolved includes pre-mating guard behavior. The male climbs onto a female pupa before the adult stage and guards it. Then, as the female starts to cast off the chrysalis, the male helps out, and then performs mating immediately. This is the spider mite's reproductive strategy.

Given this state of affairs, virgin females are a very precious asset for the males. If another male comes along, the result is a fight. Two males will enter into a violent combat that involves fencing using their stylet-like mouthparts. (These mouthparts are usually employed to suck the juices out of plants). Often the fight continues until one kills the other and while they are engaged in this battle, a third male may come along and mate with the virgin female. This kind of thing occurs quite often.

In my student days, there was a laboratory class during which we could observe these things taking place through a microscope. I first got into mite research when I saw these things. I was very impressed that mite ecology was so graphically revealing of the meaning of reproduction.

There was another experiment in which we observed what happens when one male is placed alone in a place where there are many virgin females. The point of this was to test the male mites' reproductive capabilities. Results showed that the more that virgin females are

available, the more mating the male will perform. Given the opportunity, a male will keep mating whenever and wherever it can until the point where it almost dies of exhaustion. I totally got into mites as living organisms that embody the male's fate as it is. I earned a master's degree and a PhD by studying mites and after that went to work for a pesticides manufacture where I was involved in developing miticides.

At that time too, in the battle between mites and miticides, we found that no matter how many types of miticide we produced, they would soon lose their effectiveness. We investigated many things related to spider mites including their DNA and came to the conclusion that spider mites encompass a huge range of diversity and that they maintain their resistance by continuously evolving. From this, I realized more clearly how important biodiversity is. In any case, the miticides we developed didn't sell well, and the company where I was working declined. So I left that company and joined the research institute where I am employed now taking up work on the preservation of biodiversity.

My initial research work at the institute was on the subject of alien species control, and I am still mainly involved in this work. The first alien species I worked on was the Buff-tailed Bumblebee (*Bombus terrestris*), which was imported to Japan from Europe.

This species of bee has been bred commercially for use in pollinating tomatoes and other crops in greenhouses. A species native to Europe, the Buff-tailed Bumblebee has been imported to Japan in large numbers. As you can see, the beehives are installed in greenhouses in this way, and the bees fly around carrying pollen and carrying out the pollination tasks that previously had to be performed by the farmers themselves, while significantly improving the tomatoes' productivity in the process.

However, some of these bees of European origin escaped from the greenhouses and began to live in the wild as an alien species, which had a very bad effect on the native Japanese Bumblebee (*Bombus hypocrita sapporoensis*). Because of this, use of the Buff-tailed

Bumblebee is now strictly regulated by the Ministry of the Environment to ensure that individuals of this species do not escape from greenhouses.

While I was carrying out this ecological risk evaluation I thought that, in importing the beehives from Europe, they must have also brought in some strange parasites. So I looked for parasitic mites and detected the presence of an endoparasitic mite species called the Tracheal Mite (*Locustacarus buchneri*) on the bodies of these bees. Then I surveyed how widely these mites had been introduced into Japan.

However, I discovered that the Japanese Bumblebee also carries this same endoparasitic mite. As a result of analyzing the mites' DNA, I ascertained that the Japanese and European forms have different DNA, which means that these mites also exhibit local endemism.

While conducting this research, I have paid particular attention to parasite co-evolution. Originally parasites and their hosts evolve in a kill-or-be-killed relationship, but when they continue in this over a long period, they eventually become tired out. Then they move to the next step, which is a symbiotic relationship. For example, the power of viruses becomes attenuated because their hosts are able to use their immune systems to control the viruses to a certain extent. Accordingly, symbiotic relationships represent the evolutionary end point of the battle between parasites and hosts.

What this means is that, in order to prevent pandemics and limit the disease damage that may be caused by various parasites in future, it is important to know the preexisting evolutionary histories of hosts and parasites. In other words, it is important to know where the original parasite evolved and where it lives. So in the case of the Tracheal Mite, I tried to find out about its origin and how it has evolved up to now by gathering bumblebees and the mites that parasitize them from all over the world.

I checked the DNA of each of the types of organism I found. A convenient point about molecular phylogenetic

analysis is that it allows us to guess at once the effective evolutionary times of organisms such as different mites and bees, which may be very different morphologically and taxonomically, because they have some similar genes. This method is beneficial in that we can get to know the effective evolutionary times of species by studying the genes that control identical characters in different species and calibration can be performed by means of the molecular clock method. Using this method, I located the region governing the assembly of the enzyme cytochrome c oxidase in the mitochondrial DNA of both mites and bees. By searching and analyzing the genes in this region I was able to construct an evolutionary tree for each species.

In this way, I was able to show that the evolutionary trees of both species were almost exactly the same shape. You can see that, in the case of the bees, there is a clear separation and differentiation between the bees of Eurasia and Asia on the one hand and those of America on the other. The evolutionary tree of the mites has an awkward shape as you can see. In short, the shapes of the trees of the two species do not match. What this means is that we can surmise that the bees and the mites did not evolve in the same way. In particular, we know that, compared with the Asian and Eurasian bees which have undergone a great deal of genetic differentiation, the mites that parasitize these bees exhibit only a very slight degree of genetic differentiation.

On the other hand, you can see that in America, the mites are parasitic on only one species of bee that has no mitochondrial DNA variation, but the mites themselves display very active genetic differentiation.

From the standpoint of diversity, in the case of Asia and Eurasia, the basic diversity index of the host bees is 0.04 while that of the parasitic mites is an order below that at only 0.005. On the other hand, in the case of America, the basic diversity index of the host bees is zero while that of the parasitic mites is 0.05, which is the reverse of the Asian and Eurasian situation. What this means is that these mites and bees have undergone different evolutionary processes and their origins are

also different.

Basically, a lot of evolutionary research has been carried out on the bees. They are considered to have originated in the central region of Asia and then rapidly expanded. As for the mites, from their present mitochondrial DNA tree, they are considered to have originated in America and, after spreading across that continent, they switched their hosts to bees and travelled to Eurasia.

In America this mite is parasitic on locusts so we can suppose that in some way it must have switched hosts to bees and then newly evolved into its present form of *Locustacarus buchneri*.

When a parasite switches hosts we often focus on the point that introduced alien species usually have a bad influence on native species. But actually, when alien species enter Japan, Japanese parasites also transfer to these alien species and begin evolving into new forms, and Japanese parasites are also capable of generating pandemics on occasion.

An example of this is a Japanese mite that has explosively parasitized alien bees despite the fact that this mite does not parasitize native bees very much. So parasites can utilize different strategies for living and, in many instances, they can more easily parasitize hosts that they have not co-evolved with.

After mites and bees, I turned next to stag beetles. Individuals of alien species in this family are imported into Japan in large numbers for commercial purposes, and their introduction has created problems. In Japan alone, there are many species of stag beetles, and throughout Asia the amount of speciation is greater still. The beetles shown here are all of different subspecies of a species called the Giant Stag Beetle (*Dorcus titanus*), which is distributed in Japan and across Asia. In order to know how much genetic diversity this species possesses, I made an evolutionary tree using mitochondrial DNA. As a result, I discovered that this single species encompassed a huge amount of genetic diversity. This one species exhibits clear differentiation in each region and each island throughout its range.

When we examine this in more detail, we find that it is differentiated into the 'big blue' family in the northern part and the 'big red' family in the southern part of its range, and at the root of this tree is the ancestor of all the subspecies of Giant Stag Beetle that exist today. In fact, the point of differentiation occurred 5.2 million years ago. Even species of such small insects achieve genetic differentiation over time by spreading out their ranges, and in the case of the Giant Stag Beetle this has created subspecies with a history spanning more than 5 million years. We call such a population an evolutionarily significant unit (ESU). In the near future, we will no longer be able to classify organisms merely as species, but we must also handle them as units in which we consider the evolutionary history of each genotype and its weight.

This next story concerns conservation. Again, I am going to turn my attention to mites. This time, I would like to talk about mites that parasitize beetles. There is a genus of mites called *Coleopterophagus* that live by attaching themselves thickly to the backs of beetles. I'm sure that people who keep beetles have seen these mites. Many beetle lovers hate them and brush them off the backs of their pet beetles with considerable enthusiasm. Because so many of these mites attach themselves to the back of a single beetle, the beetle looks poorly, which is why the common Japanese name of this mite is the 'kuwagatanakase', which literally translated into English means "beetle's bane".

However, these mites are merely scavengers. They are cleaners that eat the fungus that grows on the stag beetle's back or any waste matter that gets attached. So actually, these are very good mites as far as the beetles are concerned. For this reason, I would advise people who keep these beetles not to remove the mites. Moreover, the *kuwagatanakase* can only live on the backs of stag beetles. They would die even if they were placed on some other insect or given double the amount of waste to eat. For this reason, it is considered that the stag beetles and the mites that live on their backs have co-evolved.

This is a close up of one of these mites. The photo will

appeal strongly to mite lovers, but if you hate mites you probably won't be able to stand it.

I love mites more than beetles, so I want to know about the evolution of mites. Actually, these kuwagatanakase mites are not only found on Japanese stag beetles. They also live on stag beetles all over Asia. I performed a search of the beetles' DNA and, along the way, I also took a great deal of interest in the sort of trivia that only an acarologist would care about. I refer to things such as the amount of genetic diversity within the genus *Coleopterophagus*, the evolutionary history between the beetles and the mites, and place from where the kuwagatanakase mites originally came. In order to find out as much as I could I collected stag beetles from all over Asia and the mites attached to their backs as well. Using the information in their DNA I built up an evolutionary tree. This is a mitochondrial evolutionary tree of the stag beetle species living in Asia including the Giant Stag Beetle. Since the tree is so large, you can't read the writing on this slide, but please don't worry about that.

In order to complete this evolutionary tree, I had to read approximately 2,000 bases. From this evolutionary tree, we can see that the host species has a huge amount of genetic diversity. And here is the evolutionary tree of the mites that live on these beetles. Somewhat surprisingly, this species' genetic differentiation has also progressed to a great extent. Moreover, if we try to link up the relationship between the host and parasite, we can obtain a one-to-one relationship like this.

This means that the mites have not simply been drifting in their evolution and their mode of living. They have walked and lived together with their beetle hosts along an evolutionary history that stretches back for over 12 million years. The fact that different populations of these two species have been evolving together for such long time also indicates that these small mites also exist as evolutionarily significant units.

This is a computer graphic image of a Tracheal Mite. I do computer graphics as a hobby and I'm proud to say that this image is one of my own efforts. When I

have an opportunity to talk about mites, I purposefully put in a slide like this in an effort to educate people as to the splendor and beauty of mites. However, these efforts often come to grief as the bigger I make the slides, the more people say they feel sick. When I drew this image, I thought it was well done, so I sent it to the publisher of an international acarology journal in the United States and they used it on one of their cover pages. The publisher was very impressed with the image and assumed I was an artist. He invited me as an artist to attend the journal's 60th anniversary party and requested that I bring the original image with me. I felt frustrated that I wasn't recognized as an acarologist, so I told the publisher, "I am not an artist, I'm an acarologist!" and sent them one of my papers. They replied, "Never mind your paper! Just send your picture." Since the party was being held in Ohio and it was too much bother for me to travel there, in the end I sent the picture after all. This made the publisher very happy, and they turned it into a memorial postage stamp. So if you are in the United States, please look out for a memorial postage stamp with a mite pattern and buy it.

Another thing, when the COP 10 conference on biodiversity was held the year before last, the Emperor and Empress visited our research institute and I was one of the people guiding them around. At that time, I put a lot of pictures on the wall. The Empress showed interest and asked me what they were. I'm sure that up until then she hadn't seen any big pictures of mites. From that point, I became a bit hyperactive. I stopped talking about the institute and about biodiversity and instead I talked about mites for ten minutes. The Emperor and Empress seemed very impressed and they took one of the pictures back to the Imperial Palace with them. This became a historical monument as the first picture of a mite to be taken into an Imperial residence.

Recently I appeared on TV, where I had photos of myself taken with many entertainers. Among them, Shokotan (Shoko Nakagawa) took the most interest in mites. I told her a lot about how mites couple, and she responded that she would like to see mites coupling. So

I devoted all my time over Golden Week to completing a computer graphic image of two mites coupling. This is that picture. The larger mite is the female, and the other one, which is holding up its bottom, is the male. They take a very unusual sexual position. I gave this picture to Shokotan and she was very happy with it. Moreover, when former Environment Minister Ryu Matsumoto visited our institute, I also presented the same image to him, explaining, "This is a picture of two mites coupling." He casually brought it back with him, but he actually had it put up on the wall of his office. Since he later resigned in controversial circumstances, this is now a sad memory for me.

I've wandered off the subject, but to come back to point I wanted to make, even mites exhibit endemism and diversity. These days, however, with problems such as globalization and the introduction of alien species, the endemism and diversity of mites is in a critical situation.

This is a spider mite. These mites are agricultural pests. I myself researched this both in my student days and when I was working for the pesticide manufacturer. Spider mites also exhibit endemism and diversity, and this is also in a critical situation. As an example, this Two-spotted Spider Mite (*Tetranychus urticae*) is one of the many spider mite species and it is a serious agricultural pest on a worldwide scale. This species comes in two color varieties—a red variety and a green variety. This is a rare intra-species variation, which is considered to be part of an on-going process of genetic speciation. Accordingly, I have taken an interest in this process and tried to study the degree of differentiation of the intra-species variation. And to do this I have collected red and green mites from all over Japan and Europe and analyzed their DNA.

As a result, I was able to construct this evolutionary tree, and from it I found that the green mites became differentiated from the red mites, perhaps through pigment decolorization. Here, I would like to draw your attention to the fact that the red mites' genetic differentiation is unusually active even within Japan. Indeed, this differentiation has progressed almost to

the species level. What has caused this? I thought it was curious that such extensive genetic exchange was taking place in such a limited region as Japan. So I studied those individuals for which differentiation was particularly pronounced, and ascertained that they were all parasitic on carnations.

As these mites are specifically parasitizing carnations, it would be quite reasonable if they were genetically homogeneous. So why was there this much genetic variation among them? I studied this question and found out that most carnations are not grown in Japan but are cultivated abroad and imported and sold as cut flowers. These carnations are grown in many countries, and Two-spotted Spider Mites live in all of these countries. That's the reason why there is now so much genetic diversification among the Two-spotted Spider Mites that live in Japan.

In fact, I interviewed carnation farmers asking them where they purchased their carnations, and found that they imported them from many countries as shown here. Moreover, almost all the mites that exhibit genetic differentiation are of foreign origin. Clearly these mites were brought into Japan from overseas.

An even more troublesome thing is that because these mites are pests, their drug sensitivities differ from those of ordinary spider mites. When exposed to miticide, since the green type mites are very similar genetically, they all die straight away, and most of the red type monophyletic mites, which are the main variety in Japan, also die.

However, such miticides are totally ineffective against the spider mites that have been imported from abroad because these mites had sufficient genetic resistance to the miticide from the beginning. When this much genetic variation is so widely distributed within a group, there must be some mites that have resistance, and these survive and breed.

Since these mites are pests, they should have been prevented from entering the country by means of plant quarantine in the first place. So I investigated why so

many things (that should have been incinerated) were allowed to come into Japan. Now, the World Trade Organization is aiming for global free trade and a part of that effort is the Trans-Pacific Partnership (TPP). This organization, which promotes free trade, is promoting agricultural pests as well.

Originally, the Two-spotted Spider Mite was on the list of species for which importation into Japan was prohibited. However, in 2004, they were found on apples imported from the United States and the apples were incinerated. This provoked an angry response from the US side, which sued Japan through the WTO. The US argument was that since the Two-spotted Spider Mite already existed in Japan it was unreasonable to quarantine imports from the US on the grounds that they harbored the mites. Japan lost this case and as a result, was forced to abolish the quarantine measures for the Two-spotted Spider Mite, allowing this species to be imported freely into the country.

This is a very inconsistent approach. As I explained earlier, perhaps these mites do look the same but they have very different genes and their sensitivity to drugs also varies widely. Now they are freely imported. And although most of us are unaware of the fact, the liberalization of agricultural pest species has been proceeding apace.

The Plant Protection Act was finally revised in the spring of last year. Under the revised law, Japan has moved from a 'white list' system to a 'black list' system. Under the white list system, the importation of all pests that damage plants was banned in principle. Only those species of insects, etc., that were on the white list (such as stag beetles and unicorn beetles) were considered safe to import. The white list was a very strict regulation. By contrast, the black list system operates on the principle that all species can be imported unless they have been examined and specifically placed on the black list. So only those species considered to constitute a risk are prohibited from being brought into the country. Any species not on the black list can be freely imported. This is the same system as specified by the Alien Species Act. A consequence of the change

to the black list system is that the release ratio of agricultural pests has become higher. Since only those species deemed to present a risk are listed, almost all agricultural and forestry pests can now be freely imported.

The TPP, which has become a major subject of talk, presents us with the same situation. One government minister has described the TPP as the 'third opening of the country'. Whenever the country is opened the number of alien species increases. So if we think about protecting biodiversity in a true sense, in future, we should pay a lot of attention to this TPP argument from the ecological science and animal hygiene standpoints.

As a case example, I'd like to talk about a dangerous mite that is brought into Japan together with imported living organisms. Up to now I have been talking about rather mild things unrelated to human health. But what I'm going to talk about now are ixodid - hard-bodied ticks - which feed on the blood of animals and spread various diseases. The most dangerous ones can transmit zoonoses to humans.

In the current situation, there is concern that with the large numbers of reptiles being imported into Japan and with almost all of them taken from the wild, many kinds of ixodid ticks are being brought in with them. What is even more troubling is that legal protections such as guarantees on the safety of imported animals mandated under the Infectious Disease Law by the Ministry of Health, Labour and Welfare and animal quarantine provisions are limited to warm-blooded animals. Reptiles and amphibians can be brought into Japan without any guarantees at all. I was concerned about this so I began a project to study the matter jointly with Prof. Kawabata of the National Institute of Infectious Diseases. In the course of this research, as I had expected, we found ticks of the genus *Amblyomma* on turtles and many kinds of mites attached to reptiles imported into Japan.

Moreover, the number of countries from where these living things are imported is surprisingly large, including such widely separated places as Sri Lanka,

Tanzania, and Suriname. I wasn't even sure of precisely where some of the countries of origin were so, due to this research, I also had an opportunity to study geography. What especially worried me was that the mites themselves might be carrying some dangerous pathogens so I asked the NIID to do some testing. The results came back positive. We discovered the presence of the bacterial genus *Borrelia* which can infect humans with many diseases. I studied the genetic differentiation of this genus and produced an evolutionary tree. I noticed that the genus includes a species that is the agent for Lyme disease and a species that causes tick-borne relapsing fever. This information was so complete that I was able to make use of it in conjunction with the evolutionary tree. The new *Borrelia* bacteria we discovered were not members of either of the above species. They are a totally new type. This is a very dangerous situation because nothing is clear including the pathogenicity and, even more troublingly, no risk evaluation has been made of these bacteria because it has not been possible to grow them in isolation up to now. In view of this I have to say that, under the current free trade situation, people who want to keep reptiles as pets need to seriously consider the potential risks they are taking.

For my own research I previously chose only visible large alien species. But now I am paying attention to invisible alien species as well. What set me off in this direction was a disease called chytridiomycosis, an infectious disease of amphibians that is often fatal. Perhaps you have heard of it. Chytridiomycosis is an emerging disease that has spread worldwide and wiped out entire species of frogs in some places. It landed in Japan several years ago and caused considerable uproar. I dislike frogs but I worked on this theme out of necessity, and I was shocked by my results. I don't have time to go into the details now, but I'd like to tell you about it when I get an opportunity.

As Prof. Nakamura said at the beginning, the reason why such emerging diseases that are close to being zoonoses are being encountered more often now is because biodiversity is being gradually destroyed.

Parasites and their hosts establish peaceful and stable relations after a long period of co-evolution. Biodiversity is a cradle in which these organisms can grow and develop together, but it can also serve as a cradle for pathogens. When human beings destroy this biodiversity there follows a collapse of evolutionary history and co-evolution, and in its place, pandemics occur. Examples of this process at work include SARS and HIV/AIDS. They have their respective origins in horseshoe bats in China and in chimpanzees in the Cameroon, and were living quietly with their hosts. The reason why they turned into emerging diseases is that humans destroyed the habitats of the hosts, allowing the viruses to cross the species barrier. Now the viruses are desperately evolving in order to survive in their new hosts, human beings, of which there are about 7 billion individuals at present. They have already arrived in the urban jungle.

The important thing here is that, in line with the evolutionary rule that has always been followed until now, hosts that are infected either die or else they survive. Those that survive are more likely to pass on their genes to the next generation. In this way, succeeding generations have better resistance to the effects of the virus and the two species gradually enter into a symbiotic relationship. Today, however, we attempt to contain dangerous viruses by any means available including the use of antiviral drugs and other pharmaceuticals. The result is that the viruses continue to evolve rapidly in response to the drugs they are exposed to. This becomes a non-stop fight between humans and viruses. Only humans break this evolutionary rule. When we consider the evolutionary history of viruses there is a high possibility that humans will lose this fight one day.

So, if we want to stop this fight, it is important for humans to keep at a distance from biodiversity from now on. The idea of "natural symbiosis" or "living in harmony with nature" is often talked about. But this does not mean it is OK for owners to kiss their pets or something like that. Humans have evolved as naked apes who can survive in the concrete jungle so when we talk about 'natural symbiosis' it is important to apply

some kind of zoning between the wild world and the human world and to properly separate their functions.

In that sense, protecting biodiversity has another important meaning. It can serve as a means of preventing the expansion of emerging infectious diseases. Biodiversity does not exist only to be applied to beautiful living things. Even parasites are encompassed within biodiversity. So please extend your support to the mites and ticks too.

That completes my talk. Thank you very much for listening.



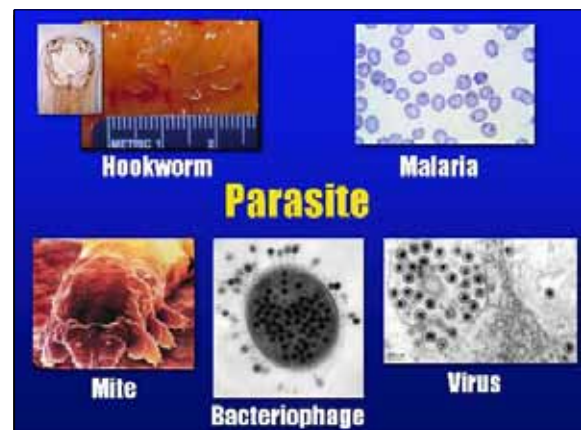
【Slide 1】



【Slide 2】



【Slide 3】



【Slide 4】

The Red Queen Hypothesis

赤の女王仮説

次々と姿を変えて襲って来るウイルスに対抗するためには、遺伝子の交換によって遺伝的多様性を維持するしかない！
In order to face the virus attack, we need to keep genetic diversity by exchanging gene!

from *Through the Looking Glass*, by Lewis Carroll

たがし、あくまでも、寄生生物のバリエーション(維持される理由)を説明しているだけで、寄生生物の起源を説明するものではないことに留意……

【Slide 5】

腐食性 Scavenger
昆虫寄生性 Insect parasitoid
海産 Marine mites
動物寄生性 Hematophagous
植物寄生性 Herbivora
捕食性 Predator
淡水性 Fresh water

【Slide 9】

すべての生物種には何らかの寄生生物種が棲息する。
All species are somehow parasitized

寄生生物種は、生物多様性の大部分を占める
Parasitic species cover most of biodiversity

【Slide 6】

私がダニの研究を始めたとき

1986年3回生実習
When and why did I start to study acarology...

【Slide 10】

I am an Acarologist!

私の専門は実はダニ学です！

【Slide 7】

世界的な重要農業害虫
ハダニ類
The serious pest
Spider mites

【Slide 11】

ダニとはどんな生き物か？

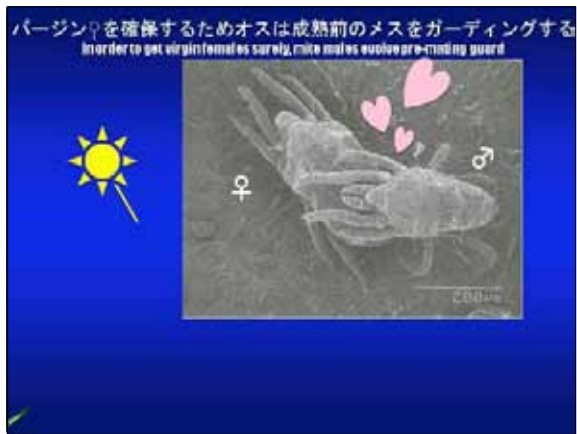
What is Acari?

- 昆虫ではない
Diferent from insects
- 体は一対で、昆虫のような脚はない
Only one body without six legs
- 足は4対8本
4 pair legs
- 食べ物も生活史も様々
Hosts and life cycles vary very much
- 種数は現時点でわかっているものが5万種
Species number already described are more than 50,000
- 未発見のものを含めると100万種はいるかも…
Total species number including un-described may be 1 million

【Slide 8】

雄成虫 (n=3) Adult male
雌成虫 (2n=6) Female adult
卵 egg (1個あたり50-200)
幼虫 larva
第一静止期 1st pupa
第一若虫 1st nymph
第二静止期 2nd pupa
第二若虫 2nd nymph
第三静止期 3rd pupa
25°Cで約10-14日間

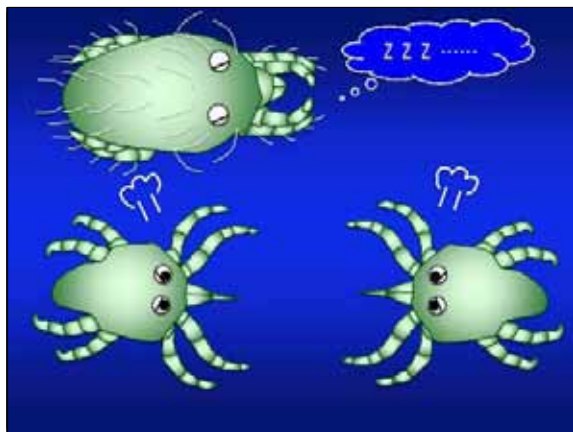
【Slide 12】



【Slide 13】



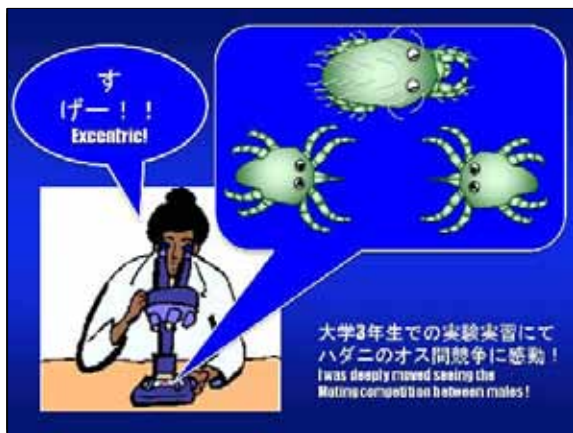
【Slide 17】



【Slide 14】



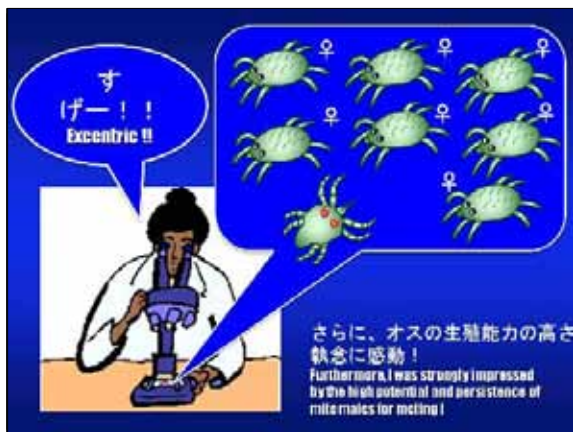
【Slide 18】



【Slide 15】



【Slide 19】



【Slide 16】



【Slide 20】



【Slide 21】

Co-Evolution of Host and Parasite

宿主-寄生物間の共進化・共通応
 Co-evolution and co-adaptation between host and parasite
 宿主と寄生物の間での敵対はコストとリスクを生み出す。
 Conflict between host and parasite would cause cost and risk.

宿主-寄生物間の共生関係
 Symbiosis between host and parasite
 宿主の免疫機構や寄生物の弱毒化が進化する。
 Host immunity and parasite virulence would evolve.

宿主-寄生物間の共種分化の歴史
 Co-speciation history between host and parasite
 寄生物の起源、本来のhabitatを知ること。
 Biogeography of origin and natural habitat of parasite.

寄生物の感染源究明と病害の予防
 Prevention of pandemic and virulence of parasite
 寄生物との共存・共生を関る上で重要。
 Significance for introduction and synthesis with parasite.

【Slide 25】

European *Bombus terrestris*
 ヨーロッパ産
 セイヨウオオマルハナバチ

Bombus diversus
 トラマルハナバチ

外来種 Native

Bombus hypocyrtus
 オオマルハナバチ

外来種 Alien

在来マルハナバチ
 に対する悪影響？
 Negative impact
 against native species?

【Slide 22】



【Slide 26】



【Slide 23】

Merit of molecular phylogenetic tree
 分子系統樹の利点

Gene regions coding a same trait
 同一形質を支配する遺伝子領域

Similarity of the rate in nucleotide substitution
 塩基置換率の近似

Quantitative comparison between speciation histories
 in host and parasite in the same time-scale
 宿主-寄生物間で種分化の歴史を同じ時間スケールで定量的比較可能

【Slide 27】

マルハナバチポリブダニ
Locustacarus buchneri

- マルハナバチ類にのみ寄生
 A parasite specific to bumblebee species
- 世界中に分布しているが感染率は低い
 Though world-wide distributed, the prevalence of infestation is generally low
- 日本のマルハナバチにも感染している
 The mite was detected also in the Japanese bumblebees (Goka et al., 2000).
- ダニのミトコンドリアDNAには地域変異がある
 There is geographic variation in mitochondria DNA of the mite (Goka et al., 2000).

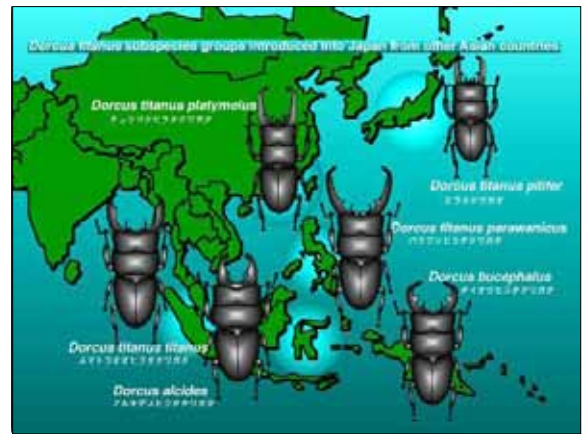
【Slide 24】



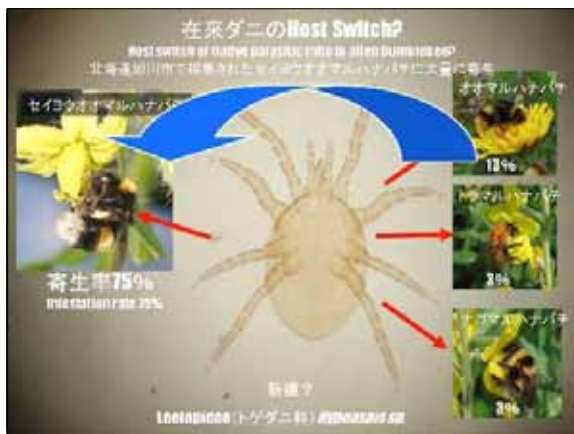
【Slide 28】



【Slide 29】



【Slide 33】



【Slide 30】



【Slide 34】



【Slide 31】



【Slide 35】



【Slide 32】



【Slide 36】



【Slide 37】



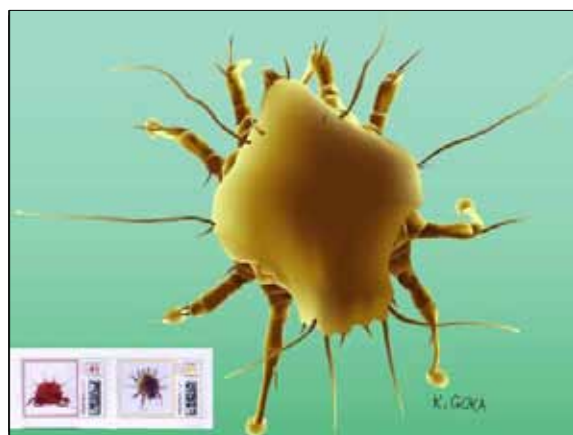
【Slide 41】



【Slide 38】



【Slide 42】



【Slide 39】



【Slide 43】



【Slide 40】



【Slide 44】

World Trade Organization : WTO

世界貿易機関 153の国と団体が加盟

- 自由貿易の促進を主たる目的とする
Main objective is promotion of free trade
- 貿易に係る国際紛争を処理する
Conducting international disputes for trade
- モノの貿易だけでなく、サービスや知的所有権を含めた世界の貿易を統括する機能を持つ
Unifying international trade not only of materials but also of services and intellectual property rights.



【Slide 45】

Conventional Plant Protection Law

White List System

Import restricted species list
Basically all alien species must be restricted



Assessment

Import permitted species list = White List
Only species decided as safe can be introduced



【Slide 49】



2004年WTO裁定
2004 WTO decision
ナミハダニはCosmopolitanであり、日本にも生息していることから検疫の必要は無い!
There is no need for restriction because the mite is cosmopolitan and actually exists also in Japan!

ハダニの侵入を許す必要はない
No need for mite introduction

ニュージーランド

【Slide 46】

Invasive Alien Species Act

Black List System

All alien species not yet assessed can be introduced freely



Assessment

All the rest species can be introduced freely

Import restricted species list = Black List

Invasive Alien Species



【Slide 50】

Nowadays, Plant Protection of Japan has released the regulations for introduction of plant pest species, one after another...

現在、植物防疫法では次々に害虫種が植物防疫措置の規制対象種から解除されている。

【Slide 47】

Trans Pacific Partnership: TPP

環太平洋戦略的経済連携協定



第3の開国!?

The 3rd opening of Japan!?

国際経済自由化のpressureから、如何にして生物多様性を守るか?

【Slide 51】

Notification of revision of the Plant Protection Law

輸入植物検疫の見直しのための植物防疫法施行規則の一部改正等について (平成23年4月1日)

Ministry of Agriculture, Forestry and Fisheries ● 農林水産省

農林産物貿易の多様化や国際流通の迅速化に伴い、
We changed the quarantine system for agricultural pests in accordance with globalization.
より一層厳格なことを旨に、平成23年4月1日に「植物防疫法施行規則」の改正等を行いました。

我が国に侵入した場合に国内農業に大きな被害をもたらす可能性のある病害虫を検疫の対象としてリストに明示します。
一方、国内に広く分布しており農林業に新たな影響を及ぼさないものは、
We removed the conventional "White List System", and newly adopted "Black List System" for Plant Protection.
その特徴や危険度に応じ、輸入禁止の対象とする地域及び植物の見直しを行う。輸出国に対し検疫措置（栽培地検査、船舶検疫及び検疫検定）を新たに要求するなど、適度な植物検疫措置を導入することにより、輸入植物検疫を強化します。このような輸入植物検疫の見直しにより、国内農林業に大きな被害を及ぼす可能性のある病害虫の侵入をより適切に防止します。

【Slide 48】

輸入爬虫類に寄生するダニ類の侵入リスクと影響

The risks of ticks parasitizing introduced pet reptiles

キララマダニ *Amblyomma sparsum*

哺乳類・鳥類・爬虫類に寄生
parasitize mammals, birds and reptiles



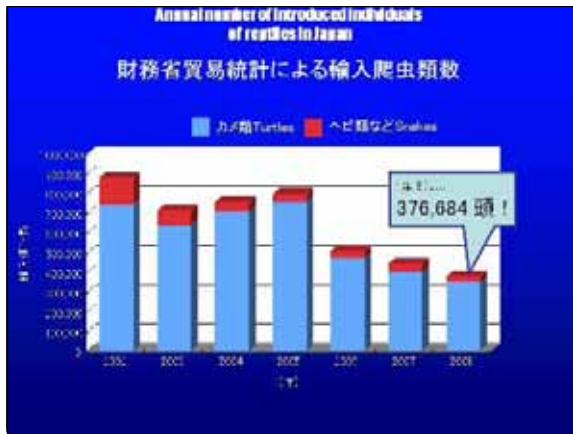
心水症ウイルス
Heartwater disease virus

Q熱リケッチア
Q fever fever borrelia

ライム病病原体 (ボレリア)
Lyme disease borrelia

Takano, A., K. Goka, Y. Ume, Y. Shimada, H. Fujita, T. Shino, H. Watanabe & H. Kawahara (2010) Environmental Microbiology, 12: 124-140.

【Slide 52】



【スライド 53】



【スライド 57】



【スライド 54】

寄生生物の多様性の危機と Pandemic
The crisis of biodiversity in pathogens and pandemic as consequence

- 近年の新興感染症Emerging Diseasesの感染爆発Pandemicの背景には生物多様性の崩壊がある。
The collapse of biodiversity has caused the pandemic of emerging diseases in these days
- 野生生物と病原体の間には永きにわたる宿主-寄生生物共進化関係が存在する。
Wildlife and pathogen have constructed host-parasite relationships through a long co-evolution
- 生物多様性は病原体微生物のゆりかごでもある。(Daszak, 2006) Diversity is a cradle for pathogenic micro-organisms
- 野生生物の生息地の破壊と生物移送が、共進化の歴史を崩壊させ、病原体微生物は新たな住処を求めて宿主転換Host SWITCHを繰り返している。
Natural habitat destruction and transportation of wild-life have caused collapse of history of co-evolution between host and parasite

【スライド 58】

病原体検査
1. PCR / Cultivation: Imported wild reptiles from foreign countries

試料名	種名	検出された病原体	検出されたウイルス	検出された細菌	検出された真菌	検出された原生動物	検出された寄生虫
001	Chelonia mydas	Chelonia mydas	None	None	None	None	None
002	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
003	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
004	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
005	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
006	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
007	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
008	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
009	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
010	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
011	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
012	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
013	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
014	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
015	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
016	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
017	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
018	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
019	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
020	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
021	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
022	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
023	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
024	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
025	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
026	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
027	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
028	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
029	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
030	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None

2. PCR / Cultivation: Wild reptiles before exporting from foreign countries
Collection (Biodiversity/Animal/3/18/07/05)

試料名	種名	検出された病原体	検出されたウイルス	検出された細菌	検出された真菌	検出された原生動物	検出された寄生虫
031	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
032	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
033	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
034	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
035	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
036	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
037	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
038	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
039	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
040	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
041	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
042	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
043	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
044	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
045	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
046	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
047	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
048	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
049	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None
050	Basiliscus vittatus	Basiliscus vittatus	None	None	None	None	None

【スライド 55】



【スライド 59】



【スライド 56】



【スライド 60】