

# **Reprinting 2 Papers on Speciation**

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Jpn. J. Environ. Entomol. Zool. 15: 189-195, 2004 (In Japanese)

**Translated from the Japanese**

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# Reprinting 2 Papers on Speciation

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## Introduction

In 1984, the author stated: “If females and males showing no genetic exchange with their original race emerge and establish genetic exchange among themselves, and their offspring maintain this characteristic, a new species will develop without geographical isolation.”, and **“The central problems in speciation mechanisms are the contents of variations preventing genetic exchange such as infertility or absence of mating and their background information in molecular genetics.”** To examine such variations, the author evaluated the possible mutations of sex pheromones, chemicals determining the sites of copulation, and their receptors (Title: On Some Ecological Problems in Japanese Butterflies 3. Mechanisms of Speciation and Directions of Character Evolution).

The paper was reported as a follow-up to 2 previous papers discussing interspecies relationships among closely related species that had interested the author since his school days (Kyoto University): On Some Ecological Problems in Japanese Butterflies 1. Presence or absence of competition among species with similar ecological niches (Tsuji, 1957), and 2. Discussion on relationships among closely related species, particularly competition and evolution (Tsuji, 1958).

Here, the 3 papers are referred to as the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> papers.

## The 3<sup>rd</sup> paper published

### by the KSK Institute for Environmental Biology

The above “Mechanisms of Speciation and Directions of Character Evolution” was a paper not related to the author’s occupation in those days (development of agricultural chemicals in a company). In addition, for paper submission, the superior’s

permission was necessary (and actually the permission was given). Therefore, 400 copies were printed at the author's own expense (KSK Institute for Environmental Biology), and about 200 copies were distributed (free or for a fee) and delivered to the National Diet Library. The 3<sup>rd</sup> paper was combined with the 1<sup>st</sup> and 2<sup>nd</sup> and related papers previously reported (such as an abstract of the author's doctoral dissertation on *Plodia interpunctella* (Hübner) and a paper on variation and pesticide resistance of aphids) to produce a booklet. This booklet has been useful for people who are interested in the ecological evolution of insects. However, particularly because the 3<sup>rd</sup> paper was not published in journals, distribution may be still inadequate to receive comments from a wide range of people.

### **The 4<sup>th</sup> paper published on a home page**

Since the 3<sup>rd</sup> paper was written for entomologists, most findings at the molecular level as the basis of the author's hypothesis were omitted. However, the hypothesis was established based on studies on insect sex pheromones or insecticides, which were most extensively performed in those days, particularly insects' resistance to insecticides and molecular genetic findings. A paper on the process from these findings to the establishment of the hypothesis had been separately written. This paper (4<sup>th</sup> paper) was also not submitted to any journal for the same reason as that for the 3<sup>rd</sup> paper, and also because it did not fulfill the requirements of various scientific societies in those days, generally requiring verified data. However, the author considered that this paper was too useful to be forgotten, and so published it on his home page from 2000 to 2001 (The 3<sup>rd</sup> paper was republished on the same homepage.

<http://homepage2.nifty.com/ksktsuji/>).

### **Reprinting in this journal (Jpn. J. Environ. Entomol. Zool.)**

Both the 3<sup>rd</sup> and 4<sup>th</sup> papers are hypothetical, and may not be appropriate for reporting in general scientific societies that attach importance to verification. However, if these papers can be reprinted in the Journal of the Japanese Society of

Environmental Entomology and Zoology, comments from more researchers will be obtained, which will contribute to the advancement of related discussions and empirical research in the future.

The following are reprints of the 3<sup>rd</sup> and 4<sup>th</sup> papers, and the author hopes to receive comments from many researchers.

Reprinted 3<sup>rd</sup> paper (Original paper without changes)

## **On Some Ecological Problems in Japanese Butterflies**

### **(3) Mechanisms of Speciation and Directions of Character Evolution**

By Hideakira Tsuji (KSK Institute for Environmental Biology)

(Published in 1984, in Japanese)

In connection with the 1<sup>st</sup> and 2<sup>nd</sup> reports, a few notes have been added, although fragmentary.

### **Mechanisms of Speciation**

#### **Geographical isolation:**

Allopatric speciation occurs when a biological population of the same species is separated into 2 or more populations due to geographical isolation preventing mixing, and they eventually diverge into different species. This mode of speciation is easy to understand. When the two geographically isolated populations meet again, genetic exchange between them should be impossible. For this, a variation in either or both regional populations during geographical isolation is necessary to occur.

However, there is no guarantee that this variation as a result of geographical adaptation always makes genetic exchange between the two populations impossible.

Therefore, it is important to determine the types of variation which make genetic interchange impossible.

#### **When geographical isolation is absent:**

If there is no genetic exchange with the original race due to a minor variation, genetic exchange is established between males and females within the population, their offspring maintain this characteristic, and a new species may emerge without geographical isolation. This possibility is evaluated.

**(Case 1)** Whether or not a “pheromone”, which is a chemical controlling mating behavior, is effective is associated with small changes in its molecular structure (for example, Ishii, 1969). There is a possibility that such changes occur due to minor

mutations of genes for pheromone production (genes for the production of enzymes involved in pheromone production) without unfavorably affecting other living functions. Similar changes may occur in the receptor molecules of sensory cells of individuals of the opposite sex receiving the pheromone. Since there has to be a correspondence between a pheromone and its receptors at the molecular level, the subtler the change, the greater the possibility of mutations corresponding to each other.

As a result, mutants mate with mutants, and do not mate with individuals of their original race. The offspring of these mutants are indistinguishable from the original race in external appearance and other characteristics, but the 2 populations (It may be more appropriate to call them 2 species.) start to compete each other without genetic exchange. The competition pressure may promote ecological, physiological, and morphological changes, leading to habitat segregation.

**(Case 2)** What happens if male and female insects select a mating space completely differing from the conventional one? There is a possibility that insects, which select mating spaces by reacting to chemicals of specific plants and lay eggs there, develop a negligible variation at the receptor molecular level of sensory cells, select a different plant, and mate and lay eggs there. In this case also, mating with the original race does not occur. If even some of the larvae can grow on the plant, the plant becomes a type of forced selection, and a host plant for larvae as secondary adaptation. Thus, the larvae will give rise another species (host plant race) with a host plant different from that for original race.

Conversely, even if a specific food plant is used to select adaptive larvae, adult insects may not always be selected to adapt to the plant to lay eggs on it.

The possibilities of 2 extreme cases were discussed above. Similarly, variations affecting visual or auditory systems may also be important in association with mating. The central problems in speciation mechanisms are the contents of variations preventing genetic exchange such as infertility and absence of mating and their background information in molecular genetics. Spatial isolation is important but only one of the environmental factors.

## **Directions of Character Evolution**

### **Aggregation of larvae:**

Some of butterfly species lay eggs in clusters, and their larvae live together in aggregations. It is unlikely that dispersed larvae begin to aggregate, and, subsequently, mothers begin to lay eggs in clusters. It can be speculated that laying eggs in clusters by mothers occurred first, and, due to the benefit, larvae continued to live in aggregations.

Based on the synchronic and sympatric growth of larvae, the primary benefit of larval aggregation may be a more effective utilization of the growth/mating period and space with narrow optimal ranges. This is supported by the fact that larvae of species and races living in colder regions show a more marked aggregation tendency.

Individual solitary rearing of larvae of such the species is frequently disadvantageous for their growth. This finding itself does not clarify the meaning of or reason for aggregation. It is rather considered to indicate secondary adaptation through the loss of characteristics needed for solitary living by species requiring aggregation.

### **Size of adult insects:**

In the relationship among related species showing overlapping host plants (4A · C · D types among the 5 types in the 2<sup>nd</sup> report) and that among geographical races within a species, the days required for growth per generation greatly differ depending on whether or not the annual number of generations is the same. As a result, the following relationships can be established:

A: The annual number of generations during the year is the same.

→ The number of days required for growth per generation is similar.

→ In low-temperature regions, early sexual maturation relative to the body size is observed.

→ The adult insect size is smaller for species and races living in lower-temperature regions.

B: The annual number of generations differs.

→ The number of days required for growth per generation differs.

→ When the annual number of generations is small, slow sexual maturation relative to the body size is possible.

→ The annual number of generation decreases, and the insect size becomes large, as it does in lower-temperature regions.

The establishment of the above relationships A and B also shows that the rate of synthesis of macroscopic components of the insect body is temperature-dependent, and does not markedly differ among related species or races at the same temperature.

Prof. Masaki (1974) performed a similar study in omnivorous crickets and provided useful information. There have also been studies in other animal species. For example, the size of long-armed prawns at the time of maturation was reported to be smaller in upper-stream than estuarine individuals (Mashiko, 1983). In butterflies, it is particularly important that there are related species that completely differ in host plants. These should be classified as different categories.

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The above is a paper on pages 23-26 of the following book:

On some Ecological Problems in Japanese Butterflies (published by the KSK Institute for Environmental Biology in 1984) 41 pp. (In Japanese)

Reprinted 4<sup>th</sup> paper (original text without changes)

## **On Some Ecological Problems in Japanese Butterflies**

### **(4) How One Species Makes Two – Facts and Considerations on Pheromone Mutations and Speciation**

By Hideakira Tsuji (KSK Institute for Environmental Biology)

(A Review of The Paper of 1984)

This paper provides discussion supporting the author's previous paper, "On Some Ecological Problems in Japanese Butterflies: 3) Mechanisms of Speciation and Directions of Character Evolution".

### **Effective insecticides and ineffective analogues**

Even analogues of effective insecticides are often ineffective. Concerning the effects of carbamate as an insecticide against rice leafhoppers, the following relationships are observed:

Substance A: R-CH<sub>3</sub> Very effective

Substance B: R-CH<sub>2</sub>CH<sub>3</sub> Ineffective

Substance C: R-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> Ineffective

The target of the A molecule (effective insecticide) is an important enzyme in the body (Note 1). This enzyme is a protein molecule. A part of the molecular structure of the insecticide binds to the active site of this protein molecule, resulting in stable inhibition of the enzyme action. The B and C molecules only slightly differ from the A molecule in the structure, but their binding to the target enzyme is difficult.

(Note 1) This enzyme is acetylcholinesterase, which degrades acetylcholine that transmits nervous excitation. When the action of this enzyme is inhibited, abnormal nervous excitation occurs, and animals (insects) die due to severe conditions such as dyspnea.

## **Emergence of leafhoppers resistant to insecticides**

When insecticides are frequently used, resistant insects often increase. Green rice leafhoppers (*Nephotettix cincticeps*) that are resistant to the A insecticide appeared. Evaluation of the insecticide-resistant green rice leafhoppers showed a genetic mutation of the enzyme as the insecticide target, preventing the A insecticide from binding (Hama and Iwata, 1971; Hama and Iwata, 1973; Iwata and Hama, 1972; and Hama, 1976).

Similar mechanisms of the emergence of resistance to insecticides are often observed and also known in spider mites and flies. An enzyme is a protein, and the sequence of amino acids forming its structure is determined by the DNA code (sequence of base pairs). Therefore, the emergence of resistance suggests a mutation in the DNA code. The mutant enzyme preserves its original function (degradation of neurotransmitters), and green rice leafhoppers proliferate, presenting a problem to farmers. Thus, the gene mutation is negligible and does not affect insects' ability to survive.

A single mutation (point mutation) in the DNA base sequence has been reported to induce tolerance to chloramphenicol as an antibiotic in yeast (Dujon, 1980) and rat and human cells (Kearsey and Craig, 1981; Blane et al., 1981). Therefore, the mutation in green rice leafhoppers can also be very small.

The mutation site of the enzyme in green rice leafhoppers is involved in the reaction with inhibitors (insecticides). However, this site is not the original site of the enzyme for degradation of the neurotransmitter (acetylcholine ester) (Hama et al., 1980). Therefore, a mutation may have occurred at the site involved in initial contact when the insecticide binds to the original site of the enzyme for inhibition.

## **The C compound is effective against the enzyme for which the A compound is ineffective**

Surprisingly, the C compound, which had been ineffective against the enzyme of green rice leafhoppers, was effective against the mutant enzyme of resistant green

rice leafhoppers. Binding of the A compound to the mutant enzyme was difficult, but the C compound bound to this enzyme, resulting in inhibition (Yamamoto et al., 1977). Since the molecular structure only slightly differs between the A and C compounds, the difference in structure between the original and mutant enzymes may also be very small.

Such an inverse relationship in effects between the A and C compounds has been observed between other insecticides. Propaphos as an organic phosphoric acid ester (P insecticide) was ineffective against the enzyme of green rice leafhoppers for which propoxur as a carbamate insecticide (A insecticide) was effective. However, as a result of using the A insecticide, green rice leafhoppers became resistant to the A insecticide, and the P insecticide was effective against the enzyme of these leafhoppers (Iwata and Hama, 1981). In not only enzymes but also insects, there was an inverse relationship between the A and P insecticides. In addition, after selection using the P insecticide, the insects return to the state of the original insects, against which the P insecticide is ineffective, and the A insecticide is effective.

### **Mixture of A and C insecticides increases effectiveness**

Concerning the relationship between A and C insecticides, it is of further interest that an A-C mixture had more marked effects on resistant green rice leafhoppers for practical use (Takahashi et al., 1977).

This suggests that not only a reaction between the compound and molecules of the organism but also that further combination effects occur even if individual mutations are very small (under certain conditions such as multiple types of mutation).

### **Substances for communication between males and females**

The above findings suggest that not only marked but also negligible differences in the molecular structure of insecticides are recognized by the protein molecules of the organism, resulting in ineffectiveness. However, even an ineffective compound becomes effective due to a small variation of the enzyme molecule of the

organism, and such the variation is hereditary. This fact is consistent with individual hereditary differences in human gustatory sensations for certain compounds and human eye perception for certain colors.

What occurs if there are differences not in the insecticide or antibiotic molecule but in physiological factors such as compounds (such as sex hormones) to recognize species and guarantee copulating and mating or acoustic sensations?

Butenandt et al. (1961) was the first to clarify the chemical structure of a substance that is released by adult female silkworms and perceived by adult male silkworms, leading to copulatory behavior. It is important that differences in the 3-dimensional structure of the compound lead to ineffectiveness, even if the 2-dimensional structure is the same (Butenandt and Hecher, 1961; Truscheit and Eiter, 1962). Subtle differences in the compound cause a marked reduction or loss of effects, which have also been observed in subsequent studies on pheromones (Ishii, 1969; Yushima, 1976; Natamura and Tamaki, 1983).

## **Mutations associated with pheromones**

It can be speculated that mutations occur in the structure of compounds such as pheromones released by organisms themselves as well as the characters such as the eye or skin color or substances and their structure determining such characters. There is a possibility that gene mutations cause slight changes in the ability to perform living activities without adverse effects.

Of course, DNA encoding genetic instructions does not directly synthesize pheromones, and a series of enzymes are involved. However, since enzyme proteins are synthesized based on the information in DNA, subtle mutations in the pheromone structure may occur due to subtle gene mutations.

The more subtle the pheromone mutations are, the more readily changeable sites in the structure may change. In other words, there should be a tendency based on the susceptibility of sites to changes (For example, isomerization,  $R-OH \rightarrow R-CHO \rightarrow R-COOH \rightarrow R-COOR$ , and  $R-CH_2-R \rightarrow R-C_2H_4-R$ ). As a result, there is a possibility

that the same mutation frequently occurs (For example, the same mutation may develop in many individuals in different regions).

Even if this mutation is very small, its consequences are serious. In the natural state, mutant individuals (population) do not mate with individuals of the opposite sex of their original population. Thus, genetic exchange disappears.

### **Mutations in individuals perceiving pheromone**

Based on the above-described mutations in the enzyme that perceives insecticides, mutations are also expected to occur at the perception sites of sensory cells (receptors, possibly the membrane structure or the molecular structure of the enzyme) in individuals of the opposite sex perceiving pheromones. In this case, there is a certain tendency based on the susceptibility of the sites to changes.

Since there may be a correspondence between pheromones and receptors functionally and structurally at the molecular level, resembling the correspondence between insecticides and the enzyme (or membrane structure) perceiving them, the more subtle mutations are, the more likely corresponding mutations in both occur. “Correspondence” means that mutant pheromones and mutant receptors effectively act with each other. Therefore, mutant individuals (population) mate with mutant individuals (population), forming a race, and do not mate with their original race.

### **Effects of a mixture of multiple pheromones**

Based on the above-described effects of a mixture of 2 types of insecticide, it seems that mutations associated with the effects of a combination of 2 types of chemical compound or more may also occur. Indeed, previous studies showed the effects of a mixture of pheromones and differences in insects’ reactions according to their mixture ratio (for example, Tamaki et al., 1971), the presence of multiple pheromones in a higher percentage of insects, and differences in the ratio of the same mixture to which related species showed maximum reactions (Yushima, 1976; Fukami, 1986).

The production of 2 or more types of sex pheromone, development of sensitivity to these pheromones, and changes in the optimal mixture ratio can result from mutations of organisms themselves without geographical isolation. Essentially, multiple pheromones can be discussed in the same way as a single pheromone. In this case, the utilization of 2 extremely similar compounds such as isomers may be a phenomenon that occurs naturally. In addition, since there are numerous combinations and mixture ratios, the types of population occurring due to speciation are expected to be abundant.

### **Characters of 2 indistinguishable populations**

Since individuals in a pheromone-associated mutant race originally belonged to the same species, they cannot be distinguished based on the external appearance or other characteristics from individuals of the original race. However, since this population does not mate with their original population, it should be regarded as another species.

Although indistinguishable, characteristics other than pheromone-associated ones cannot be strictly the same. Even in the same species, there are slight differences in genetic characters among individuals. However, in the same species, mating occurs, character similarity between individuals is maintained, and this similarity is given a direction by natural selection.

In the above 2 populations that do not mate with each other, there is no guarantee that their genetic similarity will be maintained. Although differences between the two populations may increase, they are unlikely to decrease. In addition, the presence of the other population itself becomes a pressure as a natural selection element, which is of critical importance. Thus, character differentiation including an increase in infertility between the two populations may be inevitable.

### **Superiority or inferiority of 2 populations not mating with each other in a simple environment**

The number of individual insects of one type living in a certain environment fluctuates annually, but does not infinitely increase, and remains within a certain range. When 2 apparently indistinguishable populations that do not mate with each other emerge in the same environment as a result of the above mutation, the finite number of individuals in the environment should be shared by the 2 (old and new) populations (species). What is the ratio of the two? In the new population, the general ability to perform living activities may not have changed or even be superior. It is important that genetic mutations are present in individuals, and there are minor differences between the two populations.

In the short term, the ratio of the numbers of individuals in the two populations in each environment may be determined by chance. However, in the long term, in addition to this chance, there may be inevitable influences of the above differences in characters between the two populations. When the two populations coexist, it is no wonder that the survival and proliferation rates of one population associated with factors such as environmental preference and resistance to the environment including natural enemies are better than those of the other population.

If changes in the ratio of the number of individuals due to such a relationship are expressed as “competition”, the number of individuals of either population may become dominant in a simple environment through the competition.

### **Habitat segregation in various environments**

The ratio of individuals of both populations may be determined by chance in a simple environment in some cases. However, in the long term, either population can become dominant. Even in such a case, there may also be other environments where the other population becomes more successful. There are various natural environments, and it is rational to consider that the more successful population (species) differs among environments.

Insects that can move by themselves may select different environments due to character differences between the two populations. In addition, since the environment

changes according to the season and time, individuals select the environment, and survivors are selected. In any case, differences in genetic characters between the two populations increase, allowing immediate distinction between them, and each population may begin to use each environment more exclusively, and, therefore, more effectively.

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Tokyo, (In Japanese).

The above 4<sup>th</sup> paper was published on the author's home page from 2000 to 2001 (In Japanese), The 3<sup>rd</sup> paper was re-published on the same homepage.

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