氏　　名	内山 博允
学位(専攻分野)	博士(理学)
学 位 記 番 号	総研大甲第 1697 号
学位授与の日付	平成26年3月20日
学位授与の要件	先導科学研究科 生命共生体進化学専攻 学位規則第6条第1項該当
学 位 論 文 題 目	The visual function of the 'rough' eyes of the Northeast -Asian wood white butterfly, Leptidea amurensis
論 文 審 査 委 員	主　査　教　授　佐々木 顕 教　授　蟻川 謙太郎 准教授　大田 竜也 講　師　木下 充代 准教授　Natali Hempel de Ibarra University of Exeter

(別紙様式 2)
(Separate Form 2)

論文内容の要旨

Summary of thesis contents

Hironobu Uchiyama discovered that the compound eyes of the northeast-Asian wood white, *Leptidea amurensis*, appear rough. He extensively analyzed the structure, physiology and optics of the eyes, and discussed the results in the context of evolution of butterfly eyes. The thesis begins with the General introduction, which is followed by two main chapters (Chapters I and II), and concludes with the General discussion and conclusion. Several original discoveries are described in detail in two main chapters in generally well-written English. The described results are highly convincing because several different experimental approaches are employed in a well-balanced manner: the experimental techniques include anatomy with both light and electron microscopy, intracellular and extracellular electrophysiology, molecular biology and opitical calculations.

In General introduction, the history of vision research and the properties of two main types of compound eye (apposition and superposition types) are briefly summarized. It further mentions about the variability in spectral organization of apposition type compound eyes among butterflies, including species specificity and sexual dimorphism. After summarizing the previous studies on *Pieris rapae* (Pieridae, Pierinae) and *Colias erate* (Pieridae, Coliadinae), the reason why the candidate decided to study on the eye of *Leptidea amurensis* (Pieridae, Dismorphiinae) was stated: this is because the subfamily Dismophiinae is a small, and possibly ancestral, group in the family Pieridae, and therefore crucial to understand the evolution of visual system in butterflies.

Chapter 1 is based on a published paper. In the early stage of the thesis study, he discovered that the eyes of *Leptidea amurensis* appear rough from outside. It turned out from detailed anatomical experiments, that the eye is composed of ommatidia of two different sizes, large and small. This is a very unique feature, which has not been found in any other compound eyes. Further molecular biological study, where the candidate identified three visual pigment opsins (UV, blue and green) and localized them in the eye by *in situ* hybridization, has revealed that the eye in fact contains three types of ommatidia distinguishable by opsin expression pattern. He also measured the spectral sensitivities of photoreceptor and identified their locations in these three types of ommatidia.

In Chapter II, the candidate reports about the possible function of the “roughness” of the *Leptidea* eye. Interestingly, the roughness is a property restricted to males: female eyes are smooth, constructed with the ommatidial facets with almost the same size as in regular compound eyes. Therefore, the roughness is probably evolved through sexual selection. The candidate’s hypothesis is that the roughness would expand the dynamic range in the intensity domain: the absolute sensitivity is high in large ommatidia and low in small ommatidia. By mixing the units of distinct sensitivities, the whole eye would have broader dynamic range, which has been used in latest digital camera technology. Physiological measurements indeed support the hypothesis: males have wider dynamic range than females. This difference is probably related to their mating behavior, where males have to fly back and forth among bright and shaded places to search for females, while females tend to stay around the food plants.

General discussion and conclusion remarks on the evolutionary process of eyes in the lineage of Pieridae, and possible biological meaning of the roughness in the eyes of male *Leptidea amurensis*. Finally, the contents of the entire thesis are informatively summarized.

(Separate Form 3)

博士論文の審査結果の要旨

Summary of the results of the doctoral thesis screening

The candidate, Hironobu Uchiyama, discovered that the compound eyes of the northeast-Asian wood white butterfly, *Leptidea amurensis*, appear rough. He extensively analyzed the structure, physiology and optics of the eyes, and discussed the results in the context of evolution of butterfly eyes. The thesis begins with the General introduction, which is followed by two main chapters (Chapters I and II), and concludes with the General discussion and conclusion. Several original discoveries are described in detail in two main chapters in generally well-written English. The described results are highly convincing because several different experimental approaches are employed in a well-balanced manner: the experimental techniques include anatomy with both light and electron microscopy, intracellular and extracellular electrophysiology, molecular biology and opitical calculations.

In General introduction, the history of vision research and the properties of two main types of compound eye (apposition and superposition types) are briefly summarized. It further mentions about the variability in spectral organization of apposition type compound eyes among butterflies, including species specificity and sexual dimorphism. After summarizing the previous studies on *Pieris rapae* (Pieridae, Pierinae) and *Colias erate* (Pieridae, Coliadinae), the reason why the candidate decided to study on the eye of *Leptidea amurensis* (Pieridae, Dismorphiinae) was stated: this is because the subfamily Dismophiinae is a small, and possibly ancestral, group in the family Pieridae, and therefore crucial to understand the evolution of visual system in butterflies.

Chapter 1 is based on a paper published in the Journal of Experimental Biology in 2013. In the early stage of the candidate's thesis study, he discovered that the compound eyes of *Leptidea amurensis* appear rough from outside. It turned out from detailed anatomical experiments, that the eye is composed of ommatidia of two different sizes, large and small. This is a very unique feature, which has not been found in any other compound eyes. Further molecular biological study, where the candidate identified three visual pigment opsins (UV, blue and green) and localized them in the eye by *in situ* hybridization, has revealed that the eye in fact contains three types of ommatidia distinguishable by opsin expression pattern. He also measured the spectral sensitivities of photoreceptor and identified their locations in these three types of ommatidia.

In Chapter II, the candidate reports about the possible function of the "roughness" of the *Leptidea* eye. Interestingly, the roughness is a property restricted to males: female eyes are smooth, constructed with the ommatidial facets with almost the same size as in regular compound eyes. Therefore, the roughness is probably evolved through sexual selection. The candidate's hypothesis is that the roughness would expand the dynamic range in the intensity domain: the absolute sensitivity is high in large

ommatidia and low in small ommatidia. By mixing the units of distinct sensitivities, the whole eye would have broader dynamic range, which has been used in latest digital camera technology. Physiological measurements indeed support the hypothesis: males have wider dynamic range than females. This difference is probably related to their mating behavior, where males have to fly back and forth among bright and shaded places to search for females, while females tend to stay around the food plants. The candidate is now preparing a manuscript based on the contents of Chapter II as a research paper.

General discussion and conclusion remarks on the evolutionary process of eyes in the lineage of Pieridae, and possible biological meaning of the roughness in the eyes of male *Leptidea amurensis*. Finally, the contents of the entire thesis are informatively summarized.

Based on the contents as well as the style, we conclude that the submitted thesis is of the sufficient quality as a PhD thesis of the Department of Evolutionary Studies of Biosystems, School of Advanced Sciences.