

EXPRESSION OF THE THYROID-RELATED TRANSCRIPTION FACTOR GENES, *TTF-2* AND *PAX258* IN THE ENDOSTYLE OF ADULT AMPHIOXUS

Jin Hiruta, Michio Ogasawara

Department of Biology, Faculty of Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

The endostyle is a pharyngeal organ of Urochordata, Cephalochordata and larval Cyclostomata. This organ secretes mucus-proteins for internal filter feeding, a feeding system that must have developed in the common ancestor of these subphyla. Furthermore, the endostyle has also a series of functions that parallel those of the vertebrate thyroid, such as iodine uptake and thyroid peroxidase activity. Therefore, the endostyle is a key structure to understanding the origin and evolution of thyroid as well as chordate body plan. In the present study, we investigated the expression patterns of thyroid-related transcription factor genes for *TTF-2* and *Pax258* in adult amphioxus by means of the whole-mount *in situ* hybridization. Expression of *TTF-2* was detected in the thyroid equivalent region (zone 6) of the amphioxus endostyle as same as that of *TPO* which encodes thyroid-related differentiated marker. On the other hand, *Pax258* was not expressed in the thyroid equivalent region, however, expression pattern of *Pax8* was similar to that of *TTF-1* which encodes thyroid-related transcription factor. These molecules might bring us insight into the evolution of endostyle and thyroid.

FIELD CAGE EXPERIMENTS ON THE PREMATING ISOLATION BETWEEN TWO PARAPATRIC FORMS OF FLIGHTLESS *CHRYSOLINA* LEAF BEETLESSoichi Arita¹, Norio Kobayashi², Satoru Saitoh³, Haruo Katakura¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan, ²The Hokkaido University Museum, Sapporo, Hokkaido 060-0810, Japan and ³Nippon Date Service Inc., Sapporo, Hokkaido 065-0016, Japan

Two forms of the flightless leaf beetles belonging to the *Chrysolina angusticollis* species complex are distributed in the southern suburb of Sapporo, Hokkaido, northern Japan. They are basically parapatric, feeding on the same kind of host plants. Previous studies revealed that there was rather strong sexual isolation between them, and there was reduction of fertility in males of F1 hybrids. However, allozymic analyses revealed that natural hybrids were occasionally found in very narrow hybrids zones formed at the boundary of their distribution. The present study was conducted in order to reassess the degree of premating isolation using a field cage (length 7m, width 7m, height 3m) with host plants, in which the two forms were released. In this condition, matings between the two forms were more frequent than those previously observed in the laboratory. Fate of offspring produced in the cage is also reported.

THE MONOGENIC FEMALES OF THE GYPSY MOTH ORIGINATED FROM THE HYBRIDIZATION BETWEEN TWO SUBSPECIESMichio Ishihara¹, Yasutomo Higashiura², Nanako Ono²¹Biological Laboratory, Hyogo College of Medicine, Nishinomiya 663-8501, Japan and ²School of Life Science, Tokyo University of Pharmacy and Life Science, Hachioji 192-0392, Japan

In 1996 summer, we discovered the abnormal female which produces only female progeny (We refer to such a female as a monogenic female). The daughters of the monogenic female produced only female progeny again when they crossed with sons from the normal females. The maternally inherited cytoplasmic factor killed male embryos. Bogdanowicz et al.(2000) had investigated the haplotypes of the moths in the world, which were divided into four grades based on the mitochondrial DNA haplotypes. By our investigations, the haplotypes of the monogenic females belonged to the most common haplotype obtained in Honshu subspecies, although the monogenic females were found only in the area of Hokkaido subspecies. The results become strong basis that the monogenic females brought by the crosses between subspecies Honshu female and subspecies Hokkaido male. As if proving this, Goldschmidt(1930) showed that substantially only female progeny(1245 females and one male) was produced in the backcross between F1 female (Honshu female × Hokkaido male) and Hokkaido male. The theory of Goldschmidt, who considered the maternal factor as a major determinant of female sex, deserves reevaluation.

SEASONAL REPRODUCTION OF THE DEEP-SEA OPHIUROID, *OPHIURA SARSII SARSII* LÜTKEN, 1855Maki Takeda¹, Yoichi Kogure^{1,2}, Miéko Komatsu¹¹Department of Biology, Faculty of Science, Toyama University, Toyama, Toyama 930-8555, Japan and ²Japan Sea National Fisheries Research Institute, Niigata, Niigata 951-8121, Japan

The deep-sea benthos inhabiting a relatively constant physical environment have long been considered to reproduce throughout the year. However, recent evidence suggests that benthic invertebrates have a seasonal reproductive cycle. Although *O. sarsii sarsii* is the most dominant species among the deep-sea benthos in the Sea of Japan, characterized by a deep-sea water mass, its reproductive state is unknown. We studied the reproductive biology of this ophiuroid, periodically collected at a depth of 250m north of Sado island. The annual reproductive cycle was studied by the gonad index (GI) method and by histological observations. GI for both sexes is lowest in April. From June to October, continuous growth of gonads was such as a consequence of gametogenesis. GI was highest in February, when the lumens of the testis and ovary respectively filled with a number of sperm and full-developed ova. The organic matter in the bottom sediments is increased in April, suggesting a seasonal signal of food supply for the ophiuroid in the bathyal zone. This study confirmed that *O. sarsii sarsii* exhibits a distinct annual reproductive cycle which may relate to the seasonal changes in food.

HORIZONTAL GENOME TRANSFER FROM ENDOSYMBIOTIC BACTERIUM TO HOST INSECTTakema Fukatsu¹, Natsuko Kondo¹, Nobuyuki Ijichi^{1,2}, Naruo Nikoh³¹Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan, ²Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan and ³University of the Air, Chiba 261-8586, Japan

Members of the genus *Wolbachia* are rickettsia-like endocellular bacteria associated with insects and other invertebrates. Infection with *Wolbachia* often causes a wide range of effects on the reproduction and physiology of arthropod hosts such as cytoplasmic incompatibility, parthenogenesis, feminization, male killing, etc. In the adzuki bean beetle *Callosobruchus chinensis*, we identified a strain of *Wolbachia* that was resistant to antibiotics and subjected to sex-linked inheritance. Detailed analyses revealed that the *Wolbachia* strain has no microbial entity but is a genome fragment of *Wolbachia* endosymbiont transferred to the X chromosome of the host insect. This study provided the first authentic case where horizontal gene transfer from a symbiotic microorganism to a host multicellular eukaryote occurred in nature.

ENDOSYMBIOTIC BACTERIA IN THE CLOSELY RELATED *EPILACHNA* LADYBIRD BEETLESKei Matsubayashi¹, Norio Kobayashi², Haruo Katakura¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan and ²The Hokkaido University Museum, Sapporo 060-0810, Japan

The phytophagous ladybird beetles belonging to the *Epilachna vigintioctomaculata* species complex are composed of four very closely related species, that were classified into group A (*E.vigintioctomaculata*) and group B (*E.niponica*, *E.yasutomii*, *E.pustulosa*). We determined a part of 16S rDNA of endosymbiotic bacteria obtained from all the four ladybird beetle species, and analyzed their phylogenetic relationships using Neighbor Joining method. Symbionts of each of the two beetle groups constituted monophyletic group, and those of the three species of group B shared the same sequence. The results suggest that the divergence of the symbionts followed the divergence of the two groups of the ladybird beetles.

PHYLOGEOGRAPHIC ANALYSIS OF *PHELOTRUPES LAEVISTRIATUS* BASED ON MITOCHONDRIAL COI GENEYuri Ohta¹, Norio Kobayashi², Seizi Suzuki³, Tetsuya Kato⁴, Shigehisa Hori⁵, Satoshi Yamauchi⁶, Haruo Katakura¹¹Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810 Japan, ²The Hokkaido University Museum, Sapporo, Hokkaido 060-0810 Japan, ³Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589 Japan, ⁴Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Wakayama 649-2211 Japan, ⁵Historical Museum of Hokkaido, Sapporo, Hokkaido 004-0006 Japan and ⁶Aomori Prefectural Museum, Aomori, Aomori 030-0802 Japan

Phelotrupes laevistriatus is one of the commonest dung beetles in Japan, dwelling mainly in forest habitats. Previous studies demonstrated that this species exhibited a dimorphism in flight muscle. Beetles with well developed muscle were distributed in the southern part, from the Tohoku district in the north to Kyushu district in the south, whereas those with undeveloped muscle were distributed in the northern part, Hokkaido and northern Honshu. The two forms showed a mosaic distribution in northern Honshu, with sympatric occurrence in some collecting sites. To investigate the origin of the flight muscle dimorphism in *P. laevistriatus*, we determined the sequences of mtCOI gene for 36 individuals from 11 sites and constructed gene tree and a population tree using the Neighbor-Joining method. The result suggested that *P. laevistriatus* invaded Japan from south and successively expanded their range northward. Beetles from southern Japan with developed muscle were paraphyletic, diverged near the origin, whereas all the beetles with undeveloped flight muscle were included in a derived monophyletic group comprising the northern Japan populations.