

HOST RANGE EXPANSION OF THE PHYTOPHAGOUS LADYBIRD BEETLE, *EPILACHNA VIGINTIOCTOPUNCTATA* IN SOUTHEAST ASIANorio Kobayashi¹, Hideki Ueno², Naoyuki Fujiyama³, Susumu Nakano⁴, Aryuthaka Chittima⁵, Haruo Katakura⁶¹The Hokkaido University Museum, Sapporo 060-0810, Japan, ²Faculty of Education and Human Sciences, Niigata University, Niigata 950-2181, Japan, ³Biological Laboratory, Hakodate Campus, Hakodate 040-8567, Japan, ⁴Hiroshima Shudo University, Hiroshima 731-3195, Japan, ⁵Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand and ⁶Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

The phytophagous ladybird beetle *Epilachna vigintioctopunctata* is widespread in Asia and Australia, and known as a serious pest of solanaceous crops such as potatoes, tomatoes and eggplants. However, this beetle was sometimes found occurring on a legume, *Centrosema pubescens*, at some locations in Southeast Asia. The difference in host plants might function as an ecological barrier to reproduction between the beetles on different hosts, provided that the fidelity to respective host plants is strong. It is also interesting to know whether the host shift, probably from solanaceous plants to the legume, occurred only once or not. In the present study, we determined the nucleotide sequence of a part of mtDNA COI gene of 70 individuals collected from eight populations in Thailand, Australia and Indonesia. Based on the haplotype sharing between populations, we estimated the degree of gene flow between populations on the two host plants. Furthermore, we constructed a population tree, and tested the hypothesis that the legume feeding populations originated more than once in different localities of Southeast Asia.

GENETIC DIFFERENTIATION BETWEEN TWO KARYOTYPES FOR JAPANESE LEAF BEETLE, *CHRYSOLINA AURICHALCEA*Norikazu Kitamura¹, Shizuo Fujiyama², Tadashi Aotsuka¹¹Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Hachioji 192-0397, Japan and ²Department of Biology, Graduate School of Science, Shinshu University, Matsumoto 390-8621, Japan

Chrysolina aurichalcea, leaf beetle, is distributed widely in Asia. In Japan, two karyotypes (2n = 31(male), 32(female); 2n = 41(male), 42(female)) are found in the natural population. Populations of each karyotype are usually distributed allopatrically or parapatrically. In crossing experiments, the development of reproductive isolation between the types was detected. Moreover, slight morphological differences in male genitalia and some ecological differences between the types were reported. These observations suggest that the speciation accompanied by karyotype reconstruction is now in progress in natural populations of *C. aurichalcea*. In this study, we sequenced mitochondrial and nuclear DNA sequences to elucidate the evolutionary history of the different karyotype populations.

COMPARATIVE ANALYSIS OF GENES FOR MUSCLE PROTEINS IN APPENDICULARIAN *OIKOPLEURA LONGICAUDA* (UROCHORDATA)

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Appendicularians constitute a group of free-swimming urochordates. Appendicularians retain the tail throughout their life, which contrasts with the metamorphic lifestyle of other urochordates, including ascidians. A controversy has been on the phylogenetic emergence of the non-metamorphic lifestyle of appendicularians, namely whether their lifestyle is ancestral for urochordates or a paedomorphic derivative from the metamorphic ancestor. It was revealed in ascidians that some of muscle structural protein isoforms are specifically expressed in their larval or adult stage. Here, it was intended to compare the muscle proteins in the tail of appendicularians with those in larva and adult muscles of ascidians. Several cDNAs encoding muscle proteins had been isolated, including actins, myosin heavy chains, and myosin light chains. Molecular phylogenetic relationships were examined among the urochordate muscle protein isoforms. The results obtained so far did not necessarily support the hypothesis on the paedomorphic emergence of appendicularian lifestyle. With further results, a comprehensive discussion is tried about phylogeny of the urochordate lifestyles.

EVOLUTION OF CHORDATES VIEWED FROM THE DRAFT GENOME OF THE ASCIDIAN *CIONA INTESTINALIS*

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The first chordates appear in the fossil record at the time of the Cambrian explosion, nearly 550 million years ago. To illuminate the origin and evolution of chordates, we determined a draft of the protein-coding portion of the genome of the most studied ascidian, *Ciona intestinalis*. The *Ciona* genome contains approx. 15,850 protein-coding genes, similar to the number in other invertebrates, but only half that found in vertebrates. Comparison of these *Ciona* genes with those of *C. elegans*, *Drosophila*, *Fugu*, mouse, and human made it clear the genes shared by chordates. Analyses of the chordate-specific genes give us suggestion how chordates evolved from common ancestor of deuterostomes.

RESULTS SO FAR OBTAINED FROM THE PROJECT OF WORLD-WIDE LANCELET MOLECULAR PHYLOGENYTeruaki Nishikawa¹, Masahiro Nohara², Takeshi Kon³, Mutsumi Nishida³¹The Nagoya University Museum, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan, ²Yokohama R and D Center, HITEC Co. Ltd., 12F Queen's Tower A, 2-3-1 Minatomirai, Nishi-ku, Yokohama, Kanagawa 220-6012, Japan and ³Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo 164-8639, Japan

Our project since 2001 aims at the deeper understanding of morphological and genetic changes through time in the lancelets (Cephalochordata) by covering most of the about 30 living known species in the world as OTU's of molecular phylogenetic trees. Samples so far collected from the 23 populations of 14 species have been partly analyzed for mtDNA sequences. Results thus far gained were generally summarized with emphasis on the paraphyly of the genus *Epigonichthys* with the deepest branch between *E. lucayanus* and the remainings (consisting of its congeners and the species referred to the other genus *Branchiostoma*); the genetic differentiation of the European lancelets previously called simply as *B. lanceolatum*; and the discovery of a cryptic species related to *E. lucayanus*, both of which living together in some tropical areas. Also given was a perspective of our project with future plans to complete it.

GENE REARRANGEMENTS OF THE MITOCHONDRIAL GENOME IN THE BELL-RING FROG *BUERGERIA BUERGERI* AND AN ALLIED SPECIES FROM THE FAMILY RHACOPHORIDAENaomi Sano¹, Tamotsu Fujii², Nozomi Mikami², Atsushi Kurabayashi¹, Masayuki Sumida¹¹Institute for Amphibian Biology, Hiroshima University, Higashihiroshima and ²Department of Health Science, Hiroshima Prefectural Women's University, Hiroshima

Mitochondrial DNA gene arrangements are conserved in many vertebrates. A common arrangement is found in three amphibian species: the salamander, the caecilian, and the clawed frog. However, the positions of four tRNA genes in the gene arrangement of the Japanese pond frog *Rana nigromaculata* were found to differ from those in the gene arrangement of other vertebrates. To elucidate the mitochondrial gene rearrangements in anurans in their entirety, we determined the nucleotide sequences of the mtDNA genome of two *Buergeria* species from the family Rhacophoridae. The resultant sequences showed a novel gene arrangement that has never been identified in other vertebrates. In addition to sharing the same rearrangements of four tRNA genes found in *R. nigromaculata*, the *Buergeria buergeri* and *B. japonica* species were also found to have a rearrangement of the ND5 gene. These results suggested that (1) the gene rearrangement of the four tRNA genes occurred in a common ancestor of ranids and rhacophorids after pipids branching, and that (2) the ND5 gene was translocated in a lineage of rhacophorids after ranids divergence.

EXPERIMENTALLY INDUCED AUTOTETRAPLOIDY AND AMPHIDIPOIDY IN TWO JAPANESE POND FROGS

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To clarify cytogenetic features in tetraploid amphibians, autotetraploids(AT) were produced by suppressing second polar body formation in haploid eggs of diploid R. nigro females inseminated with diploid sperm of AT R. nigro males, and amphidiiploids (AD;allotetraploids) were also produced by suppressing second polar body formation in haploid eggs of diploid R. porosa brevipoda females inseminated with diploid sperm of AT R. nigro males. In male R. nigro AT offspring, mostly quadri- and bivalents were found in metaphase I, and 26 dyads were seen in metaphase II. AT males were then mated with AT females, and most of the resultant offspring were tetraploids possessing four chromosome sets of diploid R. nigro. Male AD offspring showed 26 bivalents in metaphase I and 26 dyads in metaphase II. A large number of the offspring resulting from crosses between AD males and females were tetraploids having two chromosome sets of diploid R. nigro and two sets of diploid R. p. brevi. These results seem to indicate that if such tetraploids develop a distinguishing premating mechanism, they will continue as a sexual fertile species in one generation and coexist with its diploid parent species.

ON A SALAMANDER FROM SOUTHERN PART OF THE CHUBU DISTRICTMasafumi Matsui¹, Yasuchika Misawa², Yasuhiro Kokuryo³¹Graduate School of Human & Environmental Studies, Kyoto University, Kyoto 606-8501, Japan, ²Kensetsu-Kankyo Co. Ltd., 2-23-2 Higashi-ikebukuro, Teshima-ku, Tokyo 170-0013, Japan and ³1097-4 Fujieda, Shizuoka 426-0061, Japan

We studied the taxonomic status of a small salamander of the genus *Hynobius* found on montane regions of the Akaishi Mountains in northwestern Shizuoka and southeastern Nagano. Through electrophoretic analyses of allozymes, the species was found to be genetically highly diverged from either lentic breeding *H. tokyoensis* or lotic breeding *H. naevius* and *H. kimurae*. It is also distinguished from the other species morphologically, and is considered to be an undescribed distinct species. Although we are not successful in revealing reproductive habits of this species in our field surveys, it is estimated that the species is a lotic breeder because females have

small number of large, yolk eggs in their ovaries. Since no larvae are found in the open streams where adults are found, the species seems to lay eggs in small streams under the ground. The species is syntopic with *H. kimurae* in several known localities and the coexistence of the two species seems to be achieved through differences in the body size and the breeding site.

PHYLOGENY OF THE LIZARD SUBFAMILY LYGOSOMINAE (REPTILIA: SCINCIDAE), WITH SPECIAL REFERENCE TO THE ORIGIN OF THE NEW WORLD TAXA

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Phylogenetic relationships of the three lygosomine skink genera occurring both in the Old World and the New World (*Mabuya*, *Scincella* and *Sphenomorphus*) were inferred from mitochondrial DNA sequence of 12S and 16S rRNA genes. Results strongly suggested the non-monophyly for any of these three genera. Within the *Mabuya* group, Asian members appear to have diverged first, leaving the Neotropical and the Afro-Malagasy *Mabuya* as sister groups. These relationships, together with the absence of extant or fossil representatives of the *Mabuya* group from North America, strongly suggest the trans-Atlantic dispersals of *Mabuya* from Africa to Neotropics. Our results also indicated a closer affinity of the New World *Scincella* with the New World *Sphenomorphus* than with the Old World *Scincella*. Such relationships suggest the trans-Beringian dispersal of the common ancestor from Asia and its subsequent divergence into the North American *Scincella* and the Neotropical *Sphenomorphus*.

TEMPORAL, SPATIAL, AND ECOLOGICAL MODES OF EVOLUTION IN MUS FROM EURASIA BASED ON MITOCHONDRIAL AND NUCLEAR GENE SEQUENCES

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We sequenced mitochondrial (cytochrome *b*, 12S rRNA) and nuclear (IRBP, RAG1) genes in species of *Mus*, primarily from Eurasia. Phylogenetic trees constructed with these data illustrated the rapid divergence of four subgenera, suggesting long-distance dispersal and subsequent geographic partitioning in Africa, India, Southeast Asia, and some unknown area. Our data illustrate a subsequent radiation event in the subgenus *Mus*, which differentiated in three geographic areas: India, Southeast Asia, and Europe. The radiation of the subgenera occurred 5-6 million years ago (mya) and that within the subgenus *Mus* occurred 2-3 mya, using the IRBP and RAG1 clocks estimated from the rat-mouse divergence 12 mya. The second expansion of the subgenus *Mus* was associated with its ecological transition from forest dwelling to grassland dwelling in subtropical Eurasia, which was probably associated with global climatic changes in the late Tertiary and Quaternary. The temporal mode of evolution is similar to that seen in the evolution of the field mouse, *Apodemus*, which evolved in temperate Eurasia.

PHYLOGENETIC RELATIONSHIPS AMONG MUSTELIDS (MAMMALIA: CARNIVORA) INFERRED FROM MULTIPLE GENE SEQUENCES IN SINGLE OR COMBINED GENE ANALYSES.

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Phylogenetic relationships among mustelids, representing Mustelinae (weasels and martens etc), Lutrinae (otters), Melinae (badgers), Mephitinae (skunks), were examined with the nuclear (IRBP, RAG1) and mitochondrial (cyt *b*) genes. Our data clearly resolved the phylogenetic relationships among genera within these subfamilies and also showed the polyphyletic relationships among the meline species, in which two genera of Melinae, *Melogale* and *Meles*, appeared at different lineages. These results therefore suggest that the characters shared by the meline lineages are plesiomorphic rather than products by homoplasy. Besides, *Mephitis* (representing Mephitinae) is found to be strongly supported as an outgroup to a clade including the other Mustelidae and Procyonidae (causing Mustelidae to be paraphyletic), indicating a need of morphological reevaluation on the subfamily Mephitinae.

ANCIENT mtDNA ANALYSIS OF THE SIKA DEER IN HOKKAIDO

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The population size of the Hokkaido sika deer *Cervus nippon yessoensis* was remarkably reduced because of the heavy hunting pressure from the beginning of the Meiji era and effects of heavy snow in 1979 and 1981. After that, the number of the Hokkaido sika deer increased gradually due to the protection by the Hokkaido government and currently the deer make a great damage to the agriculture and the forestry. In the present study, to clarify bottleneck effects on the deer population, we analyzed ancient mitochondrial DNA (mtDNA) from archaeological bone remains of the Hokkaido sika deer before the Meiji era, and compared the data with genetic characters of the modern population of the Hokkaido deer. From 81 deer examined, 602 base-pair fragments of the mtDNA D-loop region were sequenced. Some new haplotypes were identified from ancient deer. Distribution patterns of ancient mtDNA haplotypes in Hokkaido were different from those of the modern deer, indicating changes of population structures through the bottleneck.

A MODEL SYSTEM FOR COLLECTING THE LOCAL BIODIVERSITY INFORMATION AT NAGAIKE PARK IN HACHIOJI, TOKYO

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Biodiversity information provided by the experts usually covers limiting area and limiting seasons. It could be possible to utilize the information from local naturalists for complementing the knowledge of local biodiversity. In this study, the open database for archiving the biodiversity information at Nagaike Park has been constructed as a model system of the local biodiversity server. The system is composed by three database tables; name table, photo table, locality table. The name table contains the name list of existing 800 species, which have been confirmed by the previous research project in 2000. The photo table has its submitting function to collect an identification report with the photo, as the evidence. Each report contains date and locality data. The locality table possesses geographical information. Using this system, more than 200 identification reports have been collected. The name list of this particular region well helped to confirm each identification data. This kind of local database may play an important role for achieving the global biodiversity information. Those data are available at <http://biodb.i.hosei.ac.jp/nagaike/> (in Japanese).

SWITCHES OF THE STRATEGY OF GRAVITACTIC REORIENTATION MECHANISM DURING THE DEVELOPMENT OF SEA URCHIN LARVAE

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Sea urchin larvae show remarkable negative gravitaxis. Analyses of the reorienting motion during sedimentation of immobilized larvae demonstrated that gastrulae oriented upwards due to the hydrodynamic torque generated on the basis of the morphological fore-aft asymmetry; anterior location of the center of drag to those of gravity and buoyancy, while plutei did so due to the torque generated by the asymmetry of the distribution of the internal density; posterior location of the center of gravity to those of buoyancy and drag (Mogami et al, 2001, Biol. Bull., 201, 26-33). We examined their swimming behavior and the resultant vertical distribution of larvae in the usual hypodensity as well as Percoll-containing hyper-density artificial sea water, and demonstrated that these physical reorientation mechanisms actually functioned in free swimming larvae of corresponding stages. In addition, it became clear in blastula that reorientation occurred based on the asymmetrical distribution of the internal density. These results indicate that there are several switches of strategy of physical reorientation mechanism during the development of sea urchin larvae.

SPERACT INDUCES A MORE ASYMMETRIC FLAGELLAR BENDING IN THE SEA URCHIN, *HEMICENTROTUS PULCHERRIMUS*

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Speract, one of sperm-activating peptides in the sea urchin, has been demonstrated to induce an increase in the intracellular Ca^{2+} concentration and also in the intracellular pH. To examine the effects of speract on the flagellar motility, we studied motility responses to speract in *Hemicentrotus pulcherrimus* sperm, by using caged speract ([Ser⁵, NB-Gly⁶]SAP) as a source of speract release and using a 5-W LED driven intermittently to freeze the flagellar waveform. We observed the swimming