

reproductive physiology. To understand the molecular basis of soldier differentiation and function in aphids, genes specifically expressed in the 2nd-instar sterile soldiers of an eusocial aphid, *Tuberaphis styraci*, were isolated by a cDNA subtraction technique. Of 29 soldier-specific cDNA clones obtained, 18 clones were of an identical nucleotide sequence which showed a significant sequence similarity to cathepsin B, a family of cysteine protease. The expression level of the protease gene was around 2,000 times higher in soldiers than that in normal nymphs. The amount of the cathepsin B protein and its protease activity in soldiers were also higher than that in normal individuals. The protease was produced in midgut. When the soldiers attacked moth larvae, the protease was injected into the body fluid of the victims through the stylet. Therefore, it is suggested that this protease is a component of attacking substances to predators.

A TRIAL TO ANALYZE LOCAL META-POPULATIONS BY THE USE OF SNIPS OF MITOCHONDRIAL ND5 GENE IN PAPILIONID BUTTERFLY *LUEHDORFIA JAPONICA*.

Kazuhiko Hirano, Kazuo Watanabe

Department of Sciences for Biospheric Coexistence, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739, Japan

In 71st annual meeting, we described geographical distribution of the single nucleotide substitution of ND5 gene by analyzing 90 individuals. We add the results of further 47 individuals (total 137), ranging from Yamaguchi prefecture (western-most limit of distribution of natural population) to Akita prefecture (north-eastern-most limit). There found 15 nucleotide substitutions among the 782 base pairs long, and their geographical distribution was categorized into 3 types, "two of widely distributed substitution", "three of semi-widely distributed substitution", and "11 of locally distributed substitution". Interestingly there existed 9 localities, in which polymorphisms at the same nucleotide position were found. By analyzing distribution range and co-existence of these nucleotide substitutions and polymorphisms at the same locality, we tried to discuss origin of constitution of the local population of the species.

IDENTIFICATION AND LOCALIZATION OF SYMBIOTIC BACTERIA IN THE MIXED SEGMENT OF THE SOIL-FEEDING TERMITE, *PERICAPRI-TERMES NITOEI*

Gaku Tokuda¹, Chie Moromizato², Shinki Kakazu²

¹Center of Molecular Biosciences, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan and ²Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

The alimentary canal of insects consists of the foregut, the midgut and the hindgut. However, many of termites belonging to the family Termitidae have the mixed segment, where the mesenteric epithelium occupies half of the gut wall and the proctodeal epithelium covers the remaining area. Our previous study has suggested that clostridia are dominant in the mixed segment of the wood-feeding termite, *Nasutitermes takasagoensis*. Here, we studied phylogeny and distribution of symbiotic bacteria in the mixed segment of the soil-feeding termite, *Pericapritermes nitoei*. Bacterial 16S rRNA genes were amplified from the mixed segment of the gut by PCR, and identified sequences were used for phylogenetic analyses. As a result, the symbiotic bacteria of the mixed segment are primarily affiliated with spirochetes of the genus *Treponema* and sulfate-reducing bacteria, which are present not only in the mixed segment but also in the midgut. PCR using primers specific for the symbiotic clostridia localized in the mixed segment of *N. takasagoensis* resulted no band, suggesting that distribution of the symbiotic bacteria is related to the food habits of the hosts rather than phylogeny of the hosts.

CHARACTERISTICS AND ANALYSIS OF SPAWNING PATTERN IN AMPHIOXUS

Takanobu Mizuta¹, Kaoru Kubokawa²

¹Department of Aquatic Bioscience, Graduate School of Agricultural and Life Science, The University of Tokyo, Bunkyo, Tokyo 113-0033, Japan and ²Ocean Research Institute, The University of Tokyo, Nakano, Tokyo 164-8639, Japan

Both mature and immature individuals of amphioxus were collected in Enshu-Nada Sea in July, 2001 and kept in tanks at Misaki Marine Biological Station, University of Tokyo until the end of August. Spontaneous spawning of adult animals in aquaria was recorded into video and analyzed. We have found in the field that the number of males was larger than that of females in ratio of 1.21, in 9 areas, where the number of matured animals collected by dredging drastically varied among areas. In the aquaria, 100 males and the same number of females were kept together and totally 89 spermiations and 33 ovipositions were observed. Spermiation preceded oviposition always at the start of every series of the spawning but no clear regular pattern was observed in timing in the following spermiations and ovipositions. The interval between spermiation and oviposition was longer than one minute. However, 60 % of all the observed ovipositions occurred within 10 min after spermiation. Furthermore, the sperm motility was held for more than 90 min. The above mentioned timing of the spermiation and ovipositions could be formed by the relatively long active period of the sperm and the male biased sex ratio.

A COMPARATIVE STUDY ON LARVAL LIFE HISTORIES IN TWO POPULATIONS OF *HYNOBIUS BOULENGERI* (AMPHIBIA: URODELA) FROM KYUSHU I.

Kanto Nishikawa¹, Masafumi Matsui¹, Shin'ichi Sato²

¹Graduate School of Human & Environmental Studies, Kyoto University, Kyoto 606-8501, Japan and ²27 Oita, Oita 870-0835, Japan

Recently, we found a population of *Hynobius boulengeri* from the Osumi Peninsula on Kyushu Island. Compared with another population from Kyushu, from the Sobo-Katamuki Mountains population, the Osumi Peninsula population has a smaller adult body size and different larval life history. In this study, we compared larval life histories between these two populations. We found significant differences in the time of hatching, growth pattern, age composition, and size at metamorphosis between them. Some of these differences are supposed to be induced by differences in environmental factors, and seem to affect differences in the adult body size between the two populations.

QUANTITATIVE AND CONTINUOUS ANALYSES OF REPRODUCTIVE BEHAVIORS IN CAPTIVE LOGGERHEAD TURTLE (*CARETTA CARETTA*) USING SUPERSENSITIVE CAMERAS

Ken Sakaoka, Makoto Yoshii, Hitoshi Nakamura, Kazuo Kureha, Itaru Uchida

Port of Nagoya Public Aquarium, Minato, Nagoya 455-0033, Japan

Quantitative and continuous monitoring of reproductive behaviors in captive loggerhead turtle was performed using supersensitive cameras. During monitoring, males displayed a seasonal reproductive cycle with a distinct spring courtship and mounting period. As to temporal relationships among reproductive behaviors, frequencies of courtship and mounting peaked before the first nest in each female and then declined gradually. No mounting after the last nesting in each female was observed. Mounting observed during monitoring ranged from within a minute to 450 minutes. It seemed appropriate to regard mounting which persisted at least about an hour as reproductively successful mating. Among nesting females, total frequencies of experienced mounting were significantly different, however it was not confirmed that these differences affected fertility in each female. Moreover, one female was inferred to have experienced no mating after her first nesting based on the monitoring record and the fact that fertility did not decline significantly among her ongoing clutches. These results indicate the potential for sperm storage in the female genital tract up through a reproductive season.