

regulatory regions have also driven gene expression in the Ci neuronal cells. Furthermore, a 3-kb 5' fragment of Ci-syt promoter has shown pan-neuronal promoter activity in both Ci and Hr embryos. These results suggest that the neuronal gene regulatory mechanism that involves syt is conserved in both Hr and Ci promoters. It is possible that some critical binding sites for neuronal transcription factors may be conserved. Some candidate binding sites are found at high frequency within the critical regulatory regions of Hr-syt promoter. Comparison of restricted regulatory regions across species may lead to further identification of conserved neuronal gene regulatory mechanisms.

ANALYSIS OF *WHITE* GENE FUNCTION IN THE SAWFLY, *ATHALIA ROSAE*, USING INTERFERING RNAS.

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The system of stable germline transformation using a transposon-derived vector was developed in the sawfly, *Athalia rosae* (Hymenoptera). For efficient screening of the transgenic individuals, use of visible marker genes is desirable. We have cloned a *white* gene homologue of *A. rosae*. In *Drosophila melanogaster*, the *white* gene encodes an ABC transporter, which is involved in uptake and storage of metabolic precursors of the eye color pigments. As the first step to utilize this gene as a marker, we analyzed *A. rosae white* gene function. RNA interference is one of the useful tools to analyze gene function through silencing the endogenous genes. The double-stranded RNAs that were synthesized based on a short sequence of the *A. rosae white* cDNA were injected into mature unfertilized eggs dissected from wild-type adult females, activated *in vitro*, and allowed development. A fraction of embryos showed non-pigmented or faintly pigmented eye coloration during embryogenesis. The eye pigmentation of these individuals was recovered in the pupal stage. The effects seem to last until metamorphosis and not to inherit to the next generation.

ISOLATION AND CHARACTERIZATION OF A NOVEL BACTERIOPHAGE THAT INFECTS *WOLBACHIA*, AN ENDOSYMBIONT OF ARTHROPODS

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Wolbachia is a group of maternally transmitted rickettsia-like bacteria found in many insects and other arthropods. These bacteria are associated with various reproductive alterations in their hosts, which result in increasing infected population. The molecular mechanisms of the reproductive manipulations are still unknown owing to lack of transformation techniques of *Wolbachia*.

We isolated mobile bacteriophages from two *Wolbachia* strains, wCauA and wCauB, derived from the same host, the almond moth, *Cadra cautella*. Interestingly, a genotypically identical phage was detected from the two distinct *Wolbachia* strains, suggesting that the phage was involved in horizontal gene transfer between different *Wolbachia* strains. The phage genome contained putative infection or replication-related genes that can be utilized for construction of phage-derived transformation vector. Some function-unknown genes showed high sequence homology with some genes located in a pathogenicity island of a bacterial pathogen, *Dichelobacter nodosus*. They can be causative genes for sexual alterations induced by *Wolbachia*, because many bacterial pathogens keep their virulence-related genes on prophages.

ORGANDY, A NEW MEMBER OF MITES OF THE SILKWORM

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The silkworm translucent-larval skin *og'* mutant is caused by a 551-bp insertion in an exon of the *og* gene. The structure of the insertion indicates that it is a MITE (miniature inverted repeat transposable element), which is named *Organdy*. We obtained PCR fragments (0.5, 1.4, and 3.0 kb) from various silkworm strains. The nucleotide sequence of the 3.0-kb fragments shows an incomplete ORF of a transposase.

CHARACTERIZATION AND TISSUE DISTRIBUTION OF *XENOPUS* MBP GENE PRODUCTS

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The mammalian myelin basic protein (MBP) gene produces two groups of structurally related proteins by differential promoter usage and alternative RNA splicing. Despite the well-established role of classical MBPs in myelination, little is known about physiological roles of the other group of proteins designated Golli. To obtain phylogenetic and functional insights into the MBP gene products, we have cloned cDNAs encoding *Xenopus* homologues of MBP and Golli. The cDNA sequence and the result of genomic PCR show that both MBP and Golli are products of a single gene. RT-PCR analyses demonstrate nervous system-specific accumulation of MBP mRNA and expression of Golli in various tissues including the thymus, kidney and testis. Monoclonal antibodies made against a peptide shared by MBP and Golli recognize multiple protein bands at about 24 kD in the brain, spinal cord, sciatic nerve, and thymus in Western blotting. The results suggest the conserved multiple roles of the MBP/Golli gene in frogs and mammals.

SUPPRESSION OF 2,4,6-TRINITROTOLUENE-INDUCED CHROMOSOME ABERRATIONS IN VITRO BY ANTIOXIDANT ENZYMES

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2,4,6-trinitrotoluene (TNT), the widely used explosive, is an important environmental contaminant, and has mutagenic properties. TNT was directly added to the culture medium of frog leucocytes at concentrations varying from 0.001 to 0.1 ppm and maintained for 24 hours, resulting in the induction of chromosome aberrations (Abs) at all concentrations. The addition of antioxidant enzymes catalase and SOD as well as the radical scavenger mannitol and ascorbic acid caused a significant decrease in Abs by TNT, however. The above results suggest the involvement of reactive oxygen species in TNT-induced Abs in frog leucocytes.

MOLECULAR EVOLUTION OF PRIMATE CCS

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Copper, zinc containing superoxide dismutase (Cu,Zn-SOD) is very important enzyme in various organisms, and copper is essential for its activity. In the body, copper is loaded into Cu,Zn-SOD by copper chaperone for superoxide dismutase (CCS). In this study, we molecular-cloned the cDNAs for CCS from five primate species. The degree of the similarities in amino acid sequences of CCS between primates including human ranged from 100-96.4%. The sequences of copper ligand were well conserved in all primate CCS. We also constructed the molecular phylogenetic tree based on various species Cu,Zn-SOD and CCS nucleotide sequences. The tree showed that CCS is diverged from Cu,Zn-SOD gene.

ANALYSIS OF CIS-REGULATORY REGIONS FOR THE DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF MEDAKA GLOBIN GENES.

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In the medaka genome, 12 globin genes were organized in 2 clusters located on different chromosomes. The expression patterns of the 12 globin genes during development were classified into 4 groups; 1) Expression in embryo 2) Expression in embryos to larva 3) Expression in post-hatching to adult fish 4) Continuous expression in embryos to adult. Some of hematopoietic transcription factor binding sites such as GATA were found within 1-2Kb regions remote from probable minimum promoters in 200bp stretches 5'-upstream respective genes. The positions of the sites were variable from gene to gene. We constructed a fusion gene of the remote region with a reporter gene, introduced it into medaka embryos, and established 4 types of lines stably expressing the reporter genes in erythroid cells. The expression of GFP or RFP in each line was developmental stage-specific. The results indicate that the 1-2Kbp sequences 5'-upstream the respective globin genes each possesses the ability to regulate the developmental stage-specific globin gene expression in medaka. This is considerably different from mammalian LCR (locus control region) playing a role in globin gene expression.

ANALYSIS OF A CIS-REGULATORY REGION FOR CHORIOGENIN GENE EXPRESSION IN THE GREEN FLUORESCENCE PROTEIN (GFP) GENE-TRANSGENIC MEDAKA

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Choriogenin (Chg) is a precursor protein of egg envelope of medaka (*Oryzias latipes*) and is synthesized in the liver of spawning female. Expression of the Chg is

estrogen-dependent. To analyze a *cis*-regulatory region responsible for the Chg gene expression, we constructed a vector having both a fusion gene of a *cis*-regulatory region of embryonic globin gene with RFP gene and a fusion gene of a 1.4Kb 5'-upstream region of the Chg-L gene with GFP gene, injected the vector into 1- or 2-cell-stage embryos, and selected the embryos expressing the RFP in erythroid cells. When exposed to 17-beta-estradiol (10ng/ml) for 3 days, one of adult fish from the RFP-positive embryos showed the liver-specific expression of the GFP. The result indicates that the 1.4Kb 5'-upstream region plays an important role in the estrogen-dependent, liver-specific expression of the Chg-L gene. The estrogen-dependent GFP expression was detected in the liver of late embryos and fully-grown adult fish. We established a line of transgenic medaka stably expressing both the RFP and the GFP. Such estrogen-responsive transgenic medaka can be used as a novel probe for detecting environmental estrogenic steroids

EVOLUTION OF VERTEBRAE: AN APPROACH FOR FOCUSING ON *CIONA INTESTINALIS PAX1/9* AND MEDAKA *PAX1* TRANSCRIPTION REGULATORY MECHANISM

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Pax1 plays an important role in the differentiation of sclerotome cells. First, we examined that expression in embryogenesis: *Ciona intestinalis Pax1/9* is expressed only in the progenitor of gill slits, while medaka *Pax1* is expressed in gill slits and sclerotomes in similar patterns of other vertebrates. Therefore we hypothesized Chordates evolved to vertebrates as a result of acquisition of *Pax1/9* expression in sclerotomes in addition to gill slits. Secondly, we tried to compare those transcription regulatory mechanisms between *Ciona intestinalis* and medaka by promoter analysis.

POPULATION STRUCTURE AND GENETIC DIVERSITY IN PACIFIC HERRING, *CLUPEA PALLASII*, BASED ON MITOCHONDRIAL DNA SEQUENCE VARIATIONS

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Pacific herring (*Clupea pallasii*), a commercially important fish, has been classified into several forms based on their breeding ecology and morphological characteristics in northern Japan. We examined mitochondrial DNA (mtDNA) sequence variations to infer their population structure and genetic diversity among six local populations corresponding to Ishikari Bay (ISH), Hokkaido-Sakhalin (SAK), Lake Furen (FUR), Lake Akkeshi (AKK), Tomakomai (TOM) and Mangoku-Ura (MAN) forms. Sequences of the first half of mtDNA control region were obtained from 30 individuals for each population. Phylogenetic analyses using maximum parsimony and neighbor-joining methods both showed that 39 haplotypes identified were closely related to each other, no cluster being supported by significant bootstrap probability. Significant genetic differentiation was detected between MAN and TOM as well as between these two and the other populations. Gene diversity observed within TOM (0.96) was significantly higher than those in the other populations (0.80-0.93). AMOVA analysis revealed that 96 % of the total genetic variation occurred within populations.

GEOGRAPHIC POPULATION STRUCTURE OF TIGER PUFFER, *TAKIFUGU RUBRIPES*, BASED ON MITOCHONDRIAL AND MICROSATELLITE DNA VARIATIONS

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Population structure and genetic diversity were investigated in wild populations of tiger puffer (*Takifugu rubripes*) collected from three major breeding sea areas near Yamaguchi, Aichi-Mie and Ishikawa prefectures. Significant population subdivision was detected among the three areas in microsatellite analysis on six loci, but was detected only between Aichi-Mie and Ishikawa prefectures in mitochondrial DNA (mtDNA) control region sequence analysis. Nested clade analysis on intraspecific mtDNA phylogeny revealed nonrandom geographical distributions of mtDNA lineages for two out of 14 nested clades examined: one was due to the restricted gene flow with isolation by distance and the other was due to the contiguous range expansion from Yamaguchi to Aichi-Mie prefectures. Estimates of observed heterozygosity for six microsatellite loci in each population were 0.85-0.92 and those of gene diversity for mtDNA were 0.80-0.90. AMOVA analysis revealed that a large portion (98% or above) of genetic variation was found within population in both markers, indicating a little population structuring.

REPRODUCTIVE ISOLATION THROUGH SIZE DIFFERENCE IN *DROSOPHILA MELANOGASTER*

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Mechanical isolation is one of sympatric isolation mechanisms, and it is caused by physical unfitness of reproductive organs of males and females. The body size is affected with mutations and environmental conditions such as temperature and rearing density. It has not been reported so far that the size difference influences the mating success. The generality of the mechanical isolation is controversial. In this study, selection experiments for adult body weight were carried out for 14 generations in *Drosophila melanogaster*. It was examined whether sexual isolation developed or not between heavy and light selected lines. At the final generation of selection, it was uncovered that the reproductive isolation occurred between females with heavy body weight and males with light one. This reproductive isolation was confirmed not to be ethological but to be mechanical. It was observed that small males have a great trouble to copulate with large females due to the difference in body size. That is, the isolation results from the fact that the penis of males with small size can not reach to the female reproductive organs.

CHARACTERIZATION OF GENETIC VARIATION AMONG INBRED STRAINS OF BRINE SHRIMP (*ARTEMIA FRANCISCANA*) USING RAPD MARKERS.

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The brine shrimp, crustacean *Artemia*, has some advantages to be a new model organism. We had established inbred strains of *Artemia franciscana*. Several strains of F₃₅-F₄₁ were kept in our laboratory and we tried to estimate genetic variations among each strains and individuals using RAPD (random amplified polymorphic DNA) markers. In preliminary tests, we utilized 10-mer primers which were used in phylogenetic study of bisexual *Artemia* species (*Badaracco et al, 1995). These analyses confirmed that genetic diversity in each strain was quite low.

*Badaracco G, Bellorini M, Landsberger N (1995) Phylogenetic study of bisexual *Artemia* using random amplified polymorphic DNA. Journal of Molecular Evolution 41: 150-154

COMPARISON OF LENTIC AQUATIC INSECTS IN OGASAWARA ISLANDS

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There are many endemic species in Ogasawara Islands. But most of them are going to decrease by the existence of the introduced species and the change of habitat in Ogasawara Islands. Aquatic insects are one of them. In this study, we provide the artificial ponds in Ogasawara Islands as one of their habitat, and the change of aquatic insects assemblage was investigated in the natural and the artificial ponds. Then the possibility of protection and multiplication of aquatic insects was examined. In chichi-jima island, one of the Ogasawara Islands, introduced species are abundant. The assemblage of aquatic insects in one natural pond of chichi-jima island was largely different from other ponds. The individual number of dragonfly was also fewer in chichi-jima island. Endemic species of dragonfly and damselfly could be collected in the natural and the artificial ponds with the exception of the ponds in chichi-jima island. Artificial ponds are considered to provide the reproductive spaces for endemic species.

FEEDING RESPONSES OF THE BENTHIC ARROW WORM, *PARASPADELLA GOTOI* (CHAETOGNATHA), TO THE CHEMICAL STIMULATION.

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Chaetognaths are carnivorous and feed on copepods and fish larvae. The feeding behavior consists of three steps; orientation to the prey, prey capture by grasping spines, and ingestion. We have found the last two responses of the feeding behavior are induced by the chemical stimuli such as extraction of food organism and amino acids using the planktonic arrow worm, *Sagitta crassa*. In the present study, we examined the feeding responses of the benthic arrow worm *Paraspadella gotoi*. The arrow worm showed movement of the grasping spines and ingestion to the extraction of *Artemia* nauplii (0.1 g/ml). However, the response percentage was lower than that of *S. crassa*. This may be due to the difference of the mouth part structure. The feeding responses of *S. crassa* were elicited by application of amino acids, such as Gly, Pro,