

GENETICS / EVOLUTION

Poster abstracts

COMPARISON OF COLLECTION AND DNA EXTRACTION METHODS FOR COMMUNITY ANALYSIS OF UNICELLULAR EUKARYOTES FROM A LACUSTRINE SYSTEM

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Unicellular eukaryotic plankton play a central role in the complex and dynamic microbial food web (MFW). The taxonomic composition of these organisms as part of fresh-water microbial communities has not been the object of intensive study in the past especially with molecular techniques that have been applied widely to marine systems. One place where the MFW has been examined is the Old Woman Creek National Estuarine Research Reserve (OWC-NERR) and long term projects continue. Because of the wealth of background ecological and taxonomic information available, we have initiated a study to catalogue the genetic diversity at the site. At present, we have generated more than 150 partial 18S ribosomal sequences from a single location in the reserve. These sequences were derived from PCR products from community genomic extractions resulting from filtrate from either 2 liters of non concentrated water sample or 150 ml pre-concentrated sample run through a 0.2 micron filter. For both of these initial samples two genomic extractions were initiated with the first involving a sonication step and the other subject to "bead-beating". As a result of these extractions, four separate sets of PCRs were done and the products cloned and sequenced separately to compare the efficiency of each sample and extraction method at capturing the community diversity. The latter was accomplished by comparing the 18S results with direct counts using traditional morphological identifications. The results demonstrate that more "rare" taxa were identified from the non pre-concentrated 2 liter samples. Furthermore, no significant differences in the diversity and relative abundance of organisms were found between samples that were sonicated versus bead-beated prior to DNA extraction.

Keywords: *Plankton, 18S, Food web*

A STUDY OF PATERNITY IN THE ALLIGATOR SNAPPING TURTLE (*MACROCHELYS TEMMINCKII*) USING MICROSATELLITE MARKERS

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The Alligator Snapping Turtle (*Macrochelys temminckii*) is the largest freshwater turtle in North America. It has been heavily exploited in the past and is protected in every state of its range. *Macrochelys* exhibits strong sexual size dimorphism suggesting that forced insemination may occur in the wild. Multiple paternity, as a possible result of forced insemination, is an important mode of reproduction because it may help to slow the loss of genetic variability through drift in threatened species. Microsatellite regions in DNA mutate at such a high rate that they are good

indicators of parentage. Two microsatellite loci have already been identified for *Macrochelys*, and more will continue to be sequenced from an enriched genomic DNA library and used to determine the parentage of at least eight clutches of turtles collected from Black Bayou Lake National Wildlife Refuge.

Keywords: *Macrochelys temminckii*, *microsatellites*, *multiple paternity*

PERVASIVE RNA EDITING AMONG MITOCHONDRIAL *NAD5* TRANSCRIPTS IN HORNWORTS

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Hornwort mitochondrial genomes have some of the highest rates of RNA editing among plants. Work to explore the phylogeny of hornworts using mitochondrial genes allowed us to infer divergent frequencies of RNA editing sites among *nad5* sequences. Comparison of nine partial mitochondrial *nad5* genomic and cDNA sequences from diverse taxa of hornworts reveal 113 edited sites in only 1107 nucleotides. No single sample has more than half of these sites though each hornwort taxon has similar total numbers of edited sites with one exception. *Leiosporoceros*, which represents a potentially sister taxa to all other hornworts, has only eight edited sites. Phylogenetic analyses of hornwort genomic and cDNA sequences reveals that 54 of 84 (64%) of the phylogenetically informative sites within the hornwort clade are edited positions. These sites are shown to play a role in determining the topology of the basal nodes of the hornwort phylogeny. In particular, *Leiosporoceros* is resolved as sister to the hornworts when edited sites are included in the analyses but when edited sites are removed the support for the *Leiosporoceros* sister topology is lost.

Keywords: *Hornworts*, *RNA editing*, *nad5*

SPECIES-DIAGNOSTIC POLYMERASE CHAIN REACTION ASSAY FOR THE IDENTIFICATION OF *ANOPHELES* VECTORS OF HUMAN *PLASMODIUM SPP.*

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Two multiplex PCR were developed to identify two sets of morphologically similar species of anopheline mosquitoes that are primary and secondary vectors of malaria in South America. Each multiplex PCR incorporates species-specific primers with a single primer that anneals to a conserved region of the rDNA. Species-specific primers were based on interspecific sequence variation in the second internal transcribed spacer (ITS2) of the nuclear ribosomal DNA. One multiplex PCR identifies *An. oswaldoi* and an undescribed species collected in Bolivia. A second multiplex PCR identifies mosquitoes as belonging to the *Albitarsis* Complex, which includes *An. deanorum*, *An. albitarsis*, *An. albitarsis B*, and *An. marajoara*. The resulting amplicon is digested with Bfa-I to identify *An. marajoara*. Identifications using multiplex PCR are useful for continuing studies on the ecological differentiation and population structure at the level of breeding sites for the aforementioned species (at least at the regional level of Bolivia) in areas where species diversity is high and the ecology is complex.

Keywords: *Nyssorhynchus*, *Anopheles*, PCR identification

THE INFLUENCE OF POPULATION STRUCTURE ON HISTORICAL DEMOGRAPHIC INFERENCE

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When analyzed in the appropriate theoretical framework, molecular genetic data can be used to infer the demographic history of a population. For example, two approaches that can detect recent reductions in population size from microsatellite data are heterozygosity excess (*Bottleneck* program) and the *M*-ratio. A key assumption of these models is population closure; new alleles can only arise from mutation and not immigration. In reality, local populations are rarely isolated enough to meet this requirement. Before performing bottleneck analyses, it is essential to determine the extent of a population and thus make informed decisions on which sampling locations should be grouped or analyzed independently. We propose to do this with natural populations of the banner-tailed kangaroo rat (*Dipodomys spectabilis*) using F_{ST} -based approaches and assignment tests. At our study site, the putative population(s) are geographically proximate (<300m) and field studies have detected low levels of dispersal among them. A thorough understanding of population structure is requisite prior to conducting demographic analyses.

Keywords: *demographic history, genetic structure, bottleneck, kangaroo rat*

MORPHOLOGICAL VARIABILITY IN INTERSPECIFIC HYBRIDS OF FLAMMULINA

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Flammulina (Basidiomycetes, Agaricales, Tricholomataceae) is a saprobic mushroom found in the wild that is cultivated commercially and marketed worldwide under the name "Enokitake." Until the early 1960's, the species epithet *velutipes* was uniformly applied to all collections in the genus. Since then, several species have been described from a variety of ecosystems on several different continents based on morphology, mating studies, and molecular data. Interspecific hybrids "fruited" in the laboratory on synthetic logs composed of tulip poplar saw dust and rice bran produced more or less normal basidiomata with viable basidiospores. Macroscopic and microscopic features were subsequently used to describe the morphology of these hybrids. To date, both macro- and micro-morphological characterization of hybrid mushrooms have been completed and revealed morphologies that mimicked one parental species or the other rather than intermediate or "hybrid" morphologies. Since the hybrid mushrooms are "fertile," the second objective of the project was to collect single basidiospore isolates and determine the mating types of each hybrid. Once the mating types have been isolated, the hybrid mating types will be crossed back to the parental mating types to determine whether or not the hybrids are reproductively isolated from the parental species. Finally, molecular characterization of the hybrids will be conducted using the ITS regions of nrDNA to accomplish the third objective of the study. The main objective of this portion of the study is to determine what kinds of modification of the hybrid ITS region occurs following karyogamy and meiosis. Results from cloning and sequencing of the ITS region are currently underway and results will follow.

Keywords: *Fungi, Hybridization, Basidiomycetes, Flammulina.*

BACTERIAL SUCCESSION ON *ACER SACCHARINUM* LEAVES IN THE ILLINOIS RIVER FLOODPLAIN

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Ecological succession of species is a well-established concept for macrobiota. The objective of this study was to monitor change in prokaryotes in the Domain *Bacteria* on and in maple leaves beginning with the microbial community colonizing leaf buds and following changes through decomposition on the flood plain of the Illinois River. In addition to tracking changes through this temporal variable, a spatial comparison was made among leaves falling on the floodplain, those on the floodplain wetted by flood waters, leaves in a drier, upland habitat, and those permanently soaked in river water. It was expected that bacterial diversity would increase as leaves mature and that a replacement community would emerge when the leaf drops to the river floodplain. Leaves were sampled over a 21-month period and change in organic mass was determined (AFDM). The microbial assemblage was monitored through 16S rDNA extraction followed by community fingerprinting on denaturing gradient gel electrophoresis (DGGE). Domain *Bacteria* DNA was examined at the bud stage, at the mature leaf stage, and on samples collected at 6 to 10 week intervals after leaf fall. DNA extracts, purified using Sephadex G-200 columns and amplified by PCR with 338F - GC clamp and 518R primers were run on DGGE (8% acrylamide, 30-70% denaturing gradient). Results show nearly twice as much decomposition in leaves exposed to flood water (70.1%) than for those not wetted by floodwaters (36.0%). Molecular results showed limited diversity before leaf fall. More bands were found after leaf fall and on leaves exposed to flood water, some unique bands not found on unexposed leaves were present. As expected, diversity in bacterial communities increases initially after leaf fall, decreases as leaves become more decomposed and introduction of new communities occurs after leaf fall and flooding.

Keywords:

CHARACTERIZATION OF DIPTERAN TESTIS PROTEINS TO DETERMINE HOW $\beta 2$ TUBULIN HAS EVOLVED WHILE MAINTAINING A FUNCTIONAL AXONEME

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Classical Darwinian theory states that competition among phenotypic variants drives both natural and sexual selection. This, however, is not always possible. One instance in which variation may no longer be able to drive evolution is in the testis specific isoform of beta tubulin. While the testis-specific beta tubulins present in Lepidopterans and some Dipterans have been able to evolve, their tubulin ortholog in Drosophilids has not evolved in 60 million years. The extreme conservation of the amino acid sequence of Drosophilid $\beta 2$ stems from its stringent structure/function relationship to other proteins in the axoneme, and raises a question of evolutionary importance: How are structures able to evolve while maintaining their function? Axoneme proteins must be co-evolving together in order to synthesize a functional sperm tail. Through comparing the testis proteins of several Dipterans on two dimensional acrylamide gels, we have been able to identify several candidates that may be co-evolving with $\beta 2$ tubulin in Drosophilids.

Keywords: *tubulin*, *Drosophila*, *axoneme*, *constraint*

