



Abstracts 15th ISDCI Congress, Wageningen, The Netherlands

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023)
104922 104923**

A NEW APPROACH TO DIAGNOSTICS AND THERAPEUTICS IN VETERINARY MEDICINE

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Veterinary research into health and diseases and the development of diagnostics and therapeutics is behind that of human medicine. This is due to the complexity of studying a wide range of organisms, including both invertebrate and vertebrate species and the available tools that are developed specifically targeting human problems. EpitoPredikt™ and EpitoGen™ are new innovative technologies that can overcome these bottlenecks, offering immediate solutions to current research, diagnostic and therapeutic problems within veterinary medicine. EpitoPredikt™ is a software that incorporates experimental data to accurately determine immunodominant epitopes and predict the corresponding antibody isotype response. The software is embedded with advanced machine and deep learning architecture, allowing it to continually evolve through the analysis of available data and can be trained to recognize epitopes that are species or disease specific. EpitoGen™ is a biological scaffold bio-engineered to express and display single epitopes (7 to 700 aa) or multiplexed epitopes (up to 50 fragments), in their native conformation, in a cost effective way. It has been bioengineered to be extremely stable and have high efficiency (99%) and yield (200mg/ml). Scaffolds expressing single epitopes can be used for immunogenicity profiling and are compatible with standard ELISA and lateral flow platforms. Furthermore, the scaffold's multiplexing capacity is unmatched and ideal for developing accurate serology tests and effective epitope-based vaccines. This presentation will look at the two technologies and highlight how they can help advance research, aid high throughput screening and the rapid development of accurate diagnostic assays and effective vaccines within the veterinary field.

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LOCAL GENOMIC INSTABILITY OF THE SPTRANSFORMER GENE FAMILY IN THE PURPLE SEA URCHIN INFERRED FROM BAC INSERT DELETIONS

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The *SpTransformer* (*SpTrf*) gene family in the purple sea urchin, *Strongylocentrotus purpuratus*, encodes diverse immune response proteins. The family has a complex genomic structure in which the genes are tightly clustered, are surrounded with short tandem repeats (STRs; GA, GAT), have

several types of intergenic repeats and patches of shared sequence, and several genes that are a product of segmental duplication. This type of genomic region that is riddled with repeats, is hypothesized to be unstable and are thought to drive gene conversion, duplication, and deletion events. To test whether the *SpTrf* gene clusters are unstable, *E. coli* harboring bacterial artificial chromosome (BAC) clones containing overlapping regions of the *SpTrf* gene family were used. After a 10 day growth period, BAC inserts from single colonies were evaluated for size and *SpTrf* gene content. Long read assemblies of four BACs inserts showed deletions ranging in size from ~5kb to ~109kb with most inserts showing more than one deletion. *SpTrf* gene loss ranged from one to six of seven and included truncations of two *SpTrf* genes. Five of nine deletions were positioned within or near STRs. An insert with a single *SpTrf* gene was stable. Deletions suggest insert instability, consistent with reports on single coelomocytes showing variations in the *SpTrf* gene types and reduced numbers of *SpTrf* genes compared to single sperm cells. Local genomic instability may be an important aspect for driving sequence diversity in the *SpTrf* gene family that may be beneficial in the arms race with marine microbes.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023)
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DUPLICATED IMMUNE GENES MAY PROVIDE A BUFFERING EFFECT ON FITNESS IN THE THREATENED GILA TROUT (*O. GILAE*)

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Genetic diversity is thought to be associated with increased fitness and long-term persistence of a species. This idea motivates conservation efforts to maintain genetic diversity as a primary goal. However, recent studies exploring the expected relationship of genetic diversity and species persistence have challenged this idea. For example, genes of the major histocompatibility complex (MHC), require high levels of allelic diversity for hosts to recognize a broad array of pathogens. Yet demographic bottlenecks can be so severe that critical diversity is lost. After a decade of drought and wildfire in the southwest United States, Gila Trout (*Oncorhynchus gilae*) has lost allelic richness and heterozygosity at the MHC-II DAB locus, and yet it persists in the wild. Here we seek to understand how gene duplications, such as those often found within immune gene families, helps to buffer losses of genetic diversity. Analyses are underway using both vaccination challenges and bioinformatic analysis to identify the effects of demographic bottlenecks on immune genes and immune response. Immune challenge experiments indicated similar specific IgM titers in serum to vaccination with enteric redmouth killed vaccine between *O. gilae* and Rainbow trout (*O. mykiss*), suggesting that overall genetic loss has not impaired specific IgM responses. Additionally, the Gila trout genome, tissue archives, and sequence libraries are being used to evaluate the diversity of innate immune gene families such as TLRs and AMPs to understand patterns of genetic variation across duplicated loci in highly bottlenecked populations.

equal contributions to the research