

International Conference on Stickleback Behaviour & Evolution 2022

- Abstracts -

Presenting Author: **Alessandra Schnider**

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Title: **Juvenile feeding ecology in threespine stickleback (*Gasterosteus aculeatus*) of Lake Mývatn Iceland**

Abstract: Interactions between organisms and their environment shape the phenotype and are usually studied in adults. However, the organismal phenotype is largely moulded during the juvenile phase and natural selection during this stage is often strong. Studying juvenile threespine stickleback from lake Mývatn (NE Iceland) offers a unique opportunity to assess organism-environment relationships. The lake is highly heterogenous and the adult stickleback population is divergent in feeding morphology among contrasting habitats. We tested whether juveniles have similar diets as adults by analysing stomach contents of juveniles from distinct locations in the lake. We saw clear differences in juvenile diet composition between contrasting habitats, similar as reported for adults, suggesting that dietary divergence occurs early in life. This is further supported by diet diverging progressively with increasing gut length. Thus, the here presented study delivers insight into diet mediated diversification.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Alexis Heckley**

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Full list of authors: Alexis Heckley, Sandra Binning, Kiyoko Gotanda, Andrew Hendry

Title: **The role of intraspecific variation in ecosystem restorations**

Abstract: Introducing organisms into degraded environments can contribute to ecosystem restoration if the introduced organisms generate cascading beneficial effects at population, community, or ecosystem levels. Threespine stickleback could be an excellent species in this regard because they are important prey for many fish and birds (generating bottom-up effects) and they consume zooplankton and macroinvertebrates

(generating top-down effects). Additionally, because stickleback are widespread across the Northern hemisphere, their value for restorations could be widespread. However, the “type” of stickleback that should be introduced in a given restoration effort has not been considered. In lakes, for example, should we introduce “benthic” or “limnetic” ecotypes (or both)? These two ecotypes (and any other stickleback ecotypes) differ in many traits and this could result in very different effects in the restoration environment. I will focus on dispersal variation in benthic and limnetic stickleback following introductions into lakes as part of two separate restoration attempts. I focus on dispersal because the rate at which the stickleback disperse following introduction will likely be associated with restoration success. For instance, whether stickleback disperse slowly or quickly will change the spatial distribution of their effects. I also focus on two factors that I think will contribute to variation in dispersal, and aim to quantify their effects on dispersal in these lakes: behavioural traits and parasitism. Understanding the factors that contribute to intraspecific variation in dispersal will help us better understand dispersal dynamics occurring during restorations.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Alice Balard**

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Title: **Parasite infection mediates intergenerational DNA methylation in the three-spined stickleback**

Abstract: Parasites are arguably among the strongest drivers of evolution. While the genetics and genomics of parasite resistance of hosts are growingly well understood, major gaps remain about the role and mechanisms of phenotypic plasticity within and across generations. Previous studies on the three-spined stickleback (*Gasterosteus aculeatus*) have shown that paternal infection with a nematode parasite is associated with increased selection in the offspring generation but also increased tolerance upon infection. The genome-wide DNA methylation pattern differed between infected and control fathers, demonstrating the link between infection and DNA methylation. In the present work, we used reduced representation bisulfite sequencing on 135 fish and asked (1) whether some parental DNA methylation induced by the infection can be transmitted to the next generation, and (2) which genes and gene networks are affected. We investigated overall fractional DNA methylation, as well as differential methylation between infected and control offspring, depending on the paternal infection status. We show that an improved body condition of offspring correlates with a lower

global DNA methylation count, possibly indicating a link between tolerance and methylation. Strikingly, the infectious status of fathers strongly correlated with change in the methylome of the offspring, regularly beyond the offspring infection status itself. This project advances our understanding of the mechanisms of phenotypic plasticity in aquatic organisms and their consequences on adaptive evolution.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Alyssa M. Yoxsimer**

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Title: **Identification and characterization of a novel herpes virus in *Gasterosteus aculeatus***

Abstract: Viruses are nearly ubiquitous in ecosystems and show interesting co-evolution with host species. In multiple fish species, alloherpesviruses can cause mortality, exerting selective pressure on host populations and resulting in severe economic losses in aquaculture industries. Here, we identify and characterize a previously undescribed alloherpesvirus infecting stickleback fish. We observed numerous prominent epithelial protrusions (“pebbles”) developing in young fry from 2 out of 9 clutches produced by crossing anadromous sticklebacks from Rabbit Slough, Alaska in June of 2021.

Shotgun sequencing of tissue samples from affected fish revealed a high proportion of non-stickleback sequences with homology to conserved ORFs shared among distantly related herpesviruses of other fish species. We developed PCR assays to test for the presence and abundance of stickleback alloherpesvirus sequences, and detect high viral DNA copy numbers in the liver, spleen, skin, and ovaries from the mothers of affected clutches. We also detect lower alloherpesviral levels in other Rabbit Slough fish, suggesting that active or latent herpesvirus infection may be relatively common in this population. We are currently screening additional stickleback populations to determine global patterns of virus presence. Additionally, we are carrying out RNA-seq studies to better understand viral and host gene expression patterns in infected fish. Sticklebacks are a well-established and powerful model for ecological and evolutionary studies; the discovery of this novel alloherpesvirus presents a valuable opportunity to gain insights into viral and host interactions in a natural system.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Amélie Cant**

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Title: **Development of a multi-biomarker approach of genotoxicity on the three spined stickleback for aquatic biomonitoring application**

Abstract: The aquatic environment is considered as the receptacle of anthropogenic pressures, including chemical contamination as genotoxic compounds, which can impact the health of aquatic species. Genotoxic substances may directly or indirectly affect the integrity of cell's genetic material, which can lead to long-term mutagenic, carcinogenic or teratogenic effects. In this context, the measure of damages at different genomic scales may be relevant and provide an integrated view of the genotoxic risk of exposed organism. The finality of our work is to propose their use as early-warning signals of potential long-term alterations to population and ecosystem health.

Genotoxicity assessment was performed on erythrocytes of the three- spined stickleback by combining the measure of DNA integrity by the alkaline and Fpg (formamidopyrimidines DNA glycosylase)-modified comet assays and the measure of variations in nuclear DNA content by flow cytometry (FCM). In this sense, blood cells of fish offer many advantages as cellular models as their ease of collection, gives a high density of erythrocytes already dissociated allowing to obtain multiple assays on the same sample. The alkaline comet assay has been largely used with erythrocytes of fish to detect alkali-labile sites, DNA-double- and single- strand breaks. The Fpg-modified comet assay has been optimized in the present study, which expand the comet assay sensitivity to oxidized pyrimidines. Furthermore, FCM may be applied to assess the variations in the nuclear DNA content of a large population of cells and has already demonstrated its relevance for detecting chromosomal damages in blood cells of fish. The presentation will detail results regarding the definition of baseline levels of genotoxicity biomarkers (DNA strand breaks, Fpg-sensitive sites, DNA content, erythrocyte mortality), which are useful in an ecotoxicological context. These biomarkers have been measured in various field context (active biomonitoring) and integrated in the existing multi-biomarker approach of the three-spined stickleback (*Gasterosteus aculeatus* L., 1758).

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Andrew Foote**

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Title: **Stickle-back in time: paleogenomic insights into chronology and tempo of adaptation**

Abstract: Adaptation is typically studied by comparing modern populations with contrasting environments. Individuals persisting in the ancestral habitat are typically used to represent the ancestral founding population; however, it has been questioned whether these individuals are good proxies for the actual ancestors. To address this, we applied a paleogenomics approach to directly access the ancestral genepool: partially sequencing the genomes of two 11- to 13,000-year-old stickleback recovered from the transitional layer between marine and freshwater sediments of two Norwegian isolation lakes and comparing them with 30 modern stickleback genomes from the same lakes and adjacent marine fjord, in addition to a global dataset of 20 genomes. The ancient stickleback shared genome-wide ancestry with the modern fjord population, whereas modern lake populations have lost substantial ancestral variation following founder effects, and subsequent drift and selection. Freshwater-adaptive alleles found in one ancient stickleback genome have not risen to high frequency in the present-day population from the same lake. Comparison to the global dataset suggested incomplete adaptation to freshwater in our modern lake populations. Our findings reveal the impact of population bottlenecks in constraining adaptation due to reduced efficacy of selection on standing variation present in founder populations.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Andrew MacColl**

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Full list of authors: Laura L. Dean, Isabel Santos Magalhaes, Daniele Agostino and Andrew D. C. MacColl

Title: **The benthic phenotype of threespine stickleback in British Columbia may have had a single origin**

Abstract: Rapid evolution of similar phenotypes in similar environments, giving rise to in situ parallel adaptation, is an important hallmark of ecological speciation. However, patterns that appear to evidence repeated, in situ, parallel adaptation can also be consistent with a single origin followed by spread of lineages into suitable habitats. We test whether two contrasting phenotypes in an archetypal example of ecological adaptive radiation: benthic – limnetic three-spined stickleback (*Gasterosteus aculeatus*) across species-pair and solitary lakes in British Columbia evolved repeatedly in parallel, or might have a single origin. We identify two genomic clusters across freshwater populations, which differ in benthic – limnetic divergent phenotypic traits and separate

benthic from limnetic individuals in species-pairs. Phylogenetic reconstruction and niche evolution modelling both suggest a single evolutionary origin for each of these clusters. We detected strong phylogenetic signal in benthic – limnetic divergent traits, suggesting they are ancestrally retained. Accounting for ancestral state retention, we identify local adaptation of body armour due to the presence of an intraguild predator, the sculpin (*Cottus asper*) and environmental effects of lake depth and pH on body size. Taken together our results imply a predominant role for retention of ancestral characteristics in driving trait distribution, with further selection imposed on some traits by environmental factors.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Andrew P. Hendry**

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Full list of authors: Many people are working on this project as will be described in the talk

Title: **Eco-Evolutionary Dynamics with Stickleback - Experiments in Nature**

Abstract: We recently started a long-term experiment studying eco-evolutionary dynamics in threespine stickleback. Our goal is to test the dynamics and predictability of ecology shaping evolutionary dynamics and evolution shaping ecological dynamics IN THE REAL WORLD. Owing to the presence of an invasive species (pike), small lakes on the Kenai Peninsula of Alaska were poisoned in fall 2018 with the chemical Rotenone. In 2019, we introduced stickleback into nine of those lakes – Mike Bell put anadromous stickleback into a tenth lake. Four lakes received limnetic stickleback from a mixture of four limnetic-type populations in the region. Three of the lakes received benthic stickleback from a mixture of four benthic-type populations in the region. Two of the lakes, received a mixture of all eight source populations. In my talk, I will outline the design and implementation of the experiment, the various researchers leveraging this experiment to answer a diversity of questions, a set of predictions that we have about how eco-evolutionary dynamics will play out in the system, and some interesting tantalizing empirical findings that have emerged thus far.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Aruna M Shankregowda**

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Title: Contribution of epigenetic variation to local adaptation in two coexisting and phylogenetically related species

Abstract: The capacity of species to respond to environmental change largely depends on evolutionary potential, which is rooted in the amount and structure of adaptive genetic variation. However, species may compensate for low evolutionary potential through phenotypic plasticity. Adaptive plastic responses to selective forces may be modulated by the regulation of gene expression, for instance via epigenetic modifications such as DNA methylation. In this study, we investigated the role of DNA methylation in the local adaptation of coexisting populations of three-spined (Gasterosteus aculeatus; 3S) and nine-spined stickleback (Pungitius pungitius; 9S) across the Belgian-Dutch lowlands. We previously showed that the two species differ markedly in the strength and nature of local adaptation, where brackish water (BW) and freshwater populations (FW) show stronger phenotypic and genomic differentiation in 3S than in 9S. We hypothesized that differential DNA methylation is most pronounced in 9S, as this may compensate for the apparent lack of genomic differentiation across populations from contrasting salinities. Correcting for sequence effort and genome qualities (i.e., number of mapped reads), our initial results revealed similar proportions of methylation levels between 3S (0.0239) and 9S (0.0245). Likewise, the proportion of DMS was similar between the two species, although 3S had more DMS than 9S. Interestingly, pairwise comparisons for both species within and among FW and BW populations showed that most DMS occurred among FW populations. Overall, our initial results partially meet our hypothesis since 9S has less genetic but similar epigenetic variation. To shed further light on this issue, we will (1) identify the function of genes near differentially methylated sites throughout the genome; and (2) compare the number of differentially methylated genomic regions with the total number of genomic regions involved in freshwater adaptation in the two species.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Bertil Borg**

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Title: **Physiological changes over the female spawning cycle in the three-spined stickleback, Gasterosteus aculeatus**

Abstract: Sticklebacks spawn multiple times during a breeding season and the inter spawning intervals are dependent on the food supply. We studied changes in hormone levels, vitellogenesis and retinal sensitivity to red over the spawning cycle in female sticklebacks of marine origin. The fish were well fed and usually spawned with an interval of three days. Both testosterone (T) and estradiol (E2) displayed higher circulating levels in the inter spawning interval than on the days of spawning. The expression of -luteinizing hormone mRNA was highest two days after spawning, whereas -follicle stimulating hormone mRNA did not change over the cycle. Vitellogenin was studied as vitellogenin mRNA in the liver and as vitellogenin levels in the plasma. Vitellogenin mRNA levels were highest in the inter spawning interval and there were positive correlations between these levels and the plasma levels of T and E2. However, changes over the cycle were small, indicating that vitellogenesis is a rather continuous process during the spawning season. It is known that both male and female three-spined sticklebacks display a higher sensitivity to red in breeding than under non-breeding conditions, which makes sense due to the need to discern rivals and partners respectively with male red breeding colours. The female sensitivity to red was studied as measurement of red colour opsin lws mRNA in the retina and by optomotor tests at different light intensities. Both measures displayed a higher sensitivity to red on spawning days, than in the inter spawning period. This makes sense functionally as the females have no interest in males as mates except when they are ripe for spawning. Unlike for vitellogenin there is no positive correlation between red sensitivity and levels of T and E2, they peak at different times.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Brenna C.M Stanford**

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Title: **Characterizing conserved and divergent molecular responses to temperature between marine- and freshwater-adapted threespine stickleback (*Gasterosteus aculeatus*) using gene expression pathway analyses.**

Abstract: Rapid environmental changes impact the global distribution and abundance of species, highlighting the urgency to understand how populations will respond. Analysis of differentially expressed genes has elucidated areas of the genome involved in adaptive divergence. However, such studies are hampered by large numbers of genes and limited knowledge of how these genes work together. Recent methods (broadly

termed “pathway analyses”) have emerged that aim to group genes that behave in a coordinated fashion to a factor of interest, aiding in functional annotation and uncovering of biological pathways. Here, we reanalyse a dataset that investigated temperature-induced changes in gene expression in marine-adapted and freshwater-adapted threespine stickleback (*Gasterosteus aculeatus*), using three complementary pathway analyses. We found six gene modules that exhibited a conserved and six a divergent response between marine and freshwater stickleback when acclimated to 7C or 22C. One divergent module showed freshwater stickleback-specific response to temperature, and the remaining divergent modules showed differences in height of reaction norms. Analysis of these modules allowed for biological processes and pathways to be uncovered. For instance, one module was enriched for metabolic processes and genes involved in the oxidative phosphorylation pathway, many of which showed differences in expression between freshwater and marine stickleback in response to temperature. This module also contained PPARAa, a transcription factor that controls metabolic genes, which has been implicated in thermal tolerance in several fish species and adaptive divergence between freshwater and marine stickleback. Pathway analyses revealed novel patterns, providing more nuanced understandings of both the organism-level effects of modified gene expression, and the targets of adaptive divergence.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Cameron Hudson**

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Title: **Environmentally independent selection for hybrids between divergent freshwater stickleback lineages in semi-natural ponds**

Abstract: Hybridization during secondary contact can enable rapid adaptation to novel environmental conditions. This can be particularly relevant for invasive, or range expanding, species if gene flow among multiple source populations facilitates spread. Therefore, performing experimental tests of hybrid fitness in contrasting environments, allows us to explore the process of adaptive introgression. Here we are interested in two divergent European threespine stickleback lineages that are vastly different with respect to their age of freshwater colonization. We tested for fitness differences between parental lineages and F1 hybrids using a semi-natural pond experiment, in ponds with contrasting eutrophication histories. We also compared morphological traits between wild-caught adults, as these lineages differ in their niche use. We found that fish from

the older lineage (Lake Geneva population), and F1 hybrids consistently outperformed fish from the younger lineage (Lake Constance population) in both growth and survival, regardless of the environmental context that they experienced. Across all ponds, F1 hybrids exhibited the highest survival. This result could have implications for the outcomes of secondary contact in natural settings. Adult populations differed significantly in a number of functionally relevant morphological characteristics (e.g. gape width, mouth protrusion, suction force), suggesting that these populations are indeed adapted to specialize on different prey types. Whether the divergence in trophic morphology is responsible for fitness differences observed in our experiment remains to be tested. This work suggests that hybridization between these divergent stickleback lineages may therefore allow the population to expand into unoccupied habitats, perhaps accelerating the invasion.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Catherine Peichel (Katie)**

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Title: **World-wide survey of inversion polymorphisms in threespine sticklebacks**

Abstract: Inversions are hypothesized to harbor multiple loci that contribute to local adaptation, particularly in the face of gene flow. Consistent with this hypothesis, three inversions on chromosomes 1, 11 and 21 harbor some of the strongest signatures of divergent selection between marine and freshwater threespine stickleback populations from both the Pacific and Atlantic basins. In a new project funded by the Swiss National Science Foundation, we are using the threespine stickleback system to address several unanswered questions about the evolution of inversions. First, we will identify the phenotypic effects and ecological factors associated with the three inversions by combining association mapping in polymorphic freshwater populations with laboratory crosses on controlled marine genetic backgrounds. Second, we will use experiments in semi-natural freshwater ponds to determine whether and which type of selection acts on these inversions. Third, we will obtain phased sequences of marine and freshwater inversion haplotypes to determine what forms of selection act on inversions and the targets of selection within the inversions. In addition to these projects, we would like to take advantage of the world-wide collections of both marine and freshwater stickleback populations made by our community. Thus, we have designed a set of PCR primers that can be used to genotype all three inversions from both Pacific and Atlantic-derived marine and freshwater populations with a simple length polymorphism visible on an

agarose gel. By distributing these primer pairs to the community, we will build a database of inversion frequencies in marine and freshwater populations around the world, and then use the phenotypic and ecological data collected on these populations to determine whether these inversion frequencies are associated with specific phenotypes or ecological factors. The results of these analyses will be published with all contributors as co-authors.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Carlos Manuel Herrera Castillo**

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Title: **Developmental genetic basis of armour reduction in acidic-adapted three-spine stickleback fish (a study in progress)**

Abstract: The three-spine stickleback (*Gasterosteus aculeatus*) has been the subject of several studies involving adaptation. Phenotypic changes have been linked with the adaptation to different environments. We focus on two populations from North Uist (Scotland) adapted to marine and acidic freshwater environments. The predominant marine phenotype is found to be covered in lateral bony plates, possesses three dorsal spines and a fully developed pelvic girdle formed by pelvic spines, pelvic process and pelvic plates. Contrasting this, the predominant acidic freshwater phenotype is found to have only one dorsal spine and none of the lateral or pelvic structures. The focus of our study is to identify the loci responsible for these phenotypic traits and identify the underlying developmental mechanisms.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Carlos Rodríguez-Ramírez**

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Title: **Molecular mechanisms of adaptation to freshwater in threespine stickleback**

Abstract: The genetic variants that underlie adaptive phenotypes have now been identified in a growing number of cases. However, in most cases, the detailed molecular and developmental mechanisms connecting genotype to phenotype remain a black box. In threespine stickleback (*Gasterosteus aculeatus*), the genes underlying several adaptive phenotypes have been identified. One of these genes is Ectodysplasin A

(Eda), which has pleiotropic effects on the loss of bony lateral plates, changes in lateral line patterning, and changes in schooling behaviour in freshwater stickleback, and is found in an 16kb haplotype that differs between marine and freshwater sticklebacks. In this study, we aimed to gain insights into the developmental pathways and regulatory mechanisms mediating the phenotypic effects of the Eda haplotype. By comparing the skin and head kidney transcriptomes of marine sticklebacks that vary only in whether they carry the marine or the freshwater haplotype of Eda, we found changes in the expression of hundreds of genes and in the patterns of alternative splicing in dozens of other genes. The genes affected by the Eda haplotype include genes involved in ossification processes, BMP signalling and neuronal development, making them strong candidates to mediate the known phenotypic effects of Eda. We also uncovered a putative role of the Eda haplotype in mediating immune phenotypes. These results also highlighted the relevance of regulatory mechanisms like alternative splicing in adaptation. As a follow-up, we are currently investigating whether alternative splicing has played a broader role in adaptation to freshwater in threespine stickleback.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Daniel Bolnick**

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Title: **Stickleback and Schistocephalus: an emerging model system to study fibrotic immune pathology**

Abstract: Evolutionary theory suggests that vertebrates' immunity to parasites reflects a compromise. The benefits of preventing infection must be weighed against the costs of activating an immune response. Yet, there is scarce evidence for costs of immunity in natural populations. If immunity were costly, we would also expect to see examples of animals evolving towards weaker immunity (to mitigate costs). The few examples of such evolutionary loss of immunity all play out over deep macroevolutionary time. Consequently, there is a clear need for microevolutionary studies of the gain and loss of costly immune function. I will present evidence that threespine stickleback have repeatedly gained immunity to a tapeworm parasite, during their replicated colonization of freshwater in the past 12,000 years. Yet, some freshwater populations have more effective immunity than others. We identify the phenotypic and genetic basis of this recently-evolved immunity. In some genotypes, tapeworm infection stimulates extensive

fibrosis throughout the body cavity, which in turn contributes to suppression of parasite growth (in both lab and wild fish), and can kill the tapeworm. However, this fibrosis response is costly, drastically reducing female and male reproductive success (in both lab and wild fish). Consistent with these costs, our quantitative genetic, population genomic, transcriptomic, and phylogenetic analyses all suggest that, in multiple freshwater populations that currently lack fibrosis and tolerate tapeworm growth, selection acted to favor the loss of this costly immune response. These results are unique in that we show the repeated gain and loss of immune adaptations across closely related conspecific populations. Moreover, our findings open exciting new directions for biomedically relevant research: fibrosis is a major pathology in humans, and we show that stickleback contain naturally evolved genetic variation in pro- and anti-fibrotic pathways, providing a new model system to better understand fibrosis and inflammation in our own species.

Presenting day: **Wednesday 27th of July 2022**

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Title: **Convergence and divergence in the evolution of stickleback sex chromosomes**

Abstract: How and why sex chromosomes evolve has fascinated biologists for over a century. But despite the considerable attention they have received, two important questions still remain unanswered: 1) why are sex chromosomes so strongly conserved in some taxa, and so labile in others? and 2) why do sex chromosomes lose recombination? In sticklebacks, some species share conserved sex linked regions, while others have evolved entirely new sex chromosomes, making them an ideal lineage in which to address the above questions. To that end, we have recently assembled, phased, and curated the Y chromosomes of two *Gasterosteus* species (*G. aculeatus*, *G. wheatlandi*). With these resources, we are able to perform high resolution comparisons of sequence evolution, gene traffic and regulatory divergence on the sex chromosomes. The similarities and differences observed in these comparisons shed light on the most important drivers of sex chromosome evolution in these species. In addition, we have now identified the sex chromosomes in several species of stickleback, and notably, we find that the same gene has been recruited twice among different species. This convergence allows us to speculate on the importance of the

architecture of the sexual development pathway in influencing sex chromosome evolution.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Daniel Shaw**

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Title: **Positive selection throughout regulatory elements on the threespine stickleback Y chromosome.**

Abstract: Gene expression evolves rapidly on heteromorphic sex chromosomes (X and Y). A common theme across independently evolved sex chromosomes is the downregulation of most Y-linked genes. Theoretical models of sex chromosome evolution suggest this occurs through the rapid accumulation of mutations within regulatory regions. However, these patterns have not been demonstrated empirically due to the limited number of completed Y chromosome assemblies available to analyze sequence evolution outside of coding regions. The threespine stickleback (*Gasterosteus aculeatus*) is an ideal model to test hypotheses about regulatory evolution because they have a recently evolved Y chromosome that has been assembled. We used this reference assembly to identify regulatory regions on the Y chromosome that were ancestrally shared with the X chromosome. We found that promoter regions and putative enhancer regions on the Y chromosome exhibited elevated substitution rates when compared to intergenic regions and synonymous sites within coding regions. This strongly suggests that many regulatory regions are under positive selection on the Y chromosome. We found that regulatory divergence was correlated with X-biased gametolog expression, suggesting the elevated substitution rate within Y-linked regulatory regions may be acting to downregulate Y-linked gametologs. Although most Y-linked gametologs may lose expression, some classes of genes can retain expression from the Y chromosome, including dosage-sensitive gametologs and genes that have acquired functions for spermatogenesis. We have performed single cell RNA sequencing on meiotically active fish to identify distinct cell types of spermatogenesis. We hypothesized that rapid evolution of regulatory regions is associated with gain of Y expression in post-meiotic cell types. Our findings provide the first evidence that sex-linked regulatory regions can exhibit signs of positive selection quickly after the suppression of recombination. This result has broad implications for understanding how Y chromosomes degenerate and how spermatogenesis evolves in species with recently evolved sex chromosomes.

Presenting day: **Monday 25th of July 2022**

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Title: **Genome-wide analysis of spined and spineless sticklebacks in sub-arctic Norway**

Abstract: Marine three-spine sticklebacks (*Gasterosteus aculeatus*) have colonized numerous freshwater lakes since the last ice age. Subsequent loss of body armor, such as reduction of pelvic spines and lateral plate numbers, is a recurrent and likely adaptive modification observed in many isolated freshwater populations. Pelvic reduction in sticklebacks has previously been associated with the paired-like homeodomain transcription factor 1 (*Pitx1*) locus of chromosome 7. Mutations on the enhancers of *Pitx1* were found responsible for pelvic spine reduction in North American populations. However, our results suggest no differences in the sequences of these enhancers (termed PelA and PelB) between spined and spineless specimens in a sub-arctic lake in North Norway. Thus, we look for genome-wide patterns of diversity and divergence involved in adaptation among the two functional phenotypes, spined and spineless sticklebacks in this sub-arctic population. Two equimolar pools of genomic DNA representing 40 spineless and 40 spined specimens were subjected to Next Generation Sequencing and the sequence data were mapped to a stickleback reference genome. Genome-wide measures of genetic diversity and differentiation, including nucleotide diversity π , Tajima's neutrality statistic D , and fixation index F_{st} , were calculated using a sliding window approach. Our analyses reveal that the overall nucleotide diversity is similar for the two phenotypes and the genomic differentiation between them is small, as expected if they belong to the same population. Overall Tajima's D index is negative for both spined and spineless specimens. Some sequences with outlier F_{st} -values are revealed, especially at chromosome 9 and 19, and more details from these outlier sequences will be presented at the conference.

Presenting day: **Wednesday 27th of July 2022**

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Title: **Threespine stickleback as a key species of the White Sea ecosystem: a review of recent studies on the project**

Abstract: Threespine stickleback now is the most numerous fish in the Arctic and subarctic White Sea due to increase of temperature recent two-three decades. They come inshore from late May - early June to mid-July for spawning, juveniles stay there up to September. Rest of their life fish spend offshore. Our recent main findings are the following. (i) During spawning period, many spawners occur outside the spawning grounds in more open waters. (ii) Females predominate in the population starting from larval stages. (iii) Stickleback are able to find their spawning site within few days if they are moving out up to about 1 km. (iv) On spawning grounds, adult and juvenile stickleback actively prey on both plankton and benthos; egg cannibalism is very typical and often approach up to a half of the diet. Offshore, stickleback prey on larger plankton organisms sometimes including herring larvae. (v) Stickleback play an important role in feeding of sculpin, cod, navaga and herring, but predators, due their low numbers, are probably do not much affect stickleback population. (vi) Trematode *Cryptocotyle* sp infect stickleback on spawning grounds and the infection is higher in more closed areas. (vii) Sexual dimorphism patterns of the White Sea population are the most similar to the marine ecotype, but is more pronounced than in most of other populations. (viii) With increase of distance between spawning grounds and wintering area, body shape of stickleback is getting more fusiform. (ix) Studies of migrations of stickleback on spawning grounds using underwater cameras and performing image analysis with neural networks allowed to describe diurnal dynamics of migrations and their day-to day differences. (x) Analysis of coloration showed that sexes differ even in non-breeding conditions. These results allowed to better understand the stickleback population biology and their ecosystem links.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Florent Sylvestre**

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Title: **Do intra-locus sexual conflicts maintain genetic diversity? Insights from the three-spined stickleback genome**

Abstract: In natural populations, the fitness optimum of a trait commonly differs between males and females. When sexes share the same genetic architecture for such a trait, intra-locus sexual conflict may emerge, with alleles beneficial in females but deleterious to males, or vice-versa. Those conflicts have the potential to maintain genetic diversity by inducing balancing selection. Yet, because of the statistical challenges of detecting such conflicts, it is still unclear to what extent intra-locus sexual conflict occurs in natural populations and maintains genetic diversity throughout genomes. Here, we investigate intra-locus sexual conflict in the three-spined stickleback, taking advantage of its high-quality reference genome and newly sequenced Y chromosome. We used whole genome sequencing (15X) of 50 males and 50 females from a panmictic population to screen the genome for signatures of intra-locus sexual conflict. Using both SNP-by-SNP and multivariate analyses, we identified ~1370 SNPs potentially associated with intra-locus sexual conflict. Comparing genomic patterns of conflicts with variation in nucleotide diversity, Tajima's D and recombination rates showed that most of putative candidates for intra-locus sexual conflict are neither associated with increased genetic diversity nor balancing selection. However, we identified five regions of increased genetic diversity that represent an intra-locus sexual conflict associated with balancing selection. Altogether, our results suggest that most intra-locus sexual conflicts do not drive long term balancing selection resulting in increased genetic diversity. They also suggest that: i) Intra-locus sexual conflict does not generate stable polymorphism or ii) the emergence of intra-locus sexual conflict is a dynamic process that may be quickly resolved or; iii) selective pressures vary through time.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Frédéric Chain**

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Title: **Evolution of newly emerged genes and their contributions to differentiation between stickleback populations and species**

Abstract: New genes can spur adaptations and the evolution of novel functions. Research in sticklebacks has revealed recently evolved genes contributing to diversification among populations and species, including an expansion of spiggin genes important in nest building, copy number increases of a metabolic gene associated with diet availability, and duplications of hemoglobin and immune genes across lineages.

The addition of these genes to stickleback genomes has potentially contributed to colonization of new habitats and ecotype differentiation by diversifying or enhancing levels of protein synthesis. We have found that new/young genes are enriched among genes displaying population differentiation in copy numbers, and a large proportion of these have dosage effects in which more copies lead to higher expression. While extra gene copies can provide rapid adaptive expression changes, most gene copy number increases in stickleback do not strongly correlate with expression increases. When new genes are not immediately beneficial via dosage, we hypothesize that epigenetic modifications like DNA methylation and histone modifications often shield genes from purifying selection by repressing transcription, allowing genes to persist in the genome before functionalization or degeneration ensues. To test this, we are studying the early evolutionary dynamics of newly emerged stickleback genes by combining analyses of copy-number polymorphic genes (non-fixed genes that are detected as CNVs) and lineage-specific genes (absent in some populations or species). We find that younger genes and CNVs have on average higher methylation and lower expression than older genes, and that this supposed transcriptional repression remains for most genes that emerged in sticklebacks even before speciation of threespine, fourspine, and ninespine sticklebacks. We also find epigenetic repression of young genes and CNVs in zebrafish, suggesting a conserved evolutionary mechanism that preserves new genes that are not immediately beneficial, extending the window of opportunity for long-term genetic diversification such as neofunctionalization or subfunctionalization.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Grant Haines**

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Title: **Threespine stickleback diversity and contemporary evolution in Eastern Canada**

Abstract: The threespine stickleback are widespread in coastal regions of the northern hemisphere, and serve as important prey for larger fish that support important fisheries, as well as many bird species. Although studied extensively as a model organism in evolutionary biology, behavioral ecology, genomics, and numerous related subfields, previous stickleback research relies heavily on populations from the Pacific coastal regions of North America and Asia, and northern Europe. However, based on the morphology of some western Atlantic populations, the different ecological context, and the evolutionary history of the species, not all of the knowledge gained from Pacific and

European lineages is likely to be fully transferrable to the populations of North America's East Coast. In particular, the work on the selection pressures and genetic mechanisms influencing lateral plate morphs and pelvic reduction appears to be of reduced applicability in eastern N. America. The limited research in eastern Canada does suggest high levels of intraspecific phenotypic diversity and local adaptation, though much of this diversity may be under threat from changing climate, altered land use patterns, and especially introduced species. These factors warrant a research program focused on broad sampling of previously identified populations, identifying previously undocumented populations, determining whether there are unique genetic mechanisms underlying the unusual trait combinations present in the region, and exploring novel community interactions. Such a research program would facilitate the documentation of phenotypic change and establish baselines for future work. In my presentation, I will provide an overview of the evolutionary and biogeographic history of threespine stickleback in eastern Canada, outline a research program to build on our knowledge of stickleback diversity and contemporary adaptation to anthropogenic changes, and to apply that knowledge to broader conservation goals. Additionally, I will discuss preliminary results from fieldwork to be conducted this summer in Québec.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Heidi I. Chen**

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Title: **Investigating the genomic basis of convergent fin evolution in percomorph fish**

Abstract: Percomorph fish have undergone dramatic evolutionary radiation over the last ~100 million years, generating thousands of natural species with recurrent changes in pelvic and caudal fin morphology. While it has been shown that repeated deletions of a pelvic enhancer (PELA) from the key developmental gene PITX1 contribute to repeated pelvic fin reduction in multiple freshwater populations of threespine stickleback, much less is known about the genomic bases of convergent fin reduction across the broader percomorph tree. We aligned whole genomes of 37 diverse percomorph fish species, and searched for recurrent deletions of conserved orthologous sequences (CONDELs) that were consistently associated with fin reduction -- i.e. genomic regions that are well-conserved in fin-complete outgroup species but entirely missing from multiple independent fin-reduced ingroup lineages. We subsequently used enhancer assays and genome editing methods to test the functions of these sequences repeatedly lost in

multiple fish species that have evolved fin reduction. Enhancer assays in transgenic stickleback and in cultured medaka fin cells suggest that deletions in PELA not only contribute to pelvic fin loss in various Gasterosteidae populations, but also across many other wild fish lineages. We also identify a novel enhancer that is conserved in the majority of percomorph fish, drives caudal fin expression in transgenic stickleback, and is missing in numerous species within Tetraodontidae, Synbranchidae, and Syngnathidae that exhibit caudal fin reduction. Recapitulation of the evolutionary CONDEL lesion using CRISPR-Cas9 editing in stickleback results in fish with dramatically altered caudal fin skeletal ray morphology. Our study contributes to a greater understanding of the patterns and types of molecular mechanisms used to modulate skeletal morphology across diverse lineages of the 100-million year old percomorph radiation.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Helen Spence-Jones**

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Title: **Do you remember? Biological memory of climate variability and extreme events in marine threespine stickleback**

Abstract: Climate change is not only leading to a warmer world, but also to an increase in temperature variability and the frequency and magnitude of extreme events such as marine heatwaves. Identifying how organisms cope with such changes is important for predicting consequences for population persistence, and ultimately, biodiversity and ecosystem services. 'Biological memory', including parental effects, (transgenerational) plasticity, and epigenetic inheritance are mechanisms where previous experiences of the individual or its ancestors affect an organism's response to environmental conditions. Such mechanisms have the potential to lead to rapid, adaptive responses to environmental change. We investigated how threespine stickleback respond to increased temperature variation and heatwaves, on both an individual and transgenerational level. We used split-clutch full-factorial experimental designs to examine mechanisms of how parental and offspring experience of temperature variation and extremes affect development – looking at survival, growth, morphology and cognition, as well as DNA methylation and gene expression patterns. Theory predicts that parental experience of environmental variability should lead to 'diversified bet-hedging' strategies, where increased inter-individual variation improves the probability of high fitness in at least a subset of offspring. We did not find evidence of

this in our experiment; neither variation in egg size nor variation in offspring size were influenced by parental exposure to temperature variation. Rather, parents exposed to predicted increases in temperature variability may instead have engaged in 'conservative bet-hedging' of offspring body shape, with offspring displaying reduced inter-individual morphological variation. As wild stickleback populations have likely experienced variable temperatures as part of their selective history, it may be that they are already equipped for environmental variability. Nevertheless, the magnitude of such variability in addition to increasing frequency of marine heatwaves may push them toward their thermal limits, with implications for adaptive potential to future climate change.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Jakob Gismann**

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Title: **Personality-dependent colonization success in three-spined sticklebacks**

Abstract: Behavioural ecologists are well aware of the fact that individuals within populations differ in behavioural expression consistently over time and across context (i.e. animal personality).

While many studies focused on the proximate mechanisms responsible for such consistent

differences in behaviour, empirical investigations of fitness consequences, especially in the

animal's natural environment, remain challenging.

By investigating whether activity and aggression of three-spined sticklebacks (*Gasterosteus*

aculeatus) – as measured in the lab – correlate with movement behaviour in a novel environment, we first examined the existence of a dispersal syndrome in the population.

Next, by remotely monitoring territory acquisition via a RFID system in a large mesocosm

consisting of several connected ponds, and subsequent male mating success, we explored

the relationship between personality differences and important proxies of fitness under semi-natural conditions.

We found that male personality predicted territory acquisition and mating success in the

mesocosm where more aggressive and more active males were more successful colonizers.

We here shine light on the often unclear relationship between personality differences and

fitness and exemplify how the use of mesocosms can be a valuable tool to bridge the gap

between studies in the laboratory and the wild.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Janette Boughman**

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Title: **Mate choice in the brain: Species differ in how male traits ‘turn on’ gene expression in female brains**

Abstract: Substantial research demonstrates the importance of sexual selection and female mate choice to speciation, yet we know very little about the cognitive mechanisms that underpin female decision-making or its genetic basis. One key question is: how does gene expression help regulate mate choice decisions? Prior research revealed that limnetic and benthic female sticklebacks use different display traits to evaluate prospective partners and they reject heterospecific males based on differences in these displays. We predicted divergent female preferences would result in divergent gene expression patterns in brains of benthic and limnetic females, and this would be especially pronounced when evaluating displays known to cause premating isolation. To test this, we coupled behavioral data from a mate choice experiment with gene expression data from female brains. We find substantial differences between species in which genes are expressed, regardless of context, suggesting general divergence in gene expression patterns in female brains. Importantly, we also find gene expression differences between females courted by conspecific versus heterospecific males, suggesting these genes are involved when females differentiate between species and are thus important for speciation. Our most novel findings are that we can connect gene expression levels to female choice behavior when evaluating specific male morphological and behavioral displays. We find genes and modules that respond to variation in male display but are expressed in opposing directions for the two species, suggesting male display variation elicits a genomic response that in turn influences female decision-making. For example, nuptial color ‘turns on’ expression of some genes and modules in limnetic females but ‘turns off’ expression in benthic females, thus linking these genes to divergent female preferences and premating isolation. Our study

helps fill gaps in our knowledge about female decision-making in how it maintains isolation between diverging species.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Jesse N. Weber**

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Title: **Found then lost: stickleback repeatedly evolve, and then lose, self-damaging immunity to cestode parasites**

Abstract: Parasites impose fitness costs on their hosts. Biologists therefore tend to assume that natural selection favors infection-resistant hosts. Yet, when the immune response itself is costly, theory suggests selection may instead favor either tolerance or loss of resistance. The evolution of heritable immune differences are difficult to document in nature, and there are few examples of adaptive loss of resistance. Here, we show that when marine threespine stickleback colonized freshwater lakes they gained resistance to the freshwater-associated tapeworm, *Schistocephalus solidus*. Extensive peritoneal fibrosis and inflammation contribute to suppression of cestode growth and viability, but also impose a substantial cost of reduced fecundity. Combining genetic mapping and population genomics, we find that opposing selection generates immune system differences between tolerant and resistant populations, consistent with divergent optimization.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Joseph S Phillips**

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Title: **Demographic basis of spatially structured fluctuations in a threespine stickleback metapopulation**

Abstract: Uncovering the demographic basis of population fluctuations is a central goal of population biology. This is particularly challenging for spatially structured populations, which require disentangling synchrony in demographic rates from coupling via dispersal. In this study, we fit a stage-structured metapopulation model to a 29-year times series of threespine stickleback (*Gasterosteus aculeatus*) abundance in the heterogeneous and productive Lake Mývatn, Iceland. The lake comprises two basins (North and South)

connected by a channel through which the stickleback may disperse. The model includes time-varying demographic rates, allowing us to disentangle the contributions of recruitment and survival, spatial coupling via dispersal, and demographic transience to the population's large fluctuations in abundance. Our analyses indicate that recruitment was only modestly synchronized between the two basins, whereas survival probabilities of adults were more strongly synchronized, contributing to cyclic fluctuations in the lake-wide population size with a period of approximately six years. The analyses further show that the two basins are coupled through dispersal, with the North Basin subsidizing the South Basin and playing a dominant role in driving the lake-wide dynamics. Our results show that cyclic fluctuations of a metapopulation can be explained in terms of the combined effects of synchronized demographic rates and spatial coupling. Moreover, these long-term data, in conjunction with ongoing phenotypic and genomic work, provide unique opportunities for understanding ecological and evolutionary processes in space and time.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Jun Kitano**

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Title: **Contingency and determinism in the genetic basis of stickleback plate evolution**

Abstract: How predictable is the genetic basis of convergent evolution? Convergent evolution can be caused by mutations at different genes, independent mutations at the same genes, and/or the repeated fixation of the same alleles. It remains elusive what kinds of deterministic and stochastic factors determine the genetic basis of convergent evolution. Armor plate evolution in stickleback is one of the most well-known examples of convergent evolution. Repeated plate reduction in several *Gasterosteus aculeatus* freshwater populations in North America and Europe is known to be caused by the repeated use of the same Ectodysplasin-A (Eda) allele. We have recently found that independent mutations on Eda and its receptor, Edar, caused plate reduction in a few Japanese freshwater *Gasterosteus* populations. Population genomic analysis shows that this may be due to the lack of standing genetic variation of the low Eda allele in the marine ancestral populations around the Japanese archipelago. Plate reduction in *Pungitius sinensis* was caused by independent mutations at Edar. Phylogenetic analysis

showed that multiple *Pungitius* species share the same alleles likely due to the ancestral polymorphism. Thus, our study showed that different species or even geographically isolated populations acquired independent mutations at *Eda* and *Edar* and these genes are the hotspot genes for the convergent plate reductions in sticklebacks. These systems provide us unique opportunities to investigate the contingency and determinism in the genetic basis of stickleback armor plate convergent evolution.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Kasha Strickland**

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Title: **Using phenomic and genomic time-series data to explore divergence through space and time**

Abstract: Studying evolution in real time in wild, spatially heterogeneous populations is a challenge, and as a result it is normally studied across either space or time.

Nevertheless, understanding the spatiotemporal dynamics of selection in nature is critical in order to understand the process of adaptive divergence – in particular in face of high gene flow and environmental fluctuations. We studied spatiotemporal genomic and phenotypic divergence in threespine stickleback (*Gasterosteus aculeatus*) from lake Mývatn, Iceland. Lake Mývatn is an exceptionally well-studied ecosystem that is both spatially heterogeneous and temporally dynamic. This suggests that stickleback experience spatiotemporally fluctuating selection. To investigate microevolutionary responses across both space and time, we measured multiple phenotypic traits of > 1000 individuals across 10 sites over a 10-year period (2010 – 2020). Then, we sequenced the genomes of 555 of these individuals to explore which regions of the genome were under selection across space and/or time, and how genomic variation was linked to observed phenotypic change. To strengthen our inferences, we used genome-wide forward simulations to isolate changes in allele frequencies that go beyond neutral expectations (e.g., genetic drift). We also explored the covariances in allele frequency change to better determine the polygenic traits that may be under selection. Time-series datasets on phenomes and genomes are extremely rare, and the dataset of wild stickleback we generated – combined with long-term monitoring of population demography - provides an unprecedented opportunity to better understand microevolutionary processes of wild populations. In particular, these data provide insight

to populations that experience dynamic environmental change across space and time, a phenomenon which is increasing in rate and frequency in ecosystems globally.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Kieran Samuk**

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Title: **Reproductive isolation (?) without ecological divergence in the white stickleback**

Abstract: In many stickleback systems, reproductive isolation is mediated by the poor performance of hybrids due to trophic niche mismatches. This suggests the evolution of ecological differences is the key early step in the formation of new species. A key alternative scenario is the evolution of sexually-selected/sexually-mediated barriers such as differences in male mating strategy, coloration, etc. I test the putative contributions of these two classes of barriers using a recently discovered "white" marine stickleback system from Nova Scotia, Canada. Using a combination of population genomic, morphological, and isotopic data, I show: (a) white sticklebacks form a unique genetic population separate from other "common" marine stickleback populations and (b) white and commons sticklebacks do not differ in any of the classic axes of ecological differentiation seen in other stickleback species pairs. Instead, white sticklebacks appear to have largely diverged in sex-specific traits, specifically those with male-biased expression (nuptial colors, parental care, mating behavior, etc). While work is ongoing, our results suggest that speciation may occur readily without geographic isolation or strong ecological differences, with sexually-selected/sexually-mediated barriers being sufficient.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Laura Dean**

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Title: TBA

Abstract: Recent advances in genomic research are beginning to reveal that secondary contact and hybridisation between divergent ecotypes plays an important role in speciation and adaptive radiation. Hybridisation introduces novel genetic material on which selection can act and allows processes such as reinforcement to strengthen

reproductive barriers. Secondary contact between two divergent populations is relatively common in nature and very well documented in three-spined stickleback (*G. aculeatus*). Contact between three divergent populations in a single location is much rarer, but offers a window into the effects of additional gene flow from a third group on a pre-existing contact zone. Such a scenario may become more common as a changing climate causes range shifts, introducing new species into previously suboptimal environments. We report a rare location in which three different ecotypes of the three-spined stickleback adapted respectively to an extreme acid freshwater environment, a low energy saline lagoon environment and a marine migratory life style all co-occur and reproduce in a single location. Lagoon resident and marine stickleback in nearby locations form strongly reproductively isolated species-pairs, but we find genomic and phenotypic evidence that where this pair occurs in the presence of the fish adapted to a freshwater extreme, reproductive isolation is no longer present. We present phenotypic and genomic analyses of these highly unusual phenomenon, and draw conclusions about the nature of reproductive isolation in three-spined stickleback.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Maria L. Rodgers**

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Title: **Evolution of Cell Types Within the Stickleback Intestine**

Abstract: The intestine plays a crucial role in physiology and immune function, but intestinal cell types have yet to be fully characterized in stickleback. To address this gap in knowledge, we performed single cell RNA sequencing (scRNAseq) on anterior and posterior portions of stickleback intestines from an estuarine population (Sayward Estuary, British Columbia, Canada). Our results reveal that the stickleback intestine includes a variety of epithelial cell types (including goblet cells, enteroendocrine cells, and enterocytes) and immune cell types (including fibroblasts, neutrophils, dendritic cells, natural killer cells, B cells, and macrophages), along with other cells such as ionocytes. We found no apparent differences in cell types between anterior and posterior portions of the intestine using scRNAseq analysis. After generating a list of cell-type diagnostic genes within the intestine from our scRNAseq analysis, we queried population genomic sequencing data from 28 stickleback populations to estimate the strength of selection acting on each gene. Focusing on marine versus lake contrasts in Alaska, and in British Columbia, we calculated all pairwise marine-lake fixation indices

(FSTs) for each single nucleotide polymorphism (SNP) in each focal gene. Averaging these provides an average marine-lake FST for each SNP, and then averaged across all SNPs within a gene to provide a gene-level mean FST. We then asked whether the strength of selection (mean FST) differed among cell types. We infer that selection is particularly weak, or absent, in erythrocyte and thrombocyte-specific genes. Selection tended to be strongest on immune cells (including neutrophils and B cells) and goblet cells. Unsurprisingly, ionocytes are also under strong evolutionary selection pressure between marine and freshwater stickleback populations, though only for one gene. These data, for the first time, demonstrate selection pressure at a cellular level in stickleback and will be useful for future work both in the intestine and within other organs.

Presenting day: **Sunday 24th of Jyly 2022**

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Title: **The Effects of Mercury Accumulation on the Gut Microbiome of the Three-Spined Stickleback (*Gasterosteus aculeatus*)**

Abstract: The anthropogenic spread of environmental contaminants represents a significant source of ecosystem disturbance worldwide. Mercury is a highly toxic heavy metal with well-documented toxicological effects on aquatic biota. Its tendency to become organometallic, volatilize, bioconcentrate, and biomagnify, allows mercury to cycle through the hydrosphere, atmosphere, and biosphere forming scattered pockets of relatively high contamination. Evidence of tolerance and local adaptation to mercury exposure in fish and aquatic bacteria suggests chronic mercury contamination can be a driver of contemporary evolution. Moreover, interactions between gut microbial flora and host fish have been found to affect the response of fish to environmental stressors. By investigating the effects of mercury accumulation on the gut microbiome of three-spined stickleback (*Gasterosteus aculeatus*) populations, this study aims to deepen the understanding of the role of microbial flora-host symbiosis in response to anthropogenic stressors. We collected sticklebacks from 21 locations across three major river basins in Flanders (Belgium). Based on mercury accumulation levels in the muscle tissue, 4 sites with low levels of accumulated mercury (22-56 ng.g⁻¹) and 4 sites with elevated levels (164-327 ng.g⁻¹) were selected. The gut and gut contents were homogenized, DNA was

extracted, and the V3 and V4 regions of the 16S bacterial rRNA gene were amplified and sequenced. The results will be used to compare the effect of mercury contamination on microbial communities in the gut across the stickleback populations from the three basins. We hypothesize that exposure to a high mercury load will select gut flora species with the capacity to tolerate or adapt to mercury, leading to a change in bacterial composition associated with contamination-driven perturbations of the gut ecosystem. Studying the effects of environmental contaminants on the gut microbiome of sticklebacks will help us understand how the composition of the microbiome responds to polluted environments.

Presenting day: **Sunday 24th of July 2022**

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Title: **Human-induced isolation causes rapid behavioral divergence with genetic underpinnings in resident and migrant sticklebacks**

Abstract: The adaptive capacity of many organisms is seriously challenged by human-induced environmental change. Water management measures in the 1970s in the Netherlands have produced a large number of land-locked 'resident' populations of three-spined sticklebacks (*Gasterosteus aculeatus*), which are cut-off from the originally 'migrant' populations. Here, we made use of this unintended field-experiment, to study the impact of human-induced isolation on behaviour and morphology. We detected differences between migrant and resident populations in virtually all phenotypic traits studied: compared to the migrants, residents were smaller in size and were significantly more active, aggressive, exploratory and bolder and showed lower shoaling and migratory tendencies. Furthermore, to investigate if these differences in wild-caught residents and migrants reflect genetic differentiation, rather than different developmental conditions, we performed a 'common-garden experiment'. We raised offspring of four crosses (migrant ♂ x migrant ♀, resident ♂ x resident ♀, migrant ♂ x resident ♀, resident ♂ x migrant ♀) under similar controlled conditions and tested for differences in phenotype as adults. We found that lab-raised resident sticklebacks exhibited lower shoaling and migratory tendencies as compared to lab-raised migrants, retaining the differences in their wild-caught parents. This indicates genetic differentiation of these traits. For all other traits, the lab-raised sticklebacks of the various crosses did not differ significantly, suggesting that the earlier-found contrast between wild-caught fish reflects

differences in their environment. Our study shows that barriers to migration can lead to rapid behavioural divergence over contemporary timescales (~50 generations), and that part of these differences reflects genetic differentiation.

Presenting day: **Thursday 28th of July 2022**

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Title: **A test for the causes and consequences of habitat selection using lake-stream stickleback**

Abstract: Adaptive population divergence is commonly ascribed to selection favoring different individuals in different habitats. Random migration between habitats, and hence gene flow between populations, should thus counteract adaptive divergence. Yet, population divergence could also occur from different individuals selecting different habitats. In case of such individual habitat selection, gene flow between habitats would not be random and could help maintain or even promote population divergence. In this project, we evaluate this overall idea by testing for the causes and consequences of habitat selection using a pair of directly adjacent but phenotypically distinct populations of lake and stream threespine stickleback. In line with earlier results, a mark-transplant-recapture experiment using wild-caught stickleback reveals phenotype-dependent native habitat selection. To test for a genetic basis of habitat selection, and to find traits and genomic regions associated with habitat selection, we then gave laboratory-raised purebred stickleback (from lake-lake and stream-stream crosses) and lake-stream F2 hybrids the choice between the same natural lake and stream habitats. Purebred stickleback showed increased preference for the native habitat of their parents, indicating a genetic basis to lake-stream habitat selection. In this talk, I will present first results from phenotypic and genetic analyses of 800 lake-stream F2 hybrids that either selected the lake or stream habitat. Furthermore, I will present our results from a fitness enclosure experiment conducted with a subset of these hybrids, in which we asked whether the selection of a certain habitat by an individual is associated with increased fitness in that habitat.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Michael A. White**

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Title: **Meiotic double strand break repair across the threespine stickleback sex chromosomes**

Abstract: Sex chromosomes (i.e. X and Y) pose unique challenges during meiotic recombination. Meiotic recombination initiates with a programmed induction of double strand breaks (DSBs) that are typically repaired using the homologous chromosome as a template. However, sex chromosomes often share reduced sequence homology between one another, limiting DSB repair to non-homologous end joining or homologous recombination with the sister chromatid. Studies in mammals have provided considerable insight into how DSB initiation and repair occurs across ancient sex chromosomes. However, we understand comparatively little about how DSBs are initiated and repaired across more recently derived X and Y chromosomes, which often share considerably more sequence homology. Threespine stickleback fish (*Gasterosteus aculeatus*) are an excellent species to study this process, as they have a relatively young Y chromosome that is fully sequenced and assembled. Using molecular cytogenetics, we found that the X and Y chromosomes fully pair during meiosis, despite exhibiting complete crossover suppression over much of their length. DSBs formed throughout the chromosomes and repaired at the same rate as autosomes. This raises the possibility that DSBs on more recently derived sex chromosomes can be repaired through inter-gametolog gene conversion before the bias against sister chromatid repair and non-homologous end joining has been lifted. To assay whether gene conversion occurs throughout the sex chromosomes, we are using a population genomics approach to identify historic signatures of genetic exchange between the X and Y. We are combining this with high-fidelity long-read sequencing of sperm DNA from a single male to estimate current rates of gene conversion. Our work will provide important insights into the generality of this repair process on newly evolving sex chromosomes, including how gene conversion may impact early sequence divergence.

Presenting day: **Monday 25th of July 2022**

Presenting Author: **Natalie Steinel**

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Title: **Threespine stickleback as a model of adaptive immune system evolution**

Abstract: Even though complex immune systems first arose in jawed fishes, our understanding of immune function chiefly comes from the study of mammals.

Mammalian B cells, the producers of antibody, are activated in the germinal centers (GCs); complex cellular aggregates of B and T cells found within lymphoid tissues. Ectotherms, including fish, lack germinal centers yet are still able to activate B cells and generate antibody. Melanomacrophage centers (MMC), aggregates of pigmented phagocytes, have been proposed as the evolutionary precursors to GCs, however this hypothesis has been developed piece-meal, from various studies across disparate fish, reptile, and amphibian species. Using threespine stickleback, we've conducted a comprehensive analysis of MMC function in a single species. We find that stickleback MMCs, like GC, are associated with the white pulp of the spleen, are found in proximity to splenic vasculature, and express the same cell surface marker (CNA42). Using RNA in situ hybridization, we show that stickleback MMCs aggregate with cells expressing B cell (IgM, IgD, CD20, CD79a) and T cell (TCRB, CD4) genes, supporting the notion of a MMCs as an organizing structure for B cell activation and antibody production. Furthermore, like GCs, MMCs increase in size in response to immunization and retain antigen. MMCs are highly conserved and have been described in over 144 fish species, however they are reported missing in fifteen spine stickleback. Our preliminary analysis of other members of the stickleback family indicate variable MMC morphology, with reduced MMC size in ninespine stickleback, and MMC absence in fourspine stickleback, suggesting an evolutionary loss among multiple stickleback species. These studies not only provide support for the hypothesis that MMCs are evolutionary precursors to GCs, but also provide important tools to broaden the study of immune evolution in stickleback and other ectotherm systems.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Nicholas Planidin**

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Title: **Behavioural responses of threespine stickleback with lateral line asymmetries to experimental mechanosensory stimuli**

Abstract: Behavioural asymmetry, typically referred to as laterality, is widespread among bilaterians and is often associated with asymmetry in brain structure. However, the influence of sensory receptor asymmetry on laterality has undergone limited investigation. Recent studies on the position and abundance of mechanosensory neuromasts on the lateral plates of *Gasterosteus aculeatus* on the Haida Gwaii archipelago, western Canada, indicate differences among plates, between sexes and

among populations, in part associated with ecological differences. In the present study, we examine experimentally whether bilateral asymmetry in neuromasts is associated with behavioural asymmetry by using simulations of three mechanosensation-dependent behaviours: predator evasion, prey localization and rheotaxis. We recorded the response of 40 adult stickleback to impacts at the water surface and water flow in daylight conditions, as well as their response to low-frequency oscillations in the dark and then compared individuals' laterality with anatomical asymmetry in the number of neuromasts on either side of their body. Stickleback hovered with their right side against the arena wall 57% of the time in illuminated surface impact trials and 56% of the time in dark low-frequency stimulation trials. Light regime modulated the effect of neuromast count on laterality, as fish with more neuromasts were more likely to hover with the wall on their right during illumination but were less likely to do so in darkness. Population-level laterality diminished in later trials across multiple behaviours and individuals did not show a consistent side bias in any behaviours. Our results demonstrate a complex relationship between sensory structure asymmetry and laterality, suggesting that laterality is modulated by multiple sensory modalities and is temporally dynamic.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Ragna Guðrún Snorradóttir**

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Title: **Foraging niche and diet variation in threespine stickleback (*Gasterosteus aculeatus*) in Lake Mývatn, Iceland**

Abstract: Resource availability is a major driver of ecological and evolutionary processes, and a good way to link these processes is to study diet specialization. Individual foraging abilities are reflected by genetic and environmental influences and shaped by morphological and behavioral traits. A well-suited study system for understanding individual specialization are threespine stickleback (*Gasterosteus aculeatus*) from Lake Mývatn. Mývatn shows considerable ecological variation, with two main basins encompassing a range of habitat types, physiochemical conditions, and invertebrate species that differ throughout the lake. Moreover, the stickleback population in Mývatn differs in densities throughout the lake, shows phenotypic variation in feeding morphology, and fluctuates in size across years. Here we assessed variation in stickleback diet (i.e. stomach content) from fish caught in June 2021 from different

habitats to investigate whether there is evidence for individual specialization. We then linked variation in diet environment (available benthic and pelagic prey species in the habitat) and individual trophic morphology (gill raker structure) to explore the relationship between diet niche, prey availability and morphology. Such divergence in individual specialization in relation to trophic morphology among environments may indicate disruptive selection. This study provides insight to the continuum of specialization - generalization of the Mývatn stickleback population and provides a baseline for future studies examining temporal changes in disruptive selection.

Presenting day: **Thursday 27th of July 2022**

Presenting Author: **Rémy Beaudouin**

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Title: **Biomarker toxicodynamics linked to BPA toxicokinetics in three-spined stickleback: a PBPK-TD model**

Abstract: Bisphenol A (BPA) is considered a substance of very serious concern due to its endocrine disruptor function, high production volume, and persistence in the aquatic environment. In this context, physiologically-based toxicokinetic models coupled with toxicodynamics (PBTK-TD) have proven to be valuable tools. They allow proposing more actual exposure scenarios as the exposure may vary over time and they also enable linking internal concentrations to various effects that are often decorrelated to external dose. Recently, a PBTK has been developed and adapted to BPA ADME (Absorption, Distribution, Metabolization, and Excretion) processes in the three-spined stickleback. Thus, this work aimed to develop a PBTK-TD model that would permit to better understand the impact of BPA on the different functions of the fish, including defense capacities and reproduction. In particular, a precise knowledge of the internal doses in the target organs would allow to i) clearly identify whether the transient biomarker responses are linked to toxicokinetics, ii) identify the compounds (parent and/or metabolites) responsible for the biomarker responses, and iii) to identify non-monotonic dose dependencies of biological responses to external exposures that would be non-monotonic. In detail, the PBTK was extended to include the toxicodynamics of various biomarkers measured during two exposures to BPA, one of seven-day exposure and seven-day depuration, and one of 21-day exposure. Biomarkers including non-specific cellular immunity, metabolic detoxification, oxidative stress, and reproductive parameters were analyzed to select a set of parameters

varying when exposed to BPA. Since it was supposed that the biomarker responses were produced by indirect mechanisms, indirect toxicodynamic models were chosen to describe the response. Consequently, mechanism-based equations describing the responses of biomarkers over time were implemented in the existing PBTK specific to BPA. Then, TD data were divided into two sets, one to allow calibration of parameters related to biomarker toxicodynamics and the other to enable evaluation on an unused collection of TD data. This study demonstrates the relevance of the three-spined stickleback as EDC sentinel species and the approach using a PBTK-TD to explore the dynamics of the EDC effects.

Presenting day: **Sunday 24th of July 2022**

Presenting Author: **Spencer Edwards**

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Title: **Shoaling in Mývatn Stickleback: Habitat divergence and social activity**

Abstract: Behavioral variation within populations has the potential to lead to novel local adaptations and population divergence. Species that can exploit new ecological niches or interact with others in a new way can have an advantage if and when environments shift. The threespine stickleback (*Gasterosteus aculeatus*) in Lake Mývatn, Iceland, is an ideal candidate to study such local adaptations; they are divergent in their morphology and physiology across the lake, in relation to ecological heterogeneity, e.g. temperature. However, we have yet to thoroughly investigate many behavioral differences, specifically social behavior, within the population. In the present study, we investigated whether variation in habitat of origin, which vary widely in temperature, predator densities, and geological features, and rearing conditions, primarily temperature, have an impact on the shoaling behavior of stickleback of lake Mývatn. Fish were crossed four ways, reared at contrasting temperatures (22 °C and 13°C) and raised for about 3000 degree-days. Shoaling was tested by placing a single fish in an arena with a shoal (10 individuals) and distractor (2 individuals) on either side. Fish were then recorded for 15 minutes, and their time spent near the shoal was examined. We will study if the tendency to shoal is related to habitat of origin and rearing conditions. We hypothesize that all fish will show a strong tendency to shoal, but that time spent shoaling (versus interacting with distractors/exploring) will be greater among fish from the North (warm) basin, due to differences in selective pressure from predators. Furthermore, we will be able to investigate parental effects on the behavior we see. This research will help us to better understand patterns of diversification across

lake Mývatn and could be replicated for other ecologically significant populations. Furthermore, understanding how behavior, morphology, and environment interact may increase our ability to predict how populations will respond to environmental change.

Presenting day: **Thursday 28th of July 2022**

Presenting Author: **Takuya K. Hosoki**

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Title: **Reversion to a species after the 2011 Tohoku tsunami-induced hybridization**

Abstract: Hybridization often occurs before speciation is completed. What would be the fate of hybridization? Hybridization may lead to the emergence of a panmictic population, hybrid speciation, or extinction of one species. It is also possible that the mixed genome is reorganized such that the hybrid population reverts to one parental species with the genome of the other parental species being purged. Despite the presence of a few examples of such purging after hybridization, it remains unanswered how rapidly purging can occur in nature and what factors contribute to purging. Here, we investigated the genomic change over nine years of a hybrid population between two stickleback species, freshwater *Gasterosteus aculeatus* and anadromous *G. nipponicus*, whose hybridization was induced by the devastating 2011 Tohoku earthquake and subsequent tsunamis. Genomic analysis of 2606 individuals showed that the first generation of backcross (BC1) to *G. aculeatus* and the second generation of intercross (F2) were observed in 2012, suggesting that interspecific hybridization occurred in 2011, the year of the tsunami. Over the nine years from 2012 to 2020, the *G. nipponicus* genome was purged and the hybrid population reverted to *G. aculeatus*. Surprisingly, the majority of purging occurred in the first few generations. Faster purging occurred in an autosome with a newly identified quantitative trait locus for seaward migration. Both ancestral and neo-sex chromosomes, which contribute to seaward migration, sexual isolation, and hybrid incompatibility, also showed higher purging rates of *G. nipponicus* alleles compared to autosomes. These results demonstrate that purging of heterospecific genome can occur within a few generations and barrier loci contribute to it.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Veronica C. Behrens**

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Title: **A stickleback approach to human genetic evolution**

Abstract: Over several decades, the threespine stickleback has emerged as one of the most important organisms for uncovering the genetic basis of evolutionary traits in vertebrates. The ability to make hybrids between recently diverged forms and map key chromosome regions and genes has made it possible to identify the genetic basis of classic evolutionary differences between stickleback populations. Could a similar approach be used to study the evolution of the human lineage? While conventional genetic crosses between humans and non-human primates are obviously not possible, we have been developing a conceptually related genetic mapping approach using human and chimpanzee stem cells studied in culture. Much like a stickleback QTL cross begins by generating a hybrid F1 generation, we begin by fusing induced pluripotent stem cells (iPSCs) from humans and chimpanzees to generate tetraploid cell lines that contain the entire human and chimpanzee genomes in the same nucleus. Similar to an F1x F1 intercross to generate a morphologically and genetically diverse F2 stickleback generation, we then use CRISPR/Cas9 targeting to generate panels of derivative cell lines that contain species-specific deletions or recombination between human and chimpanzee chromosomes. The human-chimpanzee tetraploid iPSCs retain the ability to differentiate in culture, and genome-wide expression profiling identifies thousands of gene expression differences between humans and chimpanzees. We are currently using this system to study multiple topics with direct parallels to evolutionary questions in sticklebacks, including: what types of genes are most likely to change in evolution, what fraction of species-specific gene expression differences are due to cis and trans regulatory differences, and what role might larger-scale chromosome changes play in contributing to species-specific differences? Comparison between the stickleback and human system helps illuminate several general issues in vertebrate evolution and also suggests additional genomic datasets that could prove useful for future stickleback research.

Presenting day: **Wednesday 27th of July 2022**

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Title: **Parallel evolution of the lateral line is driven by ancestry and selection in novel environments**

Abstract: The action of selection and its predictability can be inferred when populations undergo parallel evolution, providing a window into how organisms respond to rapid anthropogenic change. Sensory systems may play a key role in determining population persistence or decline, as they are important for many social and ecological interactions. The fish lateral line system, comprised of sensory cells called neuromasts, senses changes in water flow and mediates navigation, anti-predator, foraging and social behaviour. We tested for parallel evolution in neuromast counts for Icelandic threespine stickleback from lakes that differ in sensory ecology and age. Older lakes at higher elevation and younger lakes at lower elevation are either fed by glaciers and are turbid or are spring-fed and clear. We compared these freshwater populations to multiple extant 'ancestral' marine populations to infer both the direction and rate of evolutionary change. We found surprisingly that glacial fish had fewer neuromasts than both spring-fed fish and marines. The sexes differed more than lake types; males had more neuromasts and the sexes apportion neuromasts differently among lines. We expected more divergence for high elevation lakes, but found few differences in rate. We predicted parallel evolution within lake types due to shared sensory environments and selection, yet found a mixture of parallel and nonparallel evolution. By using multiple ancestral populations, we show that shared ancestry was a major driver of parallel change. Parallel evolution occurred primarily in glacial populations that shared an ancestral population. Spring-fed populations evolved mostly in parallel, but also showed a strong signature of convergent evolution. The overriding signal is that freshwater populations evolve to an optimum and that selection can overcome population history. Our study reveals the action of selection and rapid phenotypic change; although change is only partially parallel, the endpoint is largely predictable in changing environments.

Presenting day: **Wednesday 27th of July 2022**

Presenting Author: **Yo Yamasaki**

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Title: **Gene flow and the evolution of chromosomal inversion in sticklebacks**

Abstract: When parapatric populations adapt to contrasting environments, gene flow and subsequent recombination breaks up a combination of alleles beneficial for local adaptation, thereby preventing adaptive divergence. Chromosomal rearrangement, such as inversion, can reduce recombination and play an important role in divergence with gene flow. Although theoretical studies predict that chromosomal inversions would evolve more in parapatric pairs than in allopatric pairs and such inverted regions contain more adaptive loci than collinear regions, few empirical studies have yet explicitly tested these predictions. The presence of multiple marine and freshwater populations in both parapatry and allopatry makes the stickleback fish a suitable model for testing these hypotheses. Furthermore, recent technological advances in genome assembly enable us to comprehensively and reliably identify chromosomal rearrangement. Here, we newly assembled and compared genomes of multiple marine and freshwater stickleback populations. We successfully detected many inversions between populations. Regions with inversions between sympatric or parapatric population pairs showed significantly higher genetic differentiation compared to collinear regions, suggesting that inversion acts as barriers of gene flow. No such a trend was observed between allopatric pairs. Some inversions were shared among geographically isolated freshwater populations, suggesting that these inversions exist as standing genetic variations in this species. Phylogenetic analysis showed that inversions differ in the time of origination. We are now investigating whether parapatric pairs tend to accumulate more inversions than allopatric pairs and whether inverted regions contain more quantitative trait loci responsible for adaptive traits.

Presenting day: **Wednesday 27th of July 2022**

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Title: **A novel sex determination gene on an XY sex chromosome system with polymorphic inversions among populations of fourspine stickleback (*Apeltes quadracus*)**

Abstract: Different sex determination systems, sex chromosomes, and sex determination genes have independently evolved in many species. Multiple turnovers and an independent evolutionary history of sex chromosomes have been characterized in stickleback (*Gasterosteidae*) species, making them an excellent system to study sex chromosome turnover and differentiation. Previous studies in fourspine stickleback

(*Apeltes quadracus*) have suggested that there is variation among populations in the presence of heteromorphic sex chromosomes. Here, we collected samples from three populations of fourspine stickleback and used whole-genome DNA and RNA sequencing data to identify chromosome 23 as an XY sex chromosome system in all three populations. Unlike in the threespine stickleback (and other *Gasterosteus* species), which possess a highly degenerate Y chromosome, fourspine stickleback possess a pair of young and homomorphic chromosomes without extensive degeneration. The extent of differentiation between sexes varies among populations on the sex chromosome, which is likely due to a polymorphic inversion on the X chromosome. There is also region of divergence shared among all populations that contains a novel candidate sex-determination gene (*ZAR1*). We are currently using CRISPR knock-out and base-editing experiments to test the role of *ZAR1* in sex determination. Our results thus identify a novel candidate sex determination gene in fish, as well as a polymorphic sex chromosome system that provides an opportunity for further investigation of the role of inversions in sex chromosome evolution.

Presenting day: **Monday 25th of July 2022**