

**Purging of heterospecific genome in a hybrid stickleback population
created by the 2011 tsunamis**

by

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Summary of Doctoral Thesis

Introduction

Understanding speciation is crucial to comprehending understanding of evolution, but the process remains a mystery. One of the mysteries is the existence of hybridization between species in nature. Advances in genomic technology have shown that hybridization between species is common in nature. Recent empirical studies have also shown that evidence of both historical and ongoing interspecific hybridization can be found in the genomes at the various stages of speciation.

Hybridization often occurs before speciation is completed. What would be the consequences of hybridizations? 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 could be reconstructed to one of the parent genomes (i.e. purging of one species). Signatures of the purging of one species after hybridization are now found in genomes of a wide range of taxa, suggesting that this is one major outcome of hybridization. In the case of purging of one species, species that contribute to the majority of the genome of the hybrids are referred to as "major species" and species that contribute to a small fraction of hybrids are referred to as "minor species". After hybridization, most of the genome from the minor species is purged because it is often deleterious to the "major species". The negative selection against the minor species genome is hypothesized to result from ecological selection against immigrants, sexual isolation, and negative interaction between parental species alleles (i.e. hybrid incompatibility). In addition, I hypothesized that spatial isolation may play a role in the purging of the heterospecific genome. If hybridization occurs in the habitat of a major species, but the minor species have a preference for moving to other habitats, this can lead to the purging of the genome that is involved in habitat choice. While purging process is predicted to occur rapidly after hybridization, it remains unanswered: how rapidly purging can occur in nature and which factors contribute to such rapid purging.

Materials and methods

To answer these questions, I focused on an initial stage of hybridization of stickleback fishes belonging to genus *Gasterosteus*. Recently, one hybrid population between freshwater *G. aculeatus* and anadromous *G. nipponicus* was found in new stickleback habitats created by the 2011 Tōhoku earthquake and tsunami. These two stickleback species are thought to have diverged 0.68-1 million years ago. They are reproductively isolated by multiple isolating barriers, such as spatial isolation, seasonal isolation, sexual isolation, ecological selection against immigrants, hybrid male sterility, hybrid courtship dysfunction, and ecological selection against hybrids. Ecological selection against immigrants is likely achieved by divergent adaptation to diets. *G. aculeatus* and *G. nipponicus* exhibit phenotypic differentiation in gill raker, a foraging

morphological trait, and genotypic differentiation in a fatty acid desaturase gene *Fads2*, adapting to freshwater and marine environments, respectively. On 11 March 2011, a large earthquake occurred off the coast of Tohoku, Japan. The huge tsunamis as well as the earthquake-induced ground subsidence created new spring water-fed ponds in a formerly urban area and sticklebacks colonized there soon after the tsunami. Despite the existence of multiple isolating barriers between freshwater *G. aculeatus* and anadromous *G. nipponicus*, a previous microsatellite analysis showed that the new population contained hybrids. Because extensive field surveys have been conducted since one year after the tsunami (in 2012), this system enables us to monitor the evolution of the hybrid genome immediately after the hybridization event.

As a first step towards a comprehensive understanding of the factors determining the outcome of hybridization at the initial stage of hybridization, I investigated the genomic changes in the tsunami-created hybrid population. In chapter 1, I first explained my research question. In chapter 2, I summarized the environments of tsunami-created habitats. I also calculated the change in the ancestry of the hybrids from 2012 to 2020. Analyses in chapter 2 showed that *G. nipponicus* alleles had been purged from the new hybrid population, even though the genome was once admixed, suggesting that the outcome of the hybridization was the re-emergence of parental species from hybrid swarms. In chapter 3, to investigate the factors determining the genomic consequences of hybridization, I tested whether admixture patterns of *G. nipponicus* in the genome are associated with the genomic position of loci involved in reproductive isolation. First, to identify loci involved in spatial isolation, I focused on sea-run migratory behavior in juveniles and conducted QTL mapping of migratory behavior. Second, to identify phenotypes involved in the ecological selection against immigrants, I focused on phenotypes that are differentiated between *G. nipponicus* and *G. aculeatus*. In this analysis, I targeted a foraging trait (gill raker number) and the copy number of a fatty acid desaturase gene *Fads2*. I next examined whether the gill raker numbers and/or *Fads2* copies were differentiated along the environmental gradient in the new habitats. Then, to identify the genomic regions associated with gill raker numbers, I conducted a genome-wide association study (GWAS) of gill raker numbers. The genomic position of *Fads2* has been previously identified. Finally, I compared the observed purging patterns of *G. nipponicus* alleles in the new population between the genomic regions involved in reproductive isolation and others.

Results

Chapter 2 includes the results of my ecological surveys showing that the new habitats had an environmental gradient from fresh to brackish water. Genomic analysis showed that the first generation of backcrossing (BC1) to *G. aculeatus* and the second generation of intercrossing (F2) were observed in 2012, suggesting that interspecific hybridization occurred in 2011, immediately

after the tsunami. This is consistent with the hypothesis of the previous study that freshwater *G. aculeatus* brought in by the backwash of the tsunami from the Gensui River and *G. nipponicus* brought in from the sea by the tsunami formed the hybrid population. In the nine years from 2012 to 2020, I found that backcrossing towards *G. aculeatus* resulted in the purging of the *G. nipponicus* ancestry. These results suggest that *G. nipponicus* alleles have been purged from hybrid populations, even though the genome was once admixed.

In chapter 3, I show my QTL mapping using the recaptured BC1 hybrids. I detected significant QTL controlling sea-run behavior. A single-QTL scan with sex as an additive effect showed a significant peak on an autosome and LG19 (an ancestral sex chromosome). Although the new habitats had environmental variation from freshwater to brackish water, I could not find any evidence that gill raker number and *Fads2* copy number are under divergent ecological selection in the new habitats. Finally, I tested whether the rates of purging were higher at loci involved in ecological selection against immigrants and spatial isolation as well as previously identified QTLs for sexual isolation and hybrid incompatibilities. Analyses of genomic changes in the hybrid population showed that chromosomes with loci that contribute to spatial isolation, sexual isolation, and hybrid incompatibility underwent rapid purging of *G. nipponicus* alleles. Faster purging rates of *G. nipponicus* alleles were detected on both ancestral and neo-sex chromosomes, which contribute to spatial isolation, sexual isolation, and hybrid incompatibility, compared to autosomes. This purging pattern was also found in an autosome with a quantitative trait locus involved in spatial isolation. These multiple species barriers might have contributed to the rapid purging of the *G. nipponicus* genome. These results suggest that the genomic consequences of hybridization could be determined in a few generations and that multiple isolating barriers might contribute to the rapid purging in hybrid populations.

Discussion

Biologists have long been fascinated by the question of whether evolution is predictable. How predictable are the consequences of hybridization? Speciation is the evolution of reproductive isolation, a continuous process ranging from incomplete reproductive isolation with gene flow to complete reproductive isolation without any gene flow. While hybridization can occur before speciation is completed, the consequences of hybridization are varied. How many and how strong isolating barriers are necessary for preventing speciation reversal? Studies of the speciation continuum have suggested that the transition from weak to strong reproductive isolation may occur suddenly after passing a tipping point. Then, could this tipping point explain whether speciation reversal occurs or not after hybridization? To investigate these questions, it will be necessary to examine the genomic consequences after hybridization between species pairs at different stages in the speciation continuum. In stickleback, there are many species pairs with different levels of

reproductive isolation, making it possible to test this hypothesis. Additionally, the evolution of a particular type of isolating barrier, such as intrinsic hybrid incompatibility and divergence in habitat choice may be necessary. Further long-term analysis should be conducted on a variety of cases of hybridization across diverse taxa. In addition, experimentally manipulating the environments and population dynamics of various hybrid populations and monitoring these populations in semi-natural settings would allow us to better understand how predictable the consequences of hybridization are.