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生活史変異の創出・維持機構の解明

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神戸大学大学院理学研究科

上田るい

Rui Ueda

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Summary

Intraspecific life-history variation has been recognized as ecologically and evolutionarily important as interspecific variation for stabilizing population dynamics, regulating community structure, and local adaptation. However, the processes that generate and maintain life-history variation are not yet fully understood. Life-history theory explains that diverse life-history evolved to maximize fitness under the influence of extrinsic factors (i.e., physical environment and ecological factors) and intrinsic factors (i.e., trade-off among life-history traits). Previous studies have compared populations to reveal the correlations between these factors and different life-histories. Although life-history variation is observed among individuals, even within a single population, less attention has been paid to individual variation. Differences between individuals can have significant ecological and evolutionary effects. Therefore, it is important to understand how intraspecific life-history variations are maintained not only among different populations but also within a single population. In addition, life-history is characterized by a combination of various traits, such as growth, reproduction, and survival, and these traits often covary within individuals. Therefore, to understand the processes maintaining life-history variation within a population, life-history as patterns throughout the lifetime of organisms and its relationship with various factors at the individual level must be quantified.

In this dissertation, I focused on salmonid fish, red-spotted masu salmon (*Oncorhynchus masou ishikawae*), with the aim to understand the processes generating and maintaining intraspecific life-history variation within a wild population. In Chapter 1, I pointed out the importance of life-history variation within a population, which has

been overlooked in previous studies. I also described factors associated with the expression of life-history traits, such as sex, environmental factors, and genetic components.

In many species, sex-specific life-history is commonly observed. In organisms that are difficult to identify phenotypic sexes by external morphological differences, nonlethal molecular sexing is a powerful method to quantify sex-specific life-history. In Chapter 2, I investigated the applicability of two male-specific molecular markers, *OtY2* and *sdY*, in two wild masu salmon populations using PCR amplification and agarose gel electrophoresis. For both markers and in both populations, all phenotypic males were positive, while some phenotypic females were also positive. For phenotypic females, the concordance rates between phenotypic and genotypic sex in each population were 68 and 89 % in *OtY2* and 29 and 33 % in *sdY*. Additionally, I obtained DNA sequences of the positive amplification products from a few samples of both sexes and confirmed that the obtained sequences were authentic segments of the markers. Therefore, possible misidentification of sexes using these molecular marker approaches could not be excluded. However, individuals that were negative for both markers were identified as females, thus, these markers (especially *OtY2*) may be partially useful for selecting possible females from the populations of interest in life-history studies.

In Chapter 3, I examined ecosystem properties that can generate life-history variation within a single population. Seasonally recurring resource subsidies at the ecosystem boundaries are a primary driver of temporal resource dynamics for consumers. Previous studies showed that subsidies occurring early in the growing season, on average, shift consumers to fast life (i.e., fast growth and early maturation). However, utilization of the subsidies can vary among individuals via resource competition and niche variation.

Therefore, individuals that utilize the subsidies less may benefit from adopting slow life along with life-history trade-offs, such as a growth-survival trade-off. Furthermore, magnitude and seasonal timing of subsidies can vary depending on the surrounding environment, which may affect variation in life-history of consumers that rely on the subsidies. However, the effects of subsidy timing and magnitude on the life-history variation among individuals have never been explored. By integrating a large-scale field manipulation experiment and statistical analyses quantifying individual variations in life-history traits, I demonstrated that seasonal ecosystem linkage is a key property in maintaining life-history variation within a population in a forest-stream ecosystem. The proportional composition of the growth clusters differed significantly among three treatments; the early-subsidy increased the number of individuals who belonged to the fast-growth cluster, while reducing individuals of the slow-growth cluster. In contrast, most individuals in the no-subsidy control belonged to the medium-growth cluster, followed by fast-growth and slow-growth individuals. In the late-subsidy treatment, the input rate of the subsidy was the same as the early-subsidy treatment, but the proportional composition of growth clusters was similar to those found in the no-subsidy control. For the relationship between growth clusters and age at maturity, individuals who belonged to the fast-growth cluster matured at a younger age than those who belonged to the slow-growth cluster, which corresponds to the fast-slow life-history continuum. Consequently, the diversity of life-history patterns (i.e., the combination of growth cluster and maturation status at age-1) was highest in the early-subsidy treatment and lowest in the late-subsidy treatment. Meanwhile, this variation in life-history could not be explained simply by the growth-survival trade-off. Ecosystem linkages via seasonal resource subsidies are ubiquitous across ecosystem boundaries, but recent human-induced habitat

modification and global climate change are altering seasonal timing and magnitude of resource subsidies in many other ecosystems. Therefore, this study also suggests that those changes in ecosystem linkages might have caused substantial loss and shifts in intraspecific life-history variation.

Genetic variation is also crucial for the maintenance of life-history variation. Identifying whether life-history is phenotypically plastic or strongly regulated by genetic factors is important for understanding the maintenance process of life-history variation. To distinguish between genetic and environmental influences on life-history traits, for example, previous studies have performed reciprocal transplant experiments between populations in the field. However, in studies involving reciprocal transplant experiments without genetic information, it is difficult to directly evaluate the environmental influence on genetic variation responsible for the expression of life-history traits. Recent advances in molecular biology techniques, such as genome-wide association studies (GWAS), have allowed us to elucidate the genetic basis of various life-history traits in wild non-model organisms. This provides the opportunity to develop genetic markers by targeting loci and genomic regions associated with a trait of interest, and determine the genotype for each individual, which leads directly to testing whether an individual with a given genotype will express a phenotype under a given environmental condition (genotype-environment interaction). In Chapter 4, as a first step to understand the genotype-environment interaction for maintaining life-history variation, I investigated the genetic components associated with partial migration of red-spotted masu salmon in three geographically distant river systems. Partial migration is a phenomenon in which both residents and migrants coexist within a population and contribute to maintaining intraspecific life-history variation. Using whole-genome sequencing of pooled DNA samples (Pool-seq), I

obtained millions of SNPs, and found genomic regions containing several highly differentiated SNPs between residents and migrants in two out of three river systems. I found 57 genomic regions in samples from one river, and only one genomic region in another river. The genomic regions detected were unique to each river. In interpreting the results, it should be noted that there is a possibility that false positive SNPs were not completely eliminated due to the small sample size used for Pool-seq. It is also possible that genomic regions associated with migratory traits were overlooked because the reference genome used for alignment was incomplete. Therefore, further study is required to confirm whether the detected genomic regions are truly associated with partial migration by comparing the amino acid sequence or gene expression pattern between two migratory phenotypes. Despite these limitations, this is the first study attempting to understand the genetic components of partial migration in red-spotted masu salmon using contemporary sequencing methods, and it may open a future avenue for studying genotype-environment interactions for life-history variation.

Finally, in Chapter 5, I summarized the results in each chapter, and discussed the contribution of this dissertation to the field, as well as future directions for study to understand the processes that generate and maintain intraspecific life-history variation within a population. By combining a large-scale field manipulation experiment with a mark-recapture survey, and introducing a statistical method for quantifying individual variation, I revealed the key ecosystem property that contributes to maintaining life-history variation in the natural environment. However, two questions still remain: (i) What processes, including partial migration, generate life-history variation? and (ii) How are different life-histories maintained within a population across generations? By integration of experiments that manipulate the environment and molecular approach, and

performing long-term monitoring of a population, I will be able to answer these questions. With the answers we can better understand the processes that generate and maintain life-history variation in the wild from genotype to individual levels, which will help elucidate the ecological consequences for population dynamics, community structure, and ecosystem functions.

Chapter 1. General Introduction

From birth to death, organisms show various growth, maturation, and survival patterns. This series of events that happen through their life is called “life-history”, and remarkable differences in life-histories are observed within species and populations.

Intraspecific life-history variation is now recognized as important factor as well as interspecific variation for ecological dynamics and evolutionary response (Des Roches et al. 2018; Raffard et al. 2019). For example, asynchronous dynamics among diverse life-histories reduce extinction risk and stabilize population dynamics through the portfolio effect (Schindler et al. 2010; Moore et al. 2014), and different life-histories and foraging morphology regulate community structure by modifying the strength of trophic cascades (Post et al. 2008). In an evolutionary context, the variation in life-history is a significant source of adaptation to fluctuating environments (Moran et al. 2016). Although ecological and evolutionary consequences of intraspecific life-history variation have been identified, the process of generating and maintaining these variations in natural environments is not yet fully understood.

Traditionally, diversification of life-history within species is thought to be the result of differential adaptations to optimize survival and reproduction under the constraints of extrinsic factors (i.e., physical environment and ecological factors) and intrinsic factors (i.e., trade-off among life-history traits) (Stearns 1992), and different life-history strategies can coexist through frequency- or status-dependent selection (Gross 1996). Most previous studies have compared populations and revealed that different environments and/or genetic backgrounds account for life-history differences among populations (Kawecki & Ebert 2004). While variations in life-history are also observed

within a population, less attention has been paid to these variations. Differences between individuals have become the focus of studying ecological and evolutionary consequences (Clutton-Brock & Sheldon 2010; Bolnick et al. 2011). Therefore, it is important to understand how intraspecific life-history variation is maintained not only among populations but also within a population. In addition, life-history strategy is characterized by a combination of various life-history traits, such as growth, reproduction, and survival (Flatt & Heyland 2011), and these traits often covary within an individual (Promislow & Harvey 1990). Thus, it is necessary to evaluate life-history as patterns throughout the lifetime of organisms. However, it is challenging to quantify individual life-history trajectories, particularly in wild animals, thus, the processes driving intraspecific life-history variation, especially within a population, are poorly understood.

In species with sexual dimorphism, sex differences in life-history are ubiquitous across taxa (Hedrick & Temeles 1989). Typically, males and females maximize fitness in different ways. In most organisms, for example, cost of investment in gonads is relatively low in males, so that they can invest more resources in somatic growth. In contrast, in females, more resources are required for egg production, and a larger body size is advantageous for higher fecundity. As a result, males tend to express a faster growth rate, younger maturation, and shorter lifespan compared to females (Bonduriansky et al. 2008; Hämäläinen et al. 2018). Thus, sex is an important source that explains life-history variation and it is necessary to explicitly consider the effects of sex. Recent advances in molecular biology have allowed us to develop nonlethal molecular sexing methods that can quantify sex-specific life-history in species that are difficult to identify phenotypic sexes by external morphological differences (DeYoung & Honeycutt 2005).

As extrinsic factors, environment also have strong influence on the

diversification of life-history (Nylin & Gotthard 1998). Previous empirical and theoretical studies have shown that various environmental elements, such as temperature (Atkinson 1995), food availability (Abrams 1991), and predation risk (Day et al. 2002), affect life-history traits at the population level. However, environments vary temporally and spatially even within a population, and should drive life-history variation among individuals. However, the ecosystem properties that generate spatial and temporal environmental variation within a population are still unknown. Recent anthropogenic habitat alteration and global climate change may have induced rapid loss of intraspecific variation (Mimura et al. 2016; Lancaster et al. 2017). Therefore, it is important to elucidate the ecosystem properties that contribute to maintaining life-history variation to evaluate and predict the adaptive capacity of individuals and/or persistence of populations to environmental change.

Moreover, genetic components regulate life-history traits. Finding out whether the life-history is phenotypically plastic or strongly regulated by genetic factors is important for understanding the maintenance process of life-history variation, and its consequences for population dynamics and adaptation under environmental fluctuation. To distinguish between genetic and environmental influences on life-history traits, previous studies have focused on model organisms that are rich in genetic resources and easily handled in the laboratory, and/or carried out reciprocal transplant experiments between populations in the field (Kawecki & Ebert 2004). However, studies that involve reciprocal transplant experiments, without genetic information, cannot distinguish the effects of genetic variation responsible for the expression of life-history traits from plastic response to the environment. Rapidly advancing techniques in molecular biology, such as genome-wide association studies (GWAS), allow us to reveal the genetic basis of life-

history traits in wild non-model organisms (Stapley et al. 2010; Ekblom & Galindo 2011). This provides the opportunity to develop genetic markers by targeting loci and genomic regions associated with a trait of interest. It enables us to directly test whether an individual with a given marker genotype will express a phenotype under a given environmental condition (genotype-environment interaction) (Kruuk et al. 2008).

In this dissertation, I focused on red-spotted masu salmon (*Oncorhynchus masou ishikawae*), to understand the processes of generating and maintaining life-history variation within a wild population. Red-spotted masu salmon is endemic to southwestern Japan and exhibit considerable life-history variation within a population. For instance, as with the other salmonid species, sex-specific life-history is commonly observed in red-spotted masu salmon i.e., precocious maturation (i.e., age-0 mature) is common in males, whereas it is rare in females (Beamish 2018). Furthermore, age at maturity varies within and among sexes (ages 0–3), and most fish die after reproduction (semelparous), while some are iteroparous. In addition, red-spotted masu salmon is the southernmost species of salmon that exhibit partially migratory strategy (Kato 1973; Kishi & Tokuhara 2019) and both residents and migrants coexist within a population. Thus, this species can be considered as a model organism for studying life-history.

In Chapter 2, I examined the applicability of two male-specific molecular markers for sex identification in wild populations for the following population survey that aimed to test the sex-specific life-history. In Chapter 3, I focused on the environmental factors and demonstrated that seasonal ecosystem linkage is a key property to generate life-history variation within a population in a forest-stream ecosystem by using a large-scale field experiment. I also applied the sex-identification markers to examine the sex-specific effects and statistical approach that will quantify life-history trajectories.

Furthermore, as a first step to understand the genotype-environment interactions for life-history variation, I mentioned the propensity to migrate and investigated genomic regions associated with partial migration using whole-genome sequencing of pooled DNA samples in Chapter 4. Finally, I summarized each study and discuss future directions for study, to understand the processes that generate and maintain intraspecific life-history variation within a population.

Chapter 2. Difficulty in sex identification of two local populations of red-spotted masu salmon using two salmonid male-specific molecular markers

Introduction

For fishes, sex identification provides useful information for life-history studies (DeYoung & Honeycutt 2005), strategic conservation and management (DeWoody et al. 2010), and selective breeding in aquaculture (Yue 2014). Salmonid fish have a male heterogametic (XX/XY) sex determination system (Thorgaard 1977), and a number of male-specific molecular markers have been developed (Araneda et al. 2019). In Pacific salmon, belonging to genus *Oncorhynchus*, *OtY1*, *OtY2*, and *OtY3* are well known Y-chromosome-linked male-specific minisatellite markers in *Oncorhynchus tshawytscha* (Devlin et al. 1991; Brunelli & Thorgaard 2004; Brunelli et al. 2008). Among the three markers, *OtY2* can be applied to four other *Oncorhynchus* species, that is: *Oncorhynchus kisutch*, *Oncorhynchus keta*, *Oncorhynchus nerka*, and *Oncorhynchus masou* (Brunelli & Thorgaard 2004; Hsu & Gwo 2010). The pseudogene of growth hormone *GH-Ψ* has also been identified as a male-specific marker in *O. tshawytscha*, *O. kisutch*, *O. keta*, *Oncorhynchus gorbuscha*, and *Oncorhynchus masou masou*, but not in *Oncorhynchus mykiss*, *O. nerka*, and *O. masou ishikawae* (Du et al. 1993; Davidson et al. 2009). The master sex-determining gene, *sdY* (sexually dimorphic on the Y chromosome), was recently discovered in *O. mykiss* (Yano et al. 2012). It has been suggested that *sdY* is phylogenetically conserved across salmonid lineages, and thus, would be applicable as a universal male-specific marker across salmonids (Yano et al. 2013).

However, previous studies using male-specific molecular markers in *Oncorhynchus* have frequently shown that some phenotypic females were observed to be

positive for the male-specific markers *OtY1* in *O. tshawytscha* (Nagler et al. 2001; Devlin et al. 2005); *OtY2* in *O. tshawytscha*, *O. keta*, *O. nerka*, *O. gorbuscha*, *O. masou masou*, and *Oncorhynchus masou formosanus* (Brykov et al. 2010; Hsu & Gwo 2010; Podlesnykh et al. 2017); and *sdY* in *O. tshawytscha*, *O. keta*, *O. nerka*, *O. gorbuscha*, and *O. masou masou* (Cavileer et al. 2015; Larson et al. 2016; Podlesnykh et al. 2017). Therefore, the utility of each male-specific molecular marker should be tested in each species and/or population when applying these markers for male-sex identification of *Oncorhynchus* species.

Red-spotted masu salmon (*O. masou ishikawae*) is widely distributed in mountain streams in southwestern Japan; it is an ecologically, evolutionally, and economically significant fish because it is a top-predator in stream food webs (Sato et al. 2012); it expresses diverse life-history, including a southernmost sea-run form (called Satsukimasu) (Kato 1973); and are a common target for commercial fishing (Nakamura 2019). However, the difficulty in non-destructive sex identification of immature individuals has impeded a thorough understanding of their sex-dependent life-history and the making of their strategic aquaculture or resource management. In this study, I examined the validity of two male-specific molecular markers, *OtY2* and *sdY*, in two wild populations of red-spotted masu salmon. I selected these two markers because previous studies have reported their applicability in determining sex in red-spotted masu salmon (*OtY2*: Hsu & Gwo 2010) and in a sub-species, *O. masou masou* (*sdY*: Yano et al. 2013).

Materials and methods

Sample collection

Fish (21 males and 31 females, 114–226 mm in fork length; see Table 1) were captured between July and October in 2016 from the upper stream of the Arida River system, which drains watersheds within the Wakayama Forest Research Station, Field Science Education and Research Center, Kyoto University, Wakayama Prefecture, Japan (34°04′N, 135°31′E). Fish (10 males and 9 females, 103–306 mm in fork length; see Table 1) were also captured in October 2019 from the upper stream of the Nagara River system, Gifu Prefecture, Japan (35°42′N, 136°54′E) by electrofishing. The adipose fin for each fresh fish was clipped and preserved in 99 % ethanol for DNA analysis. The captured fish of the Arida population were exposed to a high dose (approximately 0.3‰) of anaesthesia (clove bud: Seimi Laboratory, Kanagawa, Japan). I considered the end point of fish when the fish did not show opercular movement (a sign of breathing) and responded to tactile stimulation. Those fish were then dissected and were determined their phenotypic sex by visual inspection of gonads and/or ovaries. In the Nagara population, phenotypic sex of the living fish was determined by pressing the abdomen and releasing the sperm or eggs. All fish were set free into the stream after the assessment.

DNA extraction, PCR amplification, and electrophoresis

Genomic DNA was extracted from the fin clip samples using Wizard ® Genomic DNA Purification kit (Promega, Madison, WI, USA). The concentration and purity of DNA were measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). For genotypic male-sex identification, multiplex polymerase chain reaction (PCR) amplification was performed using *OtY2* and *sdY*

markers, respectively. For multiplex PCR using the *OtY2* marker, two primer sets were used: *OtY2m* (forward: 5′-TCA ATC TGT GAC GTC ACT CA-3′ and reverse: 5′-GGC TTA CCG CTC CCA AGT AT-3′) and *16S rRNA* (forward: 5′-CCT GTA TGA ATG GCA TCA CG-3′ and reverse: 5′-ACA TGG GGG CTT AAT TTT CC-3′) (Hsu & Gwo 2010). In a previous study (Hsu & Gwo 2010), the *OtY2m* primers were designed from the *OtY2* of four *Oncorhynchus* species (*O. tshawytscha*, *O. nerka*, *O. keta*, and *O. kisutch*), and the *16S rRNA* primers, which were used as positive controls, were designed from the mitochondrial *16S rRNA* gene of Formosa landlocked salmon (*O. masou formosanus*) and were confirmed to be applicable for red-spotted masu salmon. For multiplex PCR using the *sdY* marker, two primer sets were used: *sdY* (*sdY* E1S1: 5′- ATG GCTGAC AGA GAG GCC AGA ATC CAA -3′ and *sdY* E2AS5: 5′- AGA GCA TCA CAG GGT CCA CAT CAC G -3′) and *18S rRNA* (*18S S*: 5′-GTY CGA AGA CGA TCA GAT ACCGT-3′ and *18S AS*: 5′-CCG CAT AAC TAG TTA GCA TGCCG-3′) (Yano et al. 2013). In a previous study (Yano et al. 2013), the *sdY* primers were successfully used to amplify the male-specific band of *O. masou masou*, which is a sub-species of red-spotted masu salmon, and the *18S rRNA* primers were used as positive controls for all salmonids. I preliminarily confirmed that the *sdY* primers amplified a single band of expected size (see below) in both singleplex and multiplex PCR, before being used for red-spotted masu salmon. For the *OtY2* marker, the multiplex PCR was performed in a total volume of 10 μL, containing 1 × GoTaq® Green Master Mix (Promega), 0.75 μM each of *OtY2m* (forward and reverse) primers, 0.1 μM each of *16S rRNA* (forward and reverse) primers, and 50–100 ng of genomic DNA. The cycling profile consisted of denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 56 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 3 min. For the *sdY* marker, the multiplex PCR was conducted in a

total volume of 12.5 μL, containing 1 × GoTaq ® Green Master Mix (Promega), 0.4 μM each of primers *sdY* E1S1 and *sdY* E2AS5, 0.1 μM each of primers *18S S* and *18S AS*, and 50–100 ng of genomic DNA. The cycling profile consisted of denaturation at 95°C for 3 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 3 min.

Multiplex PCR products from each male-specific marker were electrophoresed on 1.5 % agarose gels. Regardless of the band intensity, the positive or negative amplification of each marker was conservatively assessed through visual inspection of each band on the agarose gel electrophoresis. The expected band sizes of the PCR products were 300 bp for *OtY2* (Hsu & Gwo 2010), 200 bp for the mitochondrial *16S rRNA* gene (Hsu & Gwo 2010), and 370 bp for the nuclear *18S rRNA* gene (Yano et al. 2013). For *sdY*, I estimated the band size of 680 bp using the sequence data available in the International Nucleotide Sequence Database Collaboration (JF826023.1, DQ393568.1, KF006343.1, and AB626896.1).

DNA sequencing

Although the *OtY2* and *sdY* markers should be applicable to *O. masou ishikawae* (Hsu & Gwo 2010; Yano et al. 2013), unexpected positive amplifications were detected in some phenotypic females based on the results of the two multiplex PCRs. Therefore, the authenticity of the PCR products was confirmed for both markers using direct sequencing. For each marker, the six amplification products from phenotypic male and the six from phenotypic female were sequenced. The six phenotypic females were positive for both *OtY2* and *sdY*. PCR products were purified using ExoSAP-IT (USB, Cleveland, OH, USA) and sequenced directly on ABI PRISM 3100 Genetic Analyzer (Applied

Biosystems, Foster City, CA, USA) with the amplification primers using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) or ABI 3730xl DNA Analyzer (GENEWIZ, Saitama, Japan).

Nucleotide sequences were edited and aligned with the *OtY2* sequence from *O. masou masou* (GenBank accession number: GU181208.1) or with the *sdY* sequences from three *Oncorhynchus* species (GenBank accession number: *O. masou masou*: JF826023.1, *O. tshawytscha*: DQ393568.1 and KF006343.1, *O. mykiss*: AB626896.1), using MEGA 7.0.18 (Kumar et al. 2016).

Results

The results of multiplex PCR for each male-specific *OtY2* and *sdY* marker (Figure 1) are summarized in Table 2. All phenotypic males were *OtY2*-positive and *sdY*-positive for both the Arida and Nagara populations. On the other hand, some phenotypic females were *OtY2*-positive in both populations. Moreover, a relatively higher proportion of phenotypic females was *sdY*-positive in both populations. All females with *OtY2*-positive (32 % in the Arida population; 11 % in the Nagara population) were also *sdY*-positive (see Table 1).

For the positive amplification products from the *OtY2* marker, I obtained a sequence of a 223 bp segment for six phenotypic males and six phenotypic females (DDBJ accession number: LC612920). When the sequences were aligned with *OtY2* from *Oncorhynchus masou masou* (Hsu & Gwo 2010), no variable nucleotide site was found. This confirmed that the sequences obtained were authentic segments of the *OtY2*. In the same manner, I determined the sequence of a 516 bp segment from the partial *sdY* gene (including one intron and one exon; Yano et al. 2013) for six phenotypic males and six

phenotypic females (DDBJ accession number: LC612921). Two different sequences with three variable nucleotide sites were obtained (Figure. 2). Aligning with the genomic sequence of *sdY* from *O. tshawytscha*, 15 nucleotide substitutions and three insertion sites were detected in the intron region. When aligned with coding sequences of *sdY* from three *Oncorhynchus* species, only three nucleotide substitutions without amino acid substitutions were detected in the exon region. Therefore, I confirmed that the sequences obtained were authentic segments of *sdY*. There was no fixed nucleotide substitution in the gene region between the phenotypic males and females for *sdY* sequences.

Discussion

In the two red-spotted masu salmon populations studied, all phenotypic males were positive for *OtY2* and *sdY* markers. On the other hand, some phenotypic females were also positive for the two male-specific molecular markers (especially higher positive rates for the *sdY* marker).

In this study, I applied male-specific markers from previous studies, which reported no inconsistencies between phenotypic and genotypic sexes in red-spotted masu salmon (*OtY2*: Hsu & Gwo 2010) and in *Oncorhynchus masou masou* (*sdY*: Yano et al. 2013). However, the previous studies examined relatively few individuals (<10 individuals for each sex), and therefore, should be interpreted with caution. In fact, some studies have often reported phenotypic females that were positive for male-specific markers in other salmonid species both for *OtY2* [2.5 % in *O. masou formosanus* (Hsu & Gwo 2010); 43.3 % in *O. nerka* (Podlesnykh et al. 2017)] and *sdY* [2.4–22.0 % in *O. tshawytscha* (Yano et al. 2013; Cavileer et al. 2015); 23.1 % in *O. keta* (Podlesnykh et al. 2017); 4.2–43.3 % in *O. nerka* (Larson et al. 2016; Podlesnykh et al. 2017); 10.5 % in *O.*

gorbuscha (Podlesnykh et al. 2017); 1.0–9.7 % in *Salmo salar* (Eisbrenner et al. 2014; Brown et al. 2020)]. Those inconsistencies are presumably due to the complex molecular evolution of sex determination in salmonids (Kikuchi & Hamaguchi 2013). For instance, transposition or translocation of sex-linked regions to autosomes and/or X-chromosomes is well-known in salmonids, probably resulting in phenotypic females having male-specific sequences (*OtY1* and *GH-Ψ*: Williamson et al. 2008; *sdY*: Lubieniecki et al. 2015). Alternatively, mutations in *sdY*-coding region or downstream genes might inhibit differentiation of testicular tissue and cause sex reversal from genetic males to phenotypic females (Cavileer et al. 2015).

Environmental factors can also influence the determination of sex. Under high captivity temperatures, female-to-male sex reversal in the early life stages has been reported (Azuma et al. 2004; Valdivia et al. 2014). In this study, the fish from the two populations did not experience unusually high temperatures during the study period. Furthermore, estrogens (exogenous steroid hormones) can also cause sex reversal (Devlin & Nagahama 2002), and synthetic hormones mimicking the effects of natural estrogens have become a problem in the alteration of sex in aquatic organisms (Aris et al. 2014). However, this is unlikely in this study because there was no evidence of synthetic hormone pollution in the studied headwater streams. Taken together, I believe that environmental variations could not have caused phenotypic females that were positive for *OtY2* and *sdY* markers.

Although I found no inconsistencies in the males in this study, a few studies have also reported that phenotypic males of *S. salar* (Eisbrenner et al. 2014), *O. keta, O. tshawytscha* (Podlesnykh et al. 2017), and *O. nerka* (Larson et al. 2016; Podlesnykh et al. 2017) that were negative for male-specific markers. Yano et al. (2012) suggested that *sdY*

lacks a DNA-binding domain, which suggests that *sdY* might not be the sole master sex-determining gene and there might be other genes responsible for sex determination. Thus, the present study with a relatively small sample size (especially 10 individuals in the Nagara population) might overlook the inconsistencies.

Although I cannot completely rule out the possible inconsistencies between phenotypic and genotypic sex in phenotypic males, individuals with no male-specific amplification would be identified as females in most cases. Furthermore, I found that relatively fewer phenotypic females showed amplification of the *OtY2* marker (32 % and 11 % in the Arida and Nagara populations, respectively) than the *sdY* marker. Therefore, the *OtY2* marker could be used in practice to select possible females from the populations of interest. Female life-history traits, such as migratory tendency and/or age at maturity, largely affect population dynamics in salmonids (Sloat et al. 2014), and these traits are determined early in their life when morphological sexing is impossible (Jonsson & Jonsson 2014). Furthermore, reliable selection of females could be applied in strategic breeding programs in conservation (DeWoody et al. 2010) and to decrease the costs of fish production (Yue 2014). In such situations, it is still important to differentiate a number of females from the populations of interest; however, further research is required to develop better sex-specific markers (Feron et al. 2021).

Chapter 3. Seasonal timing of ecosystem linkage generates life-history variation in a salmonid fish population

Introduction

Maintaining life-history variation is fundamentally important for long-term persistence of populations and species (Schindler et al. 2010; Moore et al. 2014), community dynamics (Post et al. 2008; Weis & Post 2013) and ecosystem functions (El-Sabaawi et al. 2015). For example, Individuals with distinct life-histories respond differently to temporally fluctuating environments (Hallgrímsson & Hall 2005), and the consequent asynchronous dynamics among life-history groups stabilizes aggregate dynamics over time. This statistical-averaging process, known as a portfolio effect (Schindler et al. 2015), can reduce the risk of population extinction and over-exploitation (Schindler et al. 2010). Those ecological processes should depend on the magnitude of life-history variation. However, what remains poorly understood is potential drivers of life-history variation in natural populations. Given human activities are causing substantial shifts and loss of intraspecific variations worldwide (Mimura et al. 2016), it is more imperative than ever to elucidate the drivers of life-history variation not only from basic (Clutton-Brock & Sheldon 2010) but also from applied ecological perspectives (Blanchet et al. 2020).

For consumers living in connected open ecosystems, seasonal flows of energy and materials across ecosystems (i.e., resource subsidies) (Polis et al. 1997; Richardson & Sato 2015) can be a potential key driver of intraspecific life-history variation. Previous studies have demonstrated that subsidies occurring early in consumers' growing season tend to facilitate consumers to adopt a fast life; early subsidies can accelerate seasonal growth, which in turn allows juvenile consumers to mature and capture seasonally limited

reproductive windows at young (spiders: Marczak & Richardson 2008; lizards: Wright et al. 2013; stream fish: Sato et al. 2016). However, utilization of subsidies can vary among individuals via resource competition (Sato & Watanabe 2014; Sato et al. 2021) and niche variation (Kenny et al. 2017), and individuals with limited subsidy supplies may benefit from adopting a slow life if life-history trade-offs exist between growth and survival (Stearns 1989; Mangel & Stamps 2001). Yet, current debates are focused largely on the emergence of a certain life-history as a population average (Wright et al. 2013; Sato et al. 2016) but have offered limited insights into how seasonal resource subsidies maintain the variation in life-history within a consumer population.

Here, I hypothesize that for salmonid consumers utilizing terrestrial resource subsidies in a temperate forest-stream ecosystem, a greater proportion of fish individuals adopt a fast life when subsidies are supplied early in their growing season (Figure 3-I) than when late or no-subsidy is supplied (Figure 3-II); as a consequence, early-subsidy will increase life-history variation (Figure 3-III). If this is the case, I further investigated the mechanism to maintain the life-history variation by testing if there is a trade-off between growth pattern (e.g., fast vs. slow) and survival (Figure 3-IV).

Seasonal timing of terrestrial invertebrate inputs may vary among temperate streams covered by different forest types and under ongoing climate changes. Where surrounded by deciduous trees, input rates of terrestrial invertebrates commonly peak in early in the growing season (late spring toward early summer) of stream salmonid fishes (Nakano & Murakami 2001; Sato et al. 2011a; Inoue et al. 2013). On the other hand, the peak timing of terrestrial subsidies can also naturally occur late in salmonids' growing season (i.e., late summer toward early autumn) if riparian forests are dominated by conifer trees (Wipfli 1997). Moreover, terrestrial invertebrate subsidies can be seasonally shifted

or dampened by forest managements (i.e., clear-cut logging and extensive non-native conifer plantations; Inoue et al. 2013) and/or global climate change (Larsen et al. 2016).

Recent field evidence hints at the potential of subsidy timing in controlling life-history variation in subsidy consumers. In an experiment, early subsidies facilitated a population-level shift of salmonid individuals toward fast growth and young maturation (i.e., fast life), whereas late subsidies had no pronounced effects at population average (Sato et al. 2016). In addition, resource competition (Sato & Watanabe 2014; Sato et al. 2021) and niche variation (Kenny et al. 2017) can cause variable resource utilization among consumer individuals, potentially contributing to divergent life-histories in consumers in seasonally coupled ecosystems.

To test the hypothesis, I integrate a large-scale field manipulation experiment that mimics the magnitude and seasonal timing of subsidies associated with natural and artificial riparian forests with elaborate statistical analyses that can quantifying individual variations in life-history traits. Although resource subsidies is known to affect life-history expressions (Marczak & Richardson 2008; Wright et al. 2013; Sato et al. 2016, 2020), few studies have formally tested their effects on the variation in life-history in natural environments. In addition, it has been increasingly recognized that statistical approaches that can quantify individual trajectories is crucial in life-history studies (Hamel et al. 2017), but few attempts have been made for wild populations. Results of this study have provided the first empirical evidence that seasonal resource subsidies can be a key driver of life-history variation in a consumer population in seasonally coupled ecosystems.

Materials and methods

Rationale of the experimental design

I designed the experiment to test the effects of the early-subsidy, late-subsidy and reduced-subsidy on the life-history variation of red-spotted masu salmon *Oncorhynchus masou ishikawae* (hereinafter, masu salmon) in a Japanese temperate stream. In the study region, a riparian vegetation originally consists of deciduous trees, and the peak timing of the subsidy occurs from late June to early August (Sato et al. 2011a), which corresponds to the early in the growing season for the studied masu salmon. However, extensive non-native conifer plantations (*Cryptomeria japonica* and *Chamaecyparis obtusa*) have been conducted around the study stream (57.1 % conifer plantation within the drainage area), which has substantially reduced the magnitude of the subsidy in the studied stream (Sato et al. 2011b). In addition, the conifer plantation can potentially shift the subsidy timing toward late in the growing season (Wipfli 1997). The results from the early-subsidy treatment means how and in what extent the deciduous riparian forest can originally maintain life-history variation of recipient consumer fish, while their comparisons with the results for the reduced-subsidy (i.e., no-subsidy control) investigate the extent to which the extensive conifer plantation potentially cause loss of life-history variation. The comparison between the early- and late-subsidy tests whether shifts in riparian vegetation can increase or decrease the life-history variation that can be maintained within a population.

Study site and experimental procedures

The field experiment was conducted from June, 2016 to October, 2017 in a stream in an upper drainage of the Arida River system (34°04′N, 135°31′E), which drains a watershed

within the Wakayama Forest Research Station, Field Science Education and Research Center (FSERC), Kyoto University. The stream is a typical mountain stream [i.e., gradient ≈16.5 %, 3.0–5.2 m stream width, 0.067–0.112 m^3/sec in summer discharge, gravel-dominated streambed], and stream-resident masu salmon and a small number of minnows *Phoxinus oxycephalus jouyi* are the only fishes inhabiting the stream.

I created three treatment reaches (early-subsidy, late-subsidy and no-subsidy control) each with three replicates, resulting in nine experimental reaches. The treatment reaches were randomly assigned to nine 122.8 ± 28.0 m reaches, each separated by > 20 m intervals. I did not adjust the population density at the onset of the experiment. Although natural density of masu salmon varied among experimental reaches (age-0: 0.01–0.06 fish/m^2; ≥ age-1: 0.06–0.14 fish/m^2), those densities are relatively low compared with the densities reported in other populations (Kishi & Tokuhara 2012: 0.002–0.36 fish/m^2; Saito et al. 2013: 0.25–0.68 fish/m^2). Therefore, it is unlikely that these densities cause strong density-dependent effects on the life-history traits in this study. In addition, preliminary analysis indicated that they were unlikely to have strong density-dependent effects on the life-history traits in this study. The reaches were not fenced off to keep masu salmon in each treatment reach because it was not realistic to prevent movement of fish by building a fence in running water over two years. We confirmed that movements of fish across reaches were limited in the studied population [12 out of 346 (3.5 %) of fish moved across reaches]. I omitted individuals that moved across reaches from the analyses.

I emulated early- and late-subsidies of terrestrial invertebrate input by adding mealworms (larvae of beetle *Tenebrio molitor*) to natural stream reaches over two discrete 60-day time periods both in 2016 and 2017. Mealworms were added to subsidy reaches

at the same rate from June to August as an early-subsidy treatment or from August to October as a late-subsidy treatment. In each subsidy treatment reach, the mealworms were added at a rate of 100 mg/m^2/day by using 7–13 automatic fish feeders (W × H × D = 6.8 × 14.9 × 8.7 cm, ≈100 mL capacity for food; EHEIM Co. Ltd.) per reach. The natural input rates of the terrestrial invertebrates were relatively low in the study reaches (< 30 mg/m^2/day; Figure 3-I) probably due to the previous deforestation and subsequent non-native conifer plantations that were known to reduce the terrestrial invertebrate inputs (Inoue et al. 2013). The total input rate (mealworms + natural inputs of the terrestrial invertebrates) was comparable to the rates of peak terrestrial invertebrate inputs in temperate streams surrounded by natural and second-growth forests [136.1 ± 113.6 (mean ± SD; range 27–444) mg/m^2/day: Inoue et al. 2013]. Mealworms are ideal substitutes for the terrestrial invertebrate subsidy of the current study system (Sato et al. 2016; Sato et al. 2021).

Mark-recapture survey

Mark recapture survey was conducted each before and after the subsidy period (i.e., May, August, October) and early spring (i.e., March) to track the life-history traits, i.e., seasonal growth, survival, reproduction and movements, of the studied masu salmon. Fish were captured using two-pass electrofishing with units operating at 250 V pulsed DC (LR-24, Smith-Root, Vancouver, WA, Canada). Captured fish were anesthetized (clove bud: Seimi Laboratory, Kanagawa, Japan), measured (fork length to the nearest 1 mm and weighed to nearest 0.1 g) and individually marked by visible implant elastomer (North West Marine Technology, Inc. WA, USA). Captured location of each fish was recorded at 10-m interval to estimate the probability of emigration from the study reaches for survival

estimate. I pumped stomach contents of fish ($n > 5$) that were randomly selected from each study reach at the end of each subsidy period. The stomach samples were preserved in 70 % ethanol and identified in order to distinguish their sources (mealworms, terrestrial or aquatic invertebrates). Scales ($n < 10$ per fish) were sampled and preserved in 5 % buffered formalin solution for age-determination when captured fish were not known for their age. In October, just before the spawning season, captured fish were judged as matured if fish released sperm or eggs when I softly pressed the abdomen; otherwise, fish were recorded as immatured. All fish were subsequently released at their capture points after recovering from anaesthesia.

The definition of life history patterns

In this study, I focused on the growth trajectories and ages at maturity because those two life-history traits are tied to adaptive life-history variations in many animals, such as fast-slow life-history continuum (Promislow & Harvey 1990; Kozlowski 2006). In the studied population, precocious males were rarely observed (2.8 %), and almost all masu salmon died after the spawning season at age-2 (95.6 %). Fewer masu salmon (9.9 %) are iteroparous, i.e., reproduce multiple years during their lifespan. Therefore, I described the life-history of the studied masu salmon population by the combination of growth trajectory (e.g., fast vs. slow) and maturation status at age-1 (e.g., mature or immature). Sex-dependent life-history decision is well-known in salmonids (Klemetsen et al. 2003; Kendall et al. 2015; Bourret et al. 2016; Beamish 2018), but the preliminary analysis found responses to seasonal subsidies similar between male and female individuals (see Figure 10; Table 7). Therefore, I omitted sex-dependences from evaluation of life-history traits and proportional patterns of life-history variations. To strictly test the subsidy effects

on life-history of masu salmon, I confined the life-history evaluation to the 2016 cohort that received the subsidy treatments since their birth.

I could not know life-history types of individuals that died before the reproductive season at age-1. Thus, the frequency of life-history types I observed could have resulted from selection acting on intrinsic individual variations (e.g., genetic variations associated with life-history traits) and/or from plastic life-history decisions such as those based on threshold traits. Present study could not distinguish the two processes in evaluating the effects of subsidies on life-history variation.

Estimation of growth patterns using mixture model

In some animals, body size divergence with age can be explained by a single growth trajectory with random variation among individuals (Haddon 2011). In other animals, in contrast, multiple distinct growth trajectories may better explain individual variation; that is, individuals may be classified into multiple groups (i.e., growth clusters) sharing different growth trajectories. To test the two possibilities, I used a finite mixture regression model that can quantify individual variations in growth trajectories and statistically identify distinct growth clusters if present (Hamel et al. 2017). When the finite mixture regression model found multiple growth clusters, I further compared the number of growth clusters among early-, late-subsidy treatment, and no-subsidy control to test whether subsidy timing affected life-history variation in the study population.

In this study, I modeled the expected body length of masu salmon at each sampling timing for each growth cluster using the von Bertalanffy equation with seasonally varying coefficient (Cloern & Nichols 1978). In the finite mixture regression model, I assumed that each fish is assigned to a latent variable; i.e., an unobserved state

(strategy) corresponding to a growth cluster. A latent variable is a set of four parameters ($L_{\mathrm{max,cl}}$, $L_{\mathrm{min,cl}}$, $a_{1,\mathrm{cl}}$ and θ_{cl}) that fully describes the seasonal growth trajectory of a given growth cluster, as described in the following equation:

$$L_{\mathrm{cl}}(t) = L_{\mathrm{max,cl}} - (L_{\mathrm{max,cl}} - L_{\mathrm{min,cl}}) \cdot exp[-a_{1,\mathrm{cl}} \cdot (t - t_0) - \frac{180 \cdot a_{1,\mathrm{cl}}}{\pi}(cos\left\{\frac{\pi \cdot (t_0+\theta_{\mathrm{cl}})}{180}\right\} - cos\left\{\frac{\pi \cdot (t+\theta_{\mathrm{cl}})}{180}\right\})]$$

where $L_{\mathrm{cl}}(t)$ is body length at time t ($t = 0$ on January 1) for a given growth cluster (cl), $L_{\mathrm{max,cl}}$ is a maximum fork length of the growth cluster, $L_{\mathrm{min,cl}}$ is fork length at the time of recruitment (t_0) of the growth cluster, and $a_{1,\mathrm{cl}}$ and θ_{cl} are the estimated parameters of constant coefficients that determine seasonal dependence of body size growth.

To implement the model, I utilized FLXMRglm function in the R package 'flexmix', where the EM algorithm (Dempster et al. 1977) can be used for the parameter estimation. In the finite mixture regression model, I used 885 body-size data across 334 individuals [2.65 ± 1.53 (mean ± SD; range 1–7) data per individual]. In the finite mixture regression model, individuals with multiple captures were used to be classified into different mean trajectories, instead of a single mean population trajectory. In addition, I also added individuals with a single capture into the model to increase the amount of data for better estimating mean growth trajectory for the studied population. This might have led me to underestimate the individual differences in growth trajectory, such as season-specific rapid or slow growth. However, omitting individuals of a single capture (n = 103) made a model performance unstable and therefore I included those data into the finite mixture regression model.

I did not know *a priori* the number of growth clusters maintained in the studied population. Therefore, I applied model selection by developing the models with different number of growth clusters (k = 1–6). The Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were used for the model selection; models both with ΔAIC < 2 and ΔBIC < 2 was considered to be an equivalent statistical support (Hamel et al. 2017).

Association of growth clusters with maturation at age-1

Based on the previous study for the same masu salmon population (Sato et al. 2021) and along with the principle of fast-slow life-history continuum (Promislow & Harvey 1990), I hypothesize individuals of fast-growth clusters to have higher probabilities of maturation at age-1 than those of slow-growth clusters. In addition, the effect of growth cluster on the maturation-decision might be larger with the early-subsidy than with the late-subsidy and no-subsidy because of the timing dependent life-history decisions (Sato et al. 2016). Generalized linear mixed model (GLMM; glmer function in the R package 'lme4'), with experimental reach (n = 3 replicates) as a random effect, was used to test whether the probability of maturation at age-1 was related to the growth clusters, treatments and their interaction. The interaction term was excluded from the final model if it did not significantly maximize the log-likelihood of the model in the log-likelihood ratio test (Crawley 2007).

Variation in life-history patterns

I classified life-history patterns as a combination of growth clusters (slow, medium, and fast) and maturation status at age-1 (mature, M or immature, IM); e.g., individuals grew fast and matured at age-1 are denoted as "Fast-M". I tested whether the early-subsidy

treatment induced higher variation in life-history patterns using the following two complementary approaches. First, Fisher's exact test was used to examine if proportional composition of life-history patterns differed among treatments. When the treatment effect was significant, I further conducted pairwise comparisons with Bonferroni correction ($\alpha = 0.017$). This approach cannot quantify the variation in life-history patterns *per se*. Therefore, I calculated the Shannon-Wiener diversity index (*H'*) (Magurran 2004) to provide a statistically comparable metrics for the variation in life-history patterns. I pooled three replicates to calculate *H'* for each treatment due to low sample size of fish per replicate reach.

Estimation of survival probabilities

Fitness trade-off is a well-known mechanism to maintain life-history variations within a single population (Stearns 1989; Mangel & Stamps 2001). In this study, I hypothesized that individuals with faster growth experienced lower survival until their reproductive season at age-1, which underlies the generation of life-history variation of masu salmon. To test this hypothesis, true survival probabilities (i.e., corrected for permanent emigration) were estimated by a modified version of a Cormack-Jolly-Seber (CJS) model (Schaub & Royle 2014; Terui et al. 2017). Survival estimates in classical CJS models are called "apparent" survival, as it is confounded with permanent emigration from an observation area. The modified CJS model, however, can account for permanent emigration by integrating movement into observation processes. This modeling approach will be useful in the present study, because the experiment was conducted in a stream, and the permanent emigration of fish from the experimental reaches would inevitably occur. In this study, the primary objective was to test whether the variation in life-history

patterns in each treatment are explained by the trade-off between growth cluster and survival. Therefore, I estimated the survival probability separately for growth clusters and subsidy treatments.

In the observation process, I describe a process of recapture incidence. Let $Y_{i,t}$ be a binary variable denoting the recapture incidence for individual i at recapture event t (1 if recaptured; otherwise, 0). The recapture incidence must suffice the following conditions: (1) alive, (2) present in the observation area, and (3) detected by the two-pass electrofishing (see 'Mark-recapture survey' part in the Methods described above). Therefore, I assumed that $Y_{i,t}$ is drawn from the following Bernoulli distribution:

$$Y_{i,t}|z_{i,t}, q_{i,t}, \xi \sim \text{Bernoulli}\,(z_{i,t}q_{i,t}\xi)$$

where $z_{\mathrm{i,t}}$ is the state variable indicating the survival status of individual i at time t (1 if alive; otherwise, 0), $q_{i,t}$ is another state variable indicating whether the individual stays in the observation area, and ξ is the detection probability through a two-pass electrofishing. In this expression, recapture incidence occurs only for those that are alive ($z_{i,t} = 1$) and stay in the study area ($q_{i,t} = 1$) since the state variables were multiplied. Survival state $z_{i,t}$ is conditional on the survival probability and previous survival state as $z_{i,t}|s_{g(i),t-1}, z_{i,t-1} \sim \text{Bernoulli}\,(s_{g(i),t-1}z_{i,t-1})$, in which $s_{g(i),t-1}$ is the survival probability for group g ($g(i)$ is a combination of growth cluster and subsidy treatment to which individual i belongs) between time $t-1$ and t. The parameter $s_{g(i),t-1}$ was expressed as a geometric series of daily survival $\nu^{D}_{g(i),t-1}$ ($s_{g(i),t} = \nu^{D}_{g(i),t-1}$, where D is the number of dates between two consecutive recapture events) to assign consistent hyper-priors as $\text{logit}\,\nu_{g,t} \sim \text{Normal}(\text{logit}\,\mu_{\nu,g}, \sigma^2_{\nu})$. The variance parameter σ_{ν} controls temporal variability in daily survival among recapture events.

I derive the state variable $q_{i,t}$ using movement data of marked individuals (see 'Mark-recapture survey' part in the Methods described above). Specifically, I described recapture location $x_{i,t}$ [measured as the distance from the center of a recapture section (10-m each) to the downstream end of the whole study reach] as a random variable drawn from a Laplace distribution truncated at 1350 m, which generates values randomly from $-\infty$ to 1350. The truncation was made because fish were restricted upstream movement at the upstream end of the study section (i.e., 1350 m). The recapture location at time t is conditional on the mean movement distance δ_{t-1} and release location $x_{i,t-1}$ at time $t-1$:

$x_{i,t}|\delta_{t-1}, x_{i,t-1} \sim \text{Laplace}\ (\delta_{t-1}, x_{i,t-1})$

$\ln \delta_{t-1} = \alpha + \ln Interval_{t-1}$

where α is the log-transformed daily movement distance averaged over time and Interval_{t-1} is the offset term denoting the number of days between time $t-1$ and t. The truncated Laplace density function g_L has the following probability density function:

$$g_L(x_{i,t}|\delta_{t-1}, x_{i,t-1}) = \frac{f_L(x_{i,t}|\delta_{t-1}, x_{i,t-1})}{\int_{-\infty}^{1350} f_L(x_{i,t}|\delta_{t-1}, x_{i,t-1})\, dx_{i,t}}$$

$$f_L(x_{i,t}|\delta_{t-1}, x_{i,t-1}) = \frac{1}{2\delta_{t-1}} \exp\left(-\frac{|x_{i,t} - x_{i,t-1}|}{\delta_{t-1}}\right)$$

The term $x_t - x_{i,t-1}$ represents the observed movement distance in which positive and negative values represent up- and downstream movement, respectively. The state variable $q_{i,t}$ is determined by the value of $x_{i,t}$:

$$q_{i,t} = \begin{cases} 1 \text{ if } 0 \leq x_{i,t} \leq 1350 \\ 0 \text{ if } x_i < 0 \end{cases}$$

Therefore, the latent variable $q_{i,t}$ can be seen as a realization of a random variable drawn from a Bernoulli distribution with the probability of staying in the observation area, $D_{i,t} = \int_0^{1350} g_L(x_{i,t}|\delta_t, x_{i,t-1})\, dx_{i,t}$. As such, survival estimates in the modified CJS model are corrected for permanent emigration. (Schaub & Royle 2014; Terui 2020).

The model was fitted to the data using Just Another Gibbs Sampler (JAGS) ver 4.1.0. I assigned vague priors to parameters: i.e., normal distributions with large variance [Normal(0, 100)] for α, beta distributions [Beta(1, 1)] for ξ and $\mu_{v,g}$, and half-Cauchy distributions [Half-Cauchy(2.5)] for σ_v. I run three chains of Markov Chain Monte Carlo (MCMC) for 30,000 iterations (the first 10,000 iterations were discarded as burn-in). For the last 20,000 iterations, MCMC samples were saved every 40 steps to estimate posterior distributions. The primary objective was to estimate the survival probabilities of varied growth clusters exposed to different subsidy regimes over the entire experimental period (from June 2016 to October 2017). I calculated the survival probabilities as $\phi_{g(i)} = \prod_t s_{g(i),t}$ for each subsidy treatment separately using the MCMC samples.

Results

The total mass of all invertebrate consumption (i.e., aquatic and terrestrial invertebrates, and mealworms) by red-spotted masu salmon in subsidized-treatment reaches was, on average, 5.6 times higher than those in un-subsidized reaches in August [subsidized: 0.52 ± 0.34 mg/100 mg mass of fish; un-subsidized (no-subsidy control + late-subsidy treatment): 0.09 ± 0.09; Mann–Whitney *U*-test: $W = 34$, $p = 0.009$] and 3.6 times higher in October [subsidized: 0.31 ± 0.37; un-subsidized (no-subsidy control + early-subsidy

treatment): 0.09 ± 0.09; Mann–Whitney *U*-test: $W = 213$, $p = 0.020$], respectively. Mealworms respectively made up 97 ± 3 % and 62 ± 40 % of the total mass of consumption by masu salmon in August and October, and the consumption rate of mealworms was positively correlated with the total consumption rate in both periods (Spearman rank correlation, August: $r_s = 1$, $p = 0.0028$; October: $r_s = 0.95$, $p < 0.0001$).

Growth pattern and age at maturity

The specific growth rates of masu salmons did not differ between periods [(early in the growing season (June–August) vs. late in the growing season (August–October)] when they received no subsidy during the periods ($GLMM_{gaussian}$, period: $t = -1.24$, $P = 0.24$; Figure 4; Table 3). When subsidies were supplied, their growth increased by 1.6 times higher, and the increase did not depend on the period they received subsidies (subsidy × period: $t = -0.29$, $P = 0.77$). However, the specific growth rate varied substantially among individuals across experimental reaches. Three growth clusters (fast-, medium- and slow-growth clusters) best explained this variation in the finite mixture regression model (Table 4, 5; Figure 5). The proportional composition of the growth clusters differed significantly among treatments (Fisher's exact test: $P < 0.01$), with only a significant difference in a pairwise comparison between early- vs. late-subsidy treatments (adjusted $P < 0.001$) (Figure 6). Early-subsidies increased the proportion of individuals belonging to the fast-growth cluster (57 out of 117 fish, 49 %), while reducing that of the slow-growth cluster (n = 18, 15 %) (Figure 6). In no-subsidy control, individuals belonging to the medium-growth cluster were most common (51 out of 118 fish, 43 %), followed by the fast-growth (34 %) and slow-growth (23 %) individuals (Figure 6). Although the late-subsidy treatment supplied the same amount of subsidies as in the early-treatment, late-subsidies

resulted in the composition close to that of the no-subsidy control, with a slightly larger representation of the fast-growth cluster (Figure 6).

Probability of maturation at age-1 was higher for individuals belonging to fast-growth cluster; 29 out of 39 fast-growth individuals (74 %) matured at age-1, whereas only one out of 29 slow-growth individuals (3 %) did at age-1 (Figure 7). The optimal GLMM model revealed that the growth cluster solely had a significant effect on age at maturity, i.e., no significant effects of the subsidy timing nor its interaction with growth clusters on the probability of maturation at age-1 were detected (Table 6).

Variation in life-history patterns

I observed six different life-history patterns across treatment reaches (Figure 8). The proportional composition of the life-history patterns largely differed among treatments although it was not statistically significant (Fisher's exact test: $P = 0.11$) due to relatively small sample size. In the early-subsidy treatment, individuals that adopted fast life, i.e., fast-growth and matured at age-1 (Fast-M) were most common (11 out of 34 fish, 32 %), whereas the second common life-history pattern (7 fish, 21 %) was slow life, i.e., Slow-IM. Other life-history patterns, except for Slow-M (n = 1), were evenly found in the early-subsidy treatment. In no-subsidy control, three life-history patterns (Fast-M, Medium-IM, and Slow-IM; 34 out of 36 fish, 94 %) dominated over the other three patterns. Among the three life-history patterns, relatively slower life-history patterns, i.e., Medium-IM (n = 15, 33 %) and Slow-IM (n = 14, 31 %), were found in similar proportions, while 20 % of fish (n = 9) adopted Fast-M pattern. In the late-subsidy treatment, the three-dominant life-history patterns were the same as those observed in no-subsidy control, but the proportional composition differed largely between the two treatments. Specifically,

Medium-IM was most common (18 fish, 50 %), while Fast-M (9 fish, 25 %) and Slow-IM (7 fish, 19 %) were the second- and third-common life-history patterns, respectively. Overall, the diversity of life-history patterns was highest in the early-subsidy treatment and lowest in the late-subsidy treatment (Shannon's *H'*: early = 2.36, late = 1.75, control = 2.09).

Survival probability and its association with growth clusters

In no-subsidy control, the estimated survival probability was lowest in individuals belonging to the fast growth cluster, suggesting a growth-survival trade-off. However, the growth-survival trade-off became unclear in the early-subsidy treatment and even disappeared in the late-subsidy treatment; the survival probability was similar among individuals belonging to the different growth clusters in the early-subsidy treatment; the survival probability was lower in the slow-growth individuals than the fast-growth individuals in the late-subsidy treatment (Figure 9).

Discussion

Elucidating the maintenance mechanisms of life-history variation is fundamentally important in ecology and evolutionary biology (Stearns 1992; Clutton-Brock & Sheldon 2010). However, it remains poorly understood what ecosystem properties increase or decrease life-history variation in natural environments. Here, I provide empirical evidence that the ecosystem linkage via seasonal resource subsidies can maintain life-history variation of consumers in recipient ecosystems. Another key implication from this study is that human-induced environmental changes may reduce the life-history variation through the temporal shifts and/or decline of seasonal resource subsidies. The current study suggests that such an indirect process through resource subsidies may underlie the

ongoing loss of intraspecific variations in wildlife.

Timing-dependent effects on life-history decision and its variation

Although the experimental subsidy increased the individual growth rate irrespective of timing, the fast-life history (represented by the fast-growth cluster and maturation at age-1) was prevalent in the early-subsidy treatment than in the late-subsidy treatment. This timing-dependent effects may reflect physiological constraints on the seasonally-determined onset of maturity. In salmonids (Thorpe et al. 1998) including the studied species (Silverstein & Shimma 1994), individuals achieving rapid growth and gaining lipid reserves early in the growing season can initiate gonad development and mature in autumn. Yet, growth acceleration induced by late subsidies may be too late for individuals to develop gonads before the reproductive season of the year. Arguably, this timing-dependent life-history decision facilitated individuals to adopt a fast life and suppressed the proportion of slow-life individuals in the early-subsidy treatment. In contrast, the late-subsidy was inefficient in inducing a fast life, resulting in many individuals expressing medium-growth and late maturation. In no-subsidy control, the reduced subsidy was not able to accelerate the seasonal growth important for inducing a fast life. Consequently, life-history variation was higher in the early-subsidy treatment than in the late-subsidy treatment and no-subsidy control.

Temporal match between resource availabilities and life-history decision windows commonly regulates seasonal growth and maturation in many plants and animals (Durant et al. 2007). The current study highlights that those phenological matching can occur across ecosystem boundaries and strongly regulate variations in life-history expression. Given the ubiquitous nature of seasonal ecosystem linkages,

anthropogenic disturbances of seasonal resource subsidies at local and global scales (Richardson & Sato 2015; Larsen et al. 2016) may underlie the ongoing loss of intraspecific variations in wildlife.

Maintenance mechanisms of life-history variation and their potential population consequences

Trade-offs among life-history traits, such as growth, survival and reproduction, is considered a leading cause that maintains life-history variation within a population (Stearns 1989; Mangel & Stamps 2001; Christie et al. 2018). In this study, individuals belonging to the fast-growth cluster tended to have lower survival rate until age-1 in the no-subsidy control, suggesting that the growth-survival trade-off partially explains the life-history variation in the no-subsidy control. However, such a trade-off was not found in the early- and late-subsidy treatments; the survival rate tended to be lower in the slow-growth cluster in both treatments. Two possible mechanisms may explain life-history variation despite the apparent lack of the growth-survival trade-off. First, slow-life individuals might achieve fitness similar to that of fast-life individuals in the subsidy treatments via other types of life-history trade-off, such as the growth-reproduction trade-off (Christie et al. 2018); reproductive success in a same spawning ground would be higher in slow-life individuals that matured at age-2 than fast-life individuals that matured at age-1 due to a size-dependent breeding competition. If this is the case, the life-history variation is stably maintained with the early-subsidy within a population. Second, spatial variation in resource subsidies may maintain life-history variation across space; i.e., fast-life individuals dominate in deciduous forested streams with early subsidies, whereas slow-life individuals would be prevalent in coniferous forested streams with late subsidies

and/or reduced subsidies. As a consequence, life-history variation can be maintained at a basin scale (i.e., meta-population level). This can be the case in the studied masu salmon because many individuals complete their life-history within a stream (i.e., local population) that receives a given subsidy supply, but potentially move across streams (i.e., forming meta-population).

Those maintenance mechanisms of life-history variation are highly relevant to whether the life-history variation potentially contributes to stabilizing population fluctuation at local population or meta-population levels. In salmonid fishes, individuals with distinct ages at maturity can respond differently to environmental fluctuations, such as large floods (Jensen & Johnsen 1999), and the consequent asynchronous dynamics among life-history groups can stabilize population fluctuations over time (Schindler et al. 2010; Moore et al. 2014). If the early subsidy can stably maintain life-history variation within a population, this stabilizing effect is expected to function at local population level. On the other hand, when given subsidies (early, late or reduced subsidies) associated with riparian forest types maintain a certain life-history in each stream (i.e., local habitat), the asynchronous dynamics among life-history groups can stabilize meta-population dynamics across streams within a river basin. Unravelling temporal and spatial variations in seasonal subsidies and their relevance with life-history variation within and among local populations will be a crucial step to fully understand the maintenance mechanisms of life-history variation and their population consequences across time and space.

Limitation for the evaluation of life-history variation

While I successfully described the variation in life-history patterns until age-1 and its dependence with the timing and magnitude of the terrestrial subsidy, I might

underestimate some of the life-history variations in the studied population. For instance, I could not evaluate (1) fish that matured at age-2 and older, and (2) fish that repeated reproductions (i.e., iteroparous individuals) in the 2-year field experiment. For the first limitation, because fewer fish (approx. 5 %) survived until reproductive period at age-3 in the studied population, individuals that did not mature at age-1 could be assumed to mature at age-2. For the second limitation, the iteroparous life-history was relatively uncommon (approx. 9.9 %) in the studied population. Taken together, I believe that potential underestimates of the life-history patterns would minimally affect the main argument of the present study.

Conclusion

In this study, I demonstrated that seasonal timing of resource subsidies relative to consumer phenology can have pronounced effects on life-history variation in consumers in recipient ecosystems. Contrary to the most studies focusing the effects of subsidies on life-history traits as a population average, acknowledging the subsidy effects on the variation in life-history is crucial because life-history variations are fundamentally important for long-term persistence of populations and species (Schindler et al. 2010; Moore et al. 2014). Consumers' effects on species interactions and ecosystem functions should be determined by not only mean but variation of life-history traits of the consumers (Bolnick et al. 2011; Des Roches et al. 2018; Raffard et al. 2019). Furthermore, understanding relative contribution of life-history plasticity and genetic variation underlying such life-history variation can lead to answer two fundamental questions in ecology and evolution; how quickly individuals can respond to changing environments? and how it does maintain resilience at population level? Due to the technological

development in molecular biology, the genetic and epigenetic mechanisms related to growth and age at maturity are being elucidated in wild organisms (Morán & Pérez-Figueroa 2011; Barson et al. 2015; Therkildsen et al. 2019). The current study presents new insight into those studies by emphasizing the need to acknowledge environmental factors at landscape, where seasonal subsidies govern growth trajectory, age at maturity and ultimately life-history expressions of wildlife.

Chapter 4. Genomic analysis of partial migration in red-spotted masu salmon

Introduction

Intraspecific life-history variation is important in stabilizing population dynamics and local adaptation (Schindler et al. 2010; Moore et al. 2014; Moran et al. 2016). Migration is the seasonal long-distance movement of organisms with a suite of physiological and morphological changes (Dingle & Drake 2007). In many animals, both residents and migrants coexist within a population, which is called partial migration (Chapman et al. 2011). Theories suggest that maintaining both residents and migrants within a population contributes to the persistence of long-term populations in fluctuating environments (Teitelbaum & Mueller 2019). Alternative migratory phenotypes are influenced by both genetic and environmental factors (Chapman et al. 2011). In the past decade, advances of molecular biology techniques have allowed us to elucidate the genetic basis underlying various life-history traits (Stapley et al. 2010; Ekblom & Galindo 2011). This provides the opportunity to develop genetic markers by targeting loci and/or genomic regions associated with a trait of interest. Subsequently, this leads to directly testing whether an individual with a given marker genotype will express a phenotype under a given environmental condition (genotype-environment interaction) (Kruuk et al. 2008). However, the genetic basis of partial migration has only been elucidated in a few animal species (Liedvogel et al. 2011), which hinders our understanding of the interactive effects of genotypes and environments on migratory behavior, and their influence on population dynamics and local adaptation.

Salmonid fish are well-known for their partial migration. Resident individuals stay in fresh water throughout their lives, while migrant individuals move to the sea for

growth (Kendall et al. 2015; Nevoux et al. 2019). It has been suggested that both heritable genetic components and the environment influence migration propensity, and the genetic basis for this was investigated in several species (Hale et al. 2013; Hecht et al. 2013; Nichols et al. 2016). A previous study showed that a large-scale double inversion, located on chromosome 5 (*Omy05*) in *Oncorhynchus mykiss*, contains many genes related to migratory behaviour and acts as 'supergene'. It is closely associated with migratory phenotype in the coastal populations in California (Pearse et al. 2019). In contrast, other previous studies have shown that there is no significant association between *Omy05* and migratory phenotype in inland or northern populations of *O. mykiss* (Weinstein et al. 2019; Collins et al. 2022). This suggests that different genomic regions and genes are associated with maintaining partial migration within species, and each population requires an assessment of the genetic mechanisms.

Red-spotted masu salmon (*Oncorhynchus masou ishikawae*) is known as the southernmost salmon that expresses the partially migratory strategy (Kato 1973; Kishi & Tokuhara 2019). Both residents and migrants coexist within a population, they are called "amago" and "satsukimasu", respectively. Excluding precocious males, age-0 individuals that exceed a threshold in body size in late August to October become migrants and migrate out to sea from November to December (Kato 1973; Kuwada et al. 2016). Migrants grow for only half a year in coastal areas and then return to their natal stream for spawning before the water temperature rises in summer (Kato 1973; Umino et al. 2001; Noda et al. 2021). In contrast, individuals smaller than the threshold size become residents. Like other salmonids, both genetic and environmental factors may influence migration propensity. However, to the best of my knowledge, there is no study that investigated the genetic basis underlying partial migration of red-spotted masu salmon.

In this study, I explored genomic regions that are associated with partial migration in three wild populations of red-spotted masu salmon. Using an approach to sequence pooled DNA samples to cost-effectively obtain high coverage data (Pool-seq; Fuentes-Pardo & Ruzzante 2017), I examined (i) whether common genomic regions are associated with partial migration among geographically distant populations and (ii) what genes are associated with partial migration. I believe that elucidating genetic basis of complex life-history traits is the first step in studying genotype-environment interactions to understand the underlying processes of maintaining partial migration.

Materials and methods

Sample collection and DNA extraction

Samples were obtained in three geographically distant river systems where upstream migration of migrants are observed (Figure 11). In the Arida River system, both residents and migrants were captured from an upper stream population in Yukawa River (34°04′N, 135°31′E), a tributary of Arida River in 2016 and 2017 spawning season. This population is isolated from the sea because Futagawa Dam, constructed in 1967, has completely blocked the path of returning migrants. I defined matured fish as residents. Smolts are migrants that are ready to migrate to sea with morphological changes (silvery body colour, black fin, and thinner body shape; Figure 12). In the Nagara and Ohta River systems, matured residents were captured using electrofishing with 250 V pulsed DC operating units (LR-24, Smith-Root, Vancouver, WA, Canada). In the Nagara River, a population was captured in a Nabi River tributary (35°42′N, 136°54′E) in October 2019. In the Ohta River, a population was captured in a Yoro River tributary (34°37′N, 132°20′E) in November 2020. I obtained specimens of returning migrants from a local fisherman that

were caught approximately 40 km upstream from the river mouth of Nagara River in May 2019. In the Ohta River, I obtained specimens of returning migrants from anglers approximately 16–23 km upstream from the river mouth between May and June in 2020. For all specimens, adipose fins were clipped, preserved in 99 % ethanol, and stored at –20 ℃ for genomic analysis. All fish, except for the migrants from the Nagara River, were released back into the stream after sampling.

Genomic DNA was extracted from fin clips using the standard phenol-chloroform method as follows: each sample was digested overnight in lysis buffer (0.1 M EDTA, 1 % SDS, and 0.06 mg Proteinase K). DNA was then purified with phenol and chloroform, precipitated with isopropanol and 0.5 M NaCl, washed with 70 % ethanol, and dissolved in Tris-EDTA buffer. To get high-quality DNA, RNA was removed with RNase A. DNA was purified again with phenol and chloroform, precipitated with 99 % ethanol and 0.3 M sodium acetate, washed with 70 % ethanol, and dissolved in 10 mM Tris-HCl (pH 8.0). DNA concentration was measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA quality was assessed using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA integrity was also assessed by visual inspection of DNA fragmentation on agarose gel electrophoresis.

Library preparation and sequencing

Pools of genomic DNA for Pool-seq were prepared by pooling equal amounts of DNA (150 ng DNA per individual) of 10 individuals for each phenotype in each river system. In Pool-seq protocol, it is recommended that each DNA pool includes DNA samples of at least 50 individuals to minimize individual contribution bias (Fuentes-Pardo & Ruzzante

2017). However, collecting this number of samples was difficult, especially migrants, therefore, I could only pool 10 individuals. For some individuals, prior population structure information based on SNPs was obtained from MIG-seq (unpublished data), genetically similar individuals were considered to be from the same population and were pooled to minimize phylogenetic effects. Female-biased migration is common in salmonids including red-spotted masu salmon (Kato 1973), therefore, only females were used for the genomic analysis to reduce the possibility of sex-dependent effects on the results. Migrants without sex information were assayed by using male-specific molecular marker (*OtY2*) and assigned sex (Hsu & Gwo 2010). I confirmed the efficacy of this marker in identifying females in Chapter 2 (Ueda et al. 2022; Chapter 2). Library preparation and sequencing were performed at Macrogen Japan Corp. (Tokyo, Japan). Libraries were prepared using the TruSeq DNA PCR-Free Kit (Illumina) and sequenced with paired-end 150 bp reads on the NovaSeq 6000 platform (Illumina).

Data processing

Each sequenced pool was processed based on general approaches used in previous studies on salmonids (Micheletti et al. 2018; Narum et al. 2018; Kjærner-Semb et al. 2021). First, BBDuk in BBTools (http://sourceforge.net/projects/bbmap/) was used for adapter trimming and quality filtering (trimq = 20, minlength = 50). Trimmed reads were aligned to a reference genome of red-spotted masu salmon using BWA-MEM (Li & Durbin 2009) with mapping quality ≥ 20 (-q 20). This reference genome was previously constructed by reference-guided assembly approach using Chinook salmon (*Oncorhynchus tshawytscha*) genome (NCBI accession numbers: GCA_002872995.1), a species related to red-spotted masu salmon (Hashiguchi, unpublished data). For the genome assembly, I used

approximately 294 G paired-end short reads (estimated 18-fold genome coverage) that were generated on the Illumina HiSeq X Ten platform from a single wild resident male captured in Yukawa River in 2016. Then, PCR duplicates were removed using SAMblaster (Faust et al. 2014). SAM files were converted to BAM files using SAMtools view (Li et al. 2009) and removed unmapped reads (samtools view: -F 0x04) and multi-mapped reads (samtools view: -F 0x100).

I used SAMtools for SNP calling. First, filtered BAM files were combined into a pileup file using SAMtools mpileup. Then, the pileup file was converted to a sync file using PoPoolation2 (Kofler et al., 2011) with base quality ≥ 20 (mpileup2sync.pl: --min-qual 20). To remove false positive SNPs, insertions and deletions were identified and removed SNPs within 15 bp of insertion/deletion regions (identify-indel-regions.pl: --indel-window 15, filter-sync-by-gtf.pl). Finally, SNPs with minor allele counts of at least one were retained.

Estimation of genetic distance

Genetic distance was calculated to examine the population structure of the six pools. First, SNPs were further filtered to avoid genotyping error, variant sites with minor allele counts of at least two were retained. In addition, SNPs with a minimum and maximum depth of coverage of 15× and 250×, respectively, were retained. The minimum depth of coverage was used to decrease false-positive results due to low coverage while the maximum depth of coverage was used to remove SNPs that are probably aligned to paralogous regions. Pairwise F_{st} among pools was calculated for each SNP using Popoolation2 (fst-sliding.pl). F_{st} values were averaged for each pairwise pool comparison, and a distance matrix was created. Finally, a neighbor-joining tree was constructed using MEGA X (Kumar et al.

2018).

Detection of genomic regions associated with partial migration

When a new mutation spread in a population by natural selection, allele frequency change and genetic variation decreased near the site of selection (Smith & Haigh 1974). To detect the sites where a selective sweep was expected to occur, I explored genomic regions in which allele frequencies differed between residents and migrants for each river. First, SNPs were further filtered for each river system, SNPs with a minor allele count of at least two, and a minimum and maximum depth of coverage of 15× and 250×, respectively, were retained. Subsequently, absolute allele frequency difference (dAF) between residents and migrants were calculated for each SNP using the following formula:

$$\mathrm{dAF} = |p_{\mathrm{R}} - p_{\mathrm{M}}|$$

where p_{R} and p_{M} are the major allele frequency of residents and migrants for each SNP and each phenotype, respectively. In this study, genomic regions that include at least 10 SNPs with dAF > 0.7 in 100-kb sliding genomic windows were predicted as highly differentiated regions between residents and migrants (Kjærner-Semb et al. 2021). These regions were detected with 50-kb step size, and overlapping windows were merged.

Heterozygosity (H_{p}) was calculated for each candidate regions associated with the migratory phenotypes (hereinafter, referred to as the associated region) for each pool. First, H_{p} was calculated for each SNP based on the following formula described in Kjærner-Semb et al. (2021):

$$H_{\mathrm{p}} = 2p_{\mathrm{A}}(1 - p_{\mathrm{A}})$$

where p_A is the major allele frequency of each SNP. Second, H_p values were averaged for each associated region in a pool. Finally, the Wilcoxon signed-ranks test was used to compare mean heterozygosity for each associated region between phenotypes (residents vs. migrants) in each river system.

Candidate genes within associated regions were identified based on Chinook salmon genome (NCBI accession numbers: GCA_002872995.1) using the NCBI Genome Data Viewer.

Results

Number of raw paired-end reads was 463.2 ± 49.3 (mean ± SD) million reads, whereas 39.7 ± 0.2 % of the raw reads were, on average, retained as proper pairs after removing low quality reads and PCR duplicates. Depth of coverage was 20.3 ± 1.2× (mean ± SD) after filtering (Table 8).

Genetic distance

A total of 6,051,287 SNPs were shared among six pools and used to estimate genetic distance (Figure 13). In the Arida River sample, the genetic distance between residents and migrants were the closest to each other (mean F_{st} = 0.032). Similarly, the two phenotypes were closest to each other in the Nagara River sample (mean F_{st} = 0.031). In the Ohta River sample, the residents were the closest to migrants (mean F_{st} = 0.053). Migrants in the Ohta River have the closest relationship with migrants in the Nagara River (mean F_{st} = 0.036), this indicates that there is a lack of clear genetic differentiation among populations.

Genomic regions associated with partial migration

In the Arida River sample, a total of 7,001,405 SNPs were retained after filtering, 5,456 SNPs with dAF > 0.7 were detected (0.08 %). 48 regions were identified, including at least 10 SNPs with dAF > 0.7 in 100-kb sliding genomic windows on chromosomes and nine on unplaced scaffolds (Figure 14; Table 9). Mean (± SD) depth of coverage for these associated regions were 19.5 ± 4.3–31.3 ± 26.3× in residents, 22.3 ± 4.9–35.7 ± 24.1× in migrants. Mean heterozygosity in 47 of 57 regions were significantly different between residents and migrants. Heterozygosity was lower in residents than in migrants in 33 of 47 regions, whereas migrants showed lower heterozygosity in the other 14 regions (Table 9). A total of 178 protein-coding genes, involving various gene functions, were found on 48 of 57 associated regions (Table 11). It should be noted that a region with the greatest number of differentiated SNPs was found on chromosome 22 (442.3 kb, positions 10,900,494–11,342,799 bp; Table 9). Mean heterozygosity in this region was significantly lower in residents than in migrants (residents: $H_p = 0.23$, migrants: $H_p = 0.29$; Wilcoxon signed-ranks test: $Z = \text{Inf}$, $P < 0.0001$). The largest number of protein-coding genes were also contained in this region. These genes included annotated genes associated with lipid homeostasis, photosensory, and circadian rhythms (Table 11).

In the Nagara River sample, a total of 6,814,810 SNPs were retained after filtering, 1,444 SNPs with dAF > 0.7 were detected (0.02 %). In contrast to the Arida River sample, only one genomic region was identified, which included at least 10 SNPs with dAF > 0.7 in 100-kb sliding genomic windows on chromosome 19 (61.3 kb, positions 22,835,227–22,896,548 bp; Figure 14; Table 10). This genomic region was not found in the Arida River sample. Mean (± SD) depth of coverage for this associated region was 22.9 ± 8.4× in residents and 26.6 ± 10.8× in migrants. Mean heterozygosity in this

region was not significantly different between residents and migrants (residents: H_p = 0.21, migrants: H_p = 0.21; Wilcoxon signed-ranks test: Z = 0.37, P = 0.71). One protein-coding gene known to be associated with sensory neuron development was included in this region (Table 12).

In the Ohta River sample, a total of 5,851,523 SNPs were retained after filtering, 45,689 SNPs with dAF > 0.7 were detected (0.8 %). In addition, 559 SNPs were fixed (i.e., dAF = 1). Compared to the Arida and Nagara River samples, more SNPs with large allele frequency differences between residents and migrants were found across the genome (Figure 14). Considering the results from the genetic distance analysis (see above), it was difficult to separate the genetic differences between populations from those between phenotype, therefore, no further analyses were conducted for the Ohta River sample.

Discussion

Intraspecific life-history is important for population persistence and evolutionary adaptation (Schindler et al. 2010; Moore et al. 2014; Moran et al. 2016). Recent advances in molecular biology have allowed us to elucidate the genetic basis of life-history traits (Stapley et al. 2010; Ekblom & Galindo 2011). It enables us to develop molecular markers that are related to life-history traits, which assists in examining the response of marker genotypes to environments. This might facilitate our understanding of the contribution of genotype-environment interactions to maintaining life-history variation (Kruuk et al. 2008). In this study, I sequenced and analyzed genomes to identify genomic regions associated with partial migration of red-spotted masu salmon in three geographically distant river systems. I found genomic regions containing several highly differentiated

SNPs between residents and migrants in two of the three river systems. This study is an important step in understanding the interactive effects of genotype and the environment on maintaining partial migration. However, further investigation is required.

I found multiple genomic regions containing several highly differentiated SNPs between residents and migrants in the Arida River, while only one region was detected in those of the Nagara River, which was unique to the Nagara River sample. There are two possible explanations: The first explanation is that the differences in life stages of migrants may affect the results, i.e., smolts in the Arida River and returning individuals in the Nagara River. Hale et al. (2013) noted that it is possible that returning individuals may have undergone further selection for growth and/or survival in the sea. However, since returning individuals also go through smoltification, and if they have genomic regions strongly associated with migration propensity, common genomic regions should be detected in the samples from both the Arida and Nagara Rivers. Therefore, the differences in life stages may not be a fundamental issue. The second explanation is that different genomic regions contribute to maintaining partial migration for each population as a result of local adaptation. The residents in red-spotted masu salmon populations are derived from a migratory population by colonization upstream during the last glacial period (Yamamoto et al. 2020). Thus, genomic differentiation associated with partial migration among local populations might have occurred during or after colonization. In addition, high summer temperatures in the distribution ranges of red-spotted masu salmon may limit the migration range and period in the sea (Umino et al. 2001). Therefore, dispersal limitation may cause a reduction in gene flow, which facilitates genetic differentiation among populations. Therefore, to understand the process of maintaining partial migration, historical events must be considered. However, the results of genetic

structure analysis using F_{st} indicated a lack of clear correlation between genetic and geographic distance among populations. Alternative migratory phenotypes are strongly influenced by genetic and environmental factors (Olsson et al. 2006; Wysujack et al. 2008; Benjamin et al. 2013). Therefore, genomic regions detected in this study may be the regions with minor effects on migratory traits, and the local environment may affect migratory decision-making of individuals, especially in the Nagara River populations.

In the Arida River sample, multiple protein coding genes were found that involve various functions associated with genomic regions. Notably, a region spanning approximately 440 kb on chromosome 22 included several genes involved in lipid homeostasis, photosensory, and circadian rhythms (Nuckels et al. 2009; Yang et al. 2010; Tovin et al. 2012; Xie et al. 2021). Lipid reserves are strongly associated with growth and maturation (Thorpe et al. 1998), and photosensory and circadian rhythms are important for the seasonal timing of smoltification and maturation (Migaud et al. 2010; Björnsson et al. 2011). Genes with similar functions were also included in *Omy05* of *O. mykiss*, and it is considered that their functions are related to migration (Pearse et al. 2019). In addition to the abovementioned genes, *glucuronosyltransferase 2A2*, which was reported to be upregulated in the gill of smolts in Atlantic salmon (*Salmo salar*) was also included in this 440 kb region detected in this study (Johansson et al. 2016). Therefore, it is likely that this region on chromosome 22 is particularly associated with partial migration. Further study is required to examine whether there are any mutations that alter the amino acid sequence or compare gene expression profiles between residents and migrants using RNA-seq data (Manel et al. 2016). However, other genomic regions were also detected as regions that include several SNPs with different allele frequencies between residents and migrants. For example, in *O. mykiss*, strong associations between specific genomic

regions (e.g., *Omy05*) and partial migration were shown in some populations (Pearse et al. 2019), while some studies reported that multiple loci across genomes are associated with migratory behaviour in other populations (Hecht et al. 2013). Migration is a complex trait that is related to a few other traits, not only behavioral, but also morphological and physiological traits. Thus, it is quite possible that multiple genomic regions are associated with partial migration in red-spotted masu salmon. Contrary to the Arida River sample, only one genomic region was detected in the Nagara River sample, and one protein coding gene, *potassium voltage-gated channel, subfamily G, member 4a* (*kcng4a*), was included in this region. The relationship between *kcng4a* and migratory behaviour remains unknown, but *kcng4a* has been suggested to play an important role in the regulation of ventricular development in the brain in zebrafish (Shen et al. 2016).

When interpreting the results, there are two points that should be noted. First, the sample size that used for Pool-seq was small. Because of the limited samples of satsukimasu (i.e., migrant), DNA samples of 10 individuals for each phenotype for each river system were pooled. However, the smaller sample size may have more variation in individual contribution, and lead to an overestimation of allele frequency differences between residents and migrants, resulting in a false positive (Fuentes-Pardo & Ruzzante 2017). I could not separate the true positives from false positives. Therefore, future studies must increase the sample size to enhance the reliability of analysis. Second, the quality of reference genome used for alignment. Because there are no available genomes of red-spotted masu salmon in a public database, I used a genome assembled from short reads. However, perhaps due to the incompleteness, mapping rate in this study (mean 39.7 %) was relatively low compared with a previous study on salmonids (Micheletti et al. 2018: mean 76 %), which means that genomic regions associated with migratory traits

may have been overlooked. However, by removing reads mapped to nonspecific genomic regions and SNPs with a low depth of coverage, the accuracy of data after alignment and filtering were improved. Therefore, although the amount of data was limited, the reliability of analyzed data is the same as those of previous studies.

To confirm that genomic regions detected in this study are truly associated with partial migration, improving the reliability of data by increasing sample size and obtaining a high-quality genome assembly of red-spotted masu salmon as a reference genome is required. These processes are needed to examine whether these genomic regions have a phenotypic effect by comparing the amino acid sequence or gene expression pattern between two migratory phenotypes (Manel et al. 2016). If these tasks are accomplished, it would allow us to better study the effects of genotype-environment interactions on life-history variation. For example, some previous studies have shown the association between spatial distribution of life-history-linked genotypes and landscape features (Kannry et al. 2020; Kelson et al. 2020), genetic variation in key life-history genomic regions and foraging strategies (Aykanat et al. 2020), and temporal change of allele frequencies and human activities (Czorlich et al. 2022) using GT-seq, SNP array, and Rapture sequencing. However, these studies only investigated the correlation between life-history variation and factors that may influence it. They could not evaluate the relative contributions of genetic and environmental factors to life-history traits. In contrast, combining molecular marker genotyping with direct manipulation of the environment, may distinguish between the genetic and environmental influence on life-history traits, and enable us to understand the cause-and-effect relationships among genetic and environmental factors, and life-history variation.

Chapter 5. General Discussion

To date, the ecological and evolutionary importance of intraspecific life-history variation have been emphasized (Schindler et al. 2010; Moore et al. 2014; Moran et al. 2016). However, the processes that generate and maintain intraspecific life-history variation, especially within a population, are not fully understood. Differences in life-history among individuals can have significant ecological and evolutionary effects (Clutton-Brock & Sheldon 2010; Bolnick et al. 2011). Individual life-history is described by the combination of growth, maturation, and survival; therefore, it is required to assess life-history patterns and investigate the relationships between life-history patterns and factors that are associated with the expression of life-history traits at the individual level, which can help us understand the processes that maintain life-history variation within a population.

In this dissertation, I focused on red-spotted masu salmon (*Oncorhynchus masou ishikawae*) and aimed to elucidate the processes of generating and maintaining life-history variation within a population. In Chapter 2, I examined the applicability of two male-specific molecular markers for molecular sexing in two wild populations and showed that the efficacy of these markers for finding females in populations of interest. Sex-specific life-history is commonly observed in many species, including red-spotted masu salmon. However, in species such as fish, it is difficult to identify phenotypic sexes by external morphological characteristics, which limits our ability to evaluate an individuals' life-history throughout its lifetime. The method of examination used in this study allowed me to track a series of sex-specific life-history by nonlethal method and assign a sex to samples without physical sex information. In Chapter 3, I conducted a

large-scale field experiment and demonstrated that resource subsidies occurring early in consumers' growing season at terrestrial-aquatic boundaries contribute to maintaining life-history variation described by the combination of growth trajectory and maturation status at age-1 within a single population. Previous studies have shown the population level responses of consumers' life-history traits to seasonal subsidies. In contrast, by conducting continuous individual-based monitoring and using a statistical method that can evaluate differences among individuals not as random effects but as repeated measures of same individuals, I could quantify life-history patterns, and demonstrated that seasonal ecosystem linkages is key maintaining life-history variation within a single population. Finally, in Chapter 4, I explored the genomic regions associated with partial migration in three wild river systems by whole-genome sequencing and found genomic regions containing several highly differentiated SNPs between residents and migrants in at least two of the three river systems. Because it is difficult to distinguish between phenotypic plasticity and genetically based adaptation to the environment in life-history traits of wild non-model organisms, I provided future opportunities to develop markers by targeting genomic regions associated with migratory traits, and directly test whether an individual with a given genotype will express a phenotype under a given environmental condition, that is, I would investigate the genotype-environment interactions for partial migration at an individual level.

In this dissertation, using combination of a large-scale field manipulation experiment, tracking individuals by a mark-recapture survey, and a statistical method for quantifying individual variation, I revealed the part of the processes that generate and maintain life-history variation within a population. Meanwhile, for a better understanding of the processes generating and maintaining intraspecific life-history variation, two

questions remain: (i) What processes, including partial migration, generate life-history variation? and (ii) How are different life-histories maintained within a single population across generations? For the former question, I demonstrated that seasonal ecosystem linkages via resource subsidies can be a key ecosystem property to maintain life-history variation of residents (Chapter 3). Meanwhile, it is becoming apparent that these seasonal resource subsidies also contribute to maintaining migratory polymorphism by enhancing the seasonal growth of red-spotted masu salmon (Tanaka et al. 2021; Tanaka et al. under review). Therefore, if I can identify the genomic regions strongly related to migratory behaviour (discussed in Chapter 4), which will help to develop molecular markers linked to those regions. I can examine whether an individual with a given marker genotype will remain in the river as a resident or decide to migrate in response to seasonal resource subsidies. Integration of field manipulation experiments and a molecular approach provide a novel method to study the effects of genotype-environment interactions on generating and maintaining life-history variation in a forest-stream ecosystem. For the latter question, different life-histories can theoretically coexist within a population through frequency- or status-dependent selection (Gross 1996). By performing further long-term monitoring of a population and studying how the frequency of each life-history pattern varies across generations, we can elucidate which processes maintain life-history variation. Once both questions are answered, we should have a better understanding of the processes that generate and maintain life-history variation in the wild from the genotype to individual level. This will also elucidate the consequences for ecological dynamics, such as population dynamics, community structure, and ecosystem functions.

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Tables

Table 1. Individual data of 71 red-spotted masu salmon samples examined in this study. The positive/negative amplifications of the male-specific markers (*OtY2* and *sdY*) are listed; "1" indicates that positive amplification was observed for the male-specific marker, while "0" indicates no amplification. Ages were judged from scales. Hyphens indicate that no data were available for the columns.

Sample ID	Population	Captured date	Fork length (mm)	Weight (g)	Age (yr)	*OtY2*	*sdY*
Male 1	Arida	2016/7/13	155	-	-	1	1
Male 2	Arida	2016/7/13	160	-	-	1	1
Male 3	Arida	2016/7/13	158	-	-	1	1
Male 4	Arida	2016/7/13	135	-	-	1	1
Male 5	Arida	2016/7/13	153	-	-	1	1
Male 6	Arida	2016/7/13	145	-	-	1	1
Male 7	Arida	2016/7/13	142	-	-	1	1
Male 8	Arida	2016/7/13	147	-	-	1	1
Male 9	Arida	2016/7/13	158	-	-	1	1
Male 10	Arida	2016/8/25	160	52	-	1	1
Male 11	Arida	2016/8/25	165	63	-	1	1
Male 12	Arida	2016/8/25	130	27	-	1	1
Male 13	Arida	2016/9/28	142	37.2	-	1	1
Male 14	Arida	2016/9/28	225	138.4	-	1	1
Male 15	Arida	2016/10/13	226	137.6	-	1	1
Male 16	Arida	2016/10/13	123	21.9	-	1	1
Male 17	Arida	2016/10/13	114	20.2	-	1	1
Male 18	Arida	2016/10/13	144	37.8	-	1	1
Male 19	Arida	2016/10/13	169	56.1	-	1	1
Male 20	Arida	2016/10/13	178	60.7	-	1	1
Male 21	Arida	2016/10/13	146	34.9	-	1	1
Female 1	Arida	2016/7/13	175	-	-	1	1
Female 2	Arida	2016/7/13	135	-	-	1	1
Female 3	Arida	2016/7/13	145	-	-	1	1
Female 4	Arida	2016/7/13	130	-	-	0	1
Female 5	Arida	2016/7/13	135	-	-	0	1
Female 6	Arida	2016/7/13	155	-	-	0	1
Female 7	Arida	2016/7/13	140	-	-	0	1
Female 8	Arida	2016/7/13	160	-	-	0	0

Female 9	Arida	2016/7/13	140	-	-	1	1
Female 10	Arida	2016/7/13	129	-	-	0	1
Female 11	Arida	2016/7/13	140	-	-	0	0
Female 12	Arida	2016/8/25	125	23	-	0	1
Female 13	Arida	2016/8/25	158	42	-	0	1
Female 14	Arida	2016/8/25	162	51	-	0	1
Female 15	Arida	2016/8/25	130	29	-	0	1
Female 16	Arida	2016/8/25	140	41	-	0	0
Female 17	Arida	2016/8/25	115	21	-	0	1
Female 18	Arida	2016/8/25	155	55	-	0	1
Female 19	Arida	2016/9/28	179	65.8	-	0	0
Female 20	Arida	2016/9/28	162	60.9	-	0	1
Female 21	Arida	2016/9/28	143	26.4	-	0	0
Female 22	Arida	2016/9/28	165	51.2	-	0	0
Female 23	Arida	2016/9/28	182	70.4	-	0	0
Female 24	Arida	2016/9/28	176	77.4	-	0	0
Female 25	Arida	2016/10/13	149	41.4	-	1	1
Female 26	Arida	2016/10/13	145	30.4	-	1	1
Female 27	Arida	2016/10/13	124	18.9	-	1	1
Female 28	Arida	2016/10/13	174	63.7	-	1	1
Female 29	Arida	2016/10/13	153	42.1	-	1	1
Female 30	Arida	2016/10/13	171	56.7	-	0	0
Female 31	Arida	2016/10/13	167	56.8	-	1	1
Male 22	Nagara	2019/10/30	164	49.5	-	1	1
Male 23	Nagara	2019/10/30	225	96.6	-	1	1
Male 24	Nagara	2019/10/30	232	117	-	1	1
Male 25	Nagara	2019/10/30	172	51.5	-	1	1
Male 26	Nagara	2019/10/30	183	58.4	-	1	1
Male 27	Nagara	2019/10/30	166	49.5	-	1	1
Male 28	Nagara	2019/10/30	170	52.3	-	1	1
Male 29	Nagara	2019/10/30	103	10.7	-	1	1
Male 30	Nagara	2019/10/30	306	221.5	-	1	1
Male 31	Nagara	2019/10/30	127	23	-	1	1
Female 32	Nagara	2019/10/30	158	36.4	1+	0	0
Female 33	Nagara	2019/10/30	216	91.6	1+	0	1
Female 34	Nagara	2019/10/30	153	43.5	1+	0	1
Female 35	Nagara	2019/10/30	235	105.1	1+	0	0
Female 36	Nagara	2019/10/30	179	48.7	1+	0	0
Female 37	Nagara	2019/10/30	138	21.2	2+	0	1
Female 38	Nagara	2019/10/30	189	52.2	2+	1	1
Female 39	Nagara	2019/10/30	134	24	1+	0	1
Female 40	Nagara	2019/10/30	285	190	-	0	1

Table 2. Phenotypic sexes, positive/negative of the male-specific markers, and concordance rates for the expected sex of red-spotted masu salmon populations.

Population	Phenotype	n	Male-specific markers					
			OtY2			*sdY*		
			Positive	Negative	Concordance (%)	Positive	Negative	Concordance (%)
Arida	Male	21	21	0	100	21	0	100
	Female	31	10	21	68	22	9	29
Nagara	Male	10	10	0	100	10	0	100
	Female	9	1	8	89	6	3	33

Table 3. Results of the GLMM ($\chi^2 = 153.81$, df = 4, $P < 0.001$, pseudo $R^2 = -0.28$) model for the effect of subsidy, its timing and age on the specific growth rate of individuals. Specific growth rate was calculated using 269 individuals' data captured for each pulsed period (i.e., June–August, and August–October) following the equation in Marczak & Richardson (2008). In the model, I tested whether the specific growth rate of individuals was related to with or without subsidies, period, their interaction, and age. I included the age into the GLMM model because the specific growth rate was expected to be intrinsically lower in age-1 fish than in age-0 fish. Individuals and stream sections were incorporated as random effects.

Variables	Estimate	SE	*t*	*P*
(Intercept)	0.13	0.01	-	-
subsidy (subsidized)	0.12	0.02	8.01	< 0.001
period (late)	−0.01	0.01	−1.24	0.24
age (age-1)	−0.08	0.01	−8.40	< 0.001
subsidy (subsidized) × period (late)	−0.01	0.02	−0.29	0.77

Table 4. Result of the model comparisons of the mixture regression models based on Akaike's Information Criterion (AIC) and Bayesian information criterion (BIC). Bold font indicates the best model in terms of both AIC and BIC (k = 3).

Number of growth clusters (k)	AIC	BIC
1	−232.51	−208.59
2	−366.09	−318.24
3	**−399.20**	**−327.42**
4	−390.69	−294.98
5	−388.65	−269.01
6	−393.49	−273.85

Table 5. Estimated parameters for the best model (k = 3) of the finite mixture regression model. L_{min} and L_{max} are respectively fork length of red-spotted masu salmon at the time of recruitment and maximum fork length of each growth cluster; The a_1 and θ are coefficients for describing seasonal growth trajectories of each growth cluster.

Parameter	Growth cluster		
	Fast	Medium	Slow
L_{min}	39.13	37.76	33.58
L_{max}	394.57	756.84	273.37
a_1	0.00055	0.00019	0.00052
θ	−24.31	−15.67	−9.44

Table 6. Results of the GLMM (χ^2 = 47.34, df = 2, P < 0.001, pseudo R^2 = 0.33) model for the effect of growth clusters on the probability of maturation at age-1. In the model, the stream sections were incorporated as a random effect. No further clustering among growth clusters (i.e., medium + slow vs. fast; fast + slow vs. medium; fast + slow vs. slow) significantly improved the model fits (log-likelihood ratio tests: P > 0.05).

Variables	Estimate	SE	z	P
(Intercept)	1.06	0.37	-	-
growth cluster (medium)	−2.65	0.53	−4.96	< 0.001
growth cluster (slow)	−4.40	1.08	−4.07	< 0.001

Table 7. Results of the GLMM (χ^2 = 28.30, df = 3, P < 0.001, pseudo R^2 = 0.32) model for the effect of growth clusters and sexes on the probability of maturation at age-1. In the model, stream sections were incorporated as random effects.

Variables	Estimate	SE	z	P
(Intercept)	2.46	0.78	-	-
growth cluster (medium)	−2.67	0.74	−3.62	< 0.001
growth cluster (slow)	−4.30	1.24	−3.48	< 0.001
sex (male)	−0.56	0.70	−0.81	0.42

Table 8. Information and sequencing statistics for each pooled library. Aligned reads is the number of reads after aligned to the reference genome and filtered.

Library	River system	Phenotype	Raw reads	Aligned reads	Filtered depth
1	Arida	resident	438,819,300	174,267,101	20
2	Arida	migrant	525,437,978	206,816,710	22
3	Nagara	resident	427,702,490	170,219,668	19
4	Nagara	migrant	531,682,376	209,728,817	22
5	Ohta	resident	399,130,162	159,301,044	19
6	Ohta	migrant	456,176,176	183,140,717	20

Table 9. Genomic regions containing several highly differentiated SNPs between residents and migrants in the Arida River. Chromosome number is according to the reference genome of *O. tshawytscha* (NCBI accession numbers: GCA_002872995.1). Candidate genes are indicated by NCBI gene symbol.

Chromosome	Position	Region length (kbp)	Mean H_p in residents	Mean H_p in migrants	Wilcoxon *p*-value	Candidate genes
2	2,350,032－2,488,070	138.0	0.23	0.19	< 0.0001	
2	36,174,021－36,242,243	68.2	0.32	0.21	< 0.0001	LOC112262324, LOC112262336, LOC112262950, LOC112262347, pank4
3	47,156,099－47,299,476	143.3	0.25	0.23	< 0.05	LOC112235960, LOC112235968, LOC112236024, LOC112236043, LOC112236048
3	47,848,323－47,945,339	97.0	0.24	0.23	0.87	LOC112236198, LOC112224147, LOC112236218, LOC112236229
3	48,114,264－48,178,209	63.9	0.24	0.24	0.98	LOC112245527, LOC112236273, LOC112236284
3	48,563,762－48,656,032	92.3	0.26	0.18	< 0.0001	myo3b, sp5a, gad1a, LOC112236484, tlk1a
3	52,314,722－52,377,838	63.1	0.25	0.31	< 0.0001	LOC112237605, lypd6, mmadhcb
3	54,287,565－54,381,260	93.7	0.27	0.21	< 0.01	LOC112238049
3	55,055,730－55,086,921	31.1	0.28	0.24	< 0.05	LOC112238342, LOC112238348, LOC112238367, LOC112238358, LOC112245692, LOC112245708, LOC112245719
3	55,163,080－55,234,098	71.0	0.26	0.24	0.26	LOC112245768, LOC112245781, LOC112245788, LOC112224251
3	63,420,522－63,472,896	52.4	0.25	0.26	0.60	LOC112240006, LOC112240017, LOC112240051
7	18,717,540－18,774,334	56.8	0.28	0.21	< 0.0001	LOC112255187
10	25,900,817－25,996,219	95.4	0.22	0.25	< 0.0001	agxtb, LOC112261071,

						LOC112260180, LOC112259565
10	37,409,784－37,494,617	84.8	0.22	0.28	< 0.0001	LOC112260420, LOC112260421, LOC112260422
10	42,144,723－42,240,038	95.3	0.23	0.21	0.16	LOC112260496
13	2,258,610－2,344,429	85.8	0.20	0.25	< 0.0001	
13	2,671,145－3,037,073	365.9	0.21	0.23	< 0.0001	LOC112265790
13	10,606,378－10,744,308	137.9	0.22	0.20	< 0.05	pag1, LOC112264446, LOC112265874
13	11,260,503－11,485,420	224.9	0.23	0.27	< 0.001	LOC112264452, aste1b, si:ch73-345f18.3, klhl7, LOC112264458, tomm7, LOC112264462, LOC112265821, LOC112264463, LOC112264464, LOC112265822
13	11,855,587－12,046,626	191.0	0.24	0.26	< 0.05	LOC112264472, si:ch211-199g17.2, LOC112264474, LOC112264477, fkbp9, crtap
13	12,119,074－12,174,263	55.1	0.21	0.28	< 0.0001	clasp2
13	17,706,561－17,797,498	90.9	0.22	0.28	< 0.001	
13	18,573,082－18,645,368	72.2	0.19	0.31	< 0.0001	LOC112265997
13	21,652,562－21,893,503	240.9	0.24	0.28	< 0.0001	LOC112264671, LOC112264672, LOC112264673, LOC112264674, LOC112266006, LOC112265906
13	30,117,952－30,251,225	133.3	0.25	0.28	< 0.01	LOC112264898, LOC112264899, LOC112264900, LOC112264902, LOC112264904, LOC112264903
13	37,156,627－37,247,858	91.2	0.26	0.26	0.32	LOC112265042, LOC112265044, mmp28
13	37,303,570－37,384,791	81.2	0.25	0.23	< 0.05	LOC112266050
13	38,254,967－38,389,405	134.4	0.27	0.26	0.09	scn3b, LOC112265064, LOC112265940
13	59,678,922－59,690,446	115.2	0.07	0.37	< 0.0001	igsf9ba

14	7,368,156－7,493,090	124.9	0.23	0.30	< 0.0001	LOC112266477, LOC112266478, LOC112266479, LOC112266480
14	14,566,657－14,831,795	265.1	0.25	0.24	0.81	
14	33,102,563－33,192,470	89.9	0.23	0.30	< 0.0001	LOC112266999, LOC112266998, LOC112267000, LOC112267001, LOC112267415, LOC112267002
14	33,351,315－33,416,283	65.0	0.10	0.31	< 0.0001	LOC112267006
14	34,958,001－35,060,393	102.3	0.25	0.30	< 0.0001	aipl1, abhd15a
14	35,675,126－35,713,073	37.6	0.16	0.30	< 0.0001	
14	47,255,601－47,366,174	110.5	0.17	0.26	< 0.0001	LOC112267614, LOC112267615, LOC112267264, LOC112267265
14	48,038,760－48,117,576	78.8	0.12	0.29	< 0.0001	
14	48,281,654－48,325,076	43.4	0.10	0.31	< 0.0001	LOC112267459
16	32,321,619－32,408,406	86.8	0.11	0.32	< 0.0001	LOC112215712
16	33,967,307－34,013,091	45.7	0.23	0.25	0.55	LOC112215733
16	46,261,005－46,397,900	136.9	0.13	0.31	< 0.0001	LOC112215984, hck, LOC112215986, gata5
22	10,900,494－11,342,799	442.3	0.23	0.29	< 0.0001	LOC112221762, LOC112221764, LOC112221765, LOC112221766, ccn5, LOC112221770, LOC112221769, LOC112221771, LOC112221772, LOC112221774, LOC112221775, LOC112221404, LOC112221776, LOC112221777, camk1gb, mical1, src, LOC112221782, LOC112221783
23	18,533,385－18,559,530	26.1	0.29	0.17	< 0.001	LOC112223129
23	19,779,873－19,798,508	18.6	0.30	0.23	< 0.001	LOC112222640, LOC112223143
24	20,395,740－20,437,612	41.8	0.22	0.27	< 0.01	gucy2g
24	21,690,374－21,744,021	53.6	0.21	0.29	< 0.001	

24	23,403,884－23,473,471	69.5	0.25	0.29	< 0.01	sult1st6, LOC112223860, pelo, LOC112223323, cmn
24	23,935,997－23,972,712	36.7	0.34	0.22	< 0.0001	LOC112223876, LOC112223273
scaffold 020128963.1	15,079－20,6840	191.7	0.22	0.24	< 0.0001	LOC112235980, LOC112235981, LOC112235982, LOC112235979, LOC112235983, LOC112235984, LOC112235985, LOC112235987, LOC112235988
scaffold 020129089.1	16,367－146,580	130.2	0.18	0.24	< 0.0001	
scaffold 020130296.1	3,660－6,7051	63.3	0.21	0.23	0.24	LOC112240005, LOC112240000, wdyhv1
scaffold 020142496.1	459,570－478,195	18.6	0.27	0.17	< 0.001	LOC112244702, ccdc153
scaffold 020142496.1	557,241－948,683	391.4	0.25	0.27	< 0.0001	LOC112244677, LOC112244714, LOC112244704, c1qtnf5, LOC112244682, LOC112244676, pdzd3a, LOC112244689, LOC112244707, LOC112244681, LOC112244684, LOC112244719, snrpa, lg13h19orf54, LOC112244705
scaffold 020142496.1	1,361,661－1,435,796	74.1	0.23	0.25	< 0.05	clptm1
scaffold 020142520.1	2,061,396－2,163,884	102.5	0.26	0.23	< 0.01	LOC112245979
scaffold 020142520.1	2,318,175－2,444,981	126.8	0.20	0.28	< 0.0001	LOC112246006
scaffold 020142520.1	2,704,400－2,857,625	153.2	0.18	0.24	< 0.0001	

Table 10. Genomic region containing several highly differentiated SNPs between residents and migrants in the Nagara River. Chromosome number is according to the reference genome of *O. tshawytscha* (NCBI accession numbers: GCA_002872995.1). Candidate genes are indicated by NCBI gene symbol.

Chromosome	Position	Region length (kbp)	Mean H_p in residents	Mean H_p in migrants	Wilcoxon *p*-value	Candidate genes
19	22,835,227－22,896,548	61.3	0.21	0.21	0.71	kcng4a

Table 11. Protein-coding genes contained in the genomic regions that were identified as associated regions with partial migration in the Arida River.

Gene symbol	Gene description	Function	Reference
LOC112262324	transcription factor HES-5		
LOC112262336	transcription factor HES-5-like		
LOC112262950	transcription factor HES-4-A-like		
LOC112262347	transcription factor HES-5-like		
pank4	pantothenate kinase 4 (inactive)	CoA biosynthesis	Li et al. 2005
LOC112235960	CD302 antigen	cardiac differentiation	Yasuda et al. 2011
LOC112235968	RNA-binding motif, single-stranded-interacting protein 1	DNA replication, transcription, apoptosis, cell cycle progression	Yin et al. 2012
LOC112236024	TRAF family member-associated NF-kappa-B activator		
LOC112236043	T-box brain protein 1-like	cortical development	Dwyer et al. 2001
LOC112236048	sodium-driven chloride bicarbonate exchanger		
LOC112236198	sodium channel protein type 2 subunit alpha		
LOC112224147	tetratricopeptide repeat protein 21B-like		
LOC112236218	polypeptide N-acetylgalactosaminyltransferase 3		
LOC112236229	cysteine/serine-rich nuclear protein 3	apoptosis	Vincent et al. 2015
LOC112245527	beta-1,3-galactosyltransferase 1-like		
LOC112236273	STE20/SPS1-related proline-alanine-rich protein kinase		
LOC112236284	ceramide synthase 6-like		
myo3b	myosin IIIB		
sp5a	Sp5 transcription factor a	neural development	Tan et al. 2022
gad1a	glutamate decarboxylase 1a		
LOC112236484	Golgi reassembly-stacking protein 2		
tlk1a	tousled-like kinase 1a		
LOC112237605	ly6/PLAUR domain-containing protein 6B		
lypd6	LY6/PLAUR domain containing 6	embryogenesis	Özhan et al. 2013
mmadhcb	metabolism of cobalamin associated Db		
LOC112238049	potassium voltage-gated channel subfamily H member 8		
LOC112238342	gamma-crystallin M2-like		
LOC112238348	gamma-crystallin S-1-like		
LOC112238367	gamma-crystallin M2-like		

LOC112238358	gamma-crystallin M2-like		
LOC112245692	gamma-crystallin M2-like		
LOC112245708	gamma-crystallin M1-like		
LOC112245719	gamma-crystallin S-1-like		
LOC112245768	gamma-crystallin M2-like		
LOC112245781	gamma-crystallin S-1-like		
LOC112245788	gamma-crystallin M2-like		
LOC112224251	diacylglycerol kinase gamma	regulation of cell cycle	Matsubara et al. 2006
LOC112240006	BTB/POZ domain-containing protein KCTD4-like		
LOC112240017	E3 SUMO-protein ligase RanBP2	visual signal transduction	Campello et al. 2013
LOC112240051	LIM and senescent cell antigen-like-containing domain protein 1		
LOC112255187	low-density lipoprotein receptor-related protein 1B		
agxtb	alanine--glyoxylate and serine--pyruvate aminotransferase b		
LOC112261071	uncharacterized LOC112261071		
LOC112260180	kinesin-like protein KIF1A	axonal transport	Hotchkiss et al. 2016
LOC112259565	solute carrier organic anion transporter family member 2A1-like		
LOC112260420	ATP-dependent 6-phosphofructokinase, platelet type		
LOC112260421	double-stranded RNA-specific editase B2		
LOC112260422	WD repeat-containing protein 37		
LOC112260496	disks large-associated protein 1		
LOC112265790	extensin-like		
pag1	phosphoprotein membrane anchor with glycosphingolipid microdomains 1		
LOC112264446	fatty acid-binding protein, heart		
LOC112265874	protein FAM8A1		
LOC112264452	SWI/SNF complex subunit SMARCC1		
aste1b	asteroid homolog 1b		
si:ch73-345f18.3	uncharacterized si:ch73-345f18.3		
klhl7	kelch-like family member 7		
LOC112264458	hyccin	synthesis of phosphatidylinositol 4-phosphate	Baskin et al. 2016
tomm7	translocase of outer mitochondrial membrane 7 homolog (yeast)		
LOC112264462	interleukin-6-like	immune response	Savan et al. 2006
LOC112265821	rap guanine nucleotide exchange factor 5	embryonic development	Griffin et al. 2018

LOC112264463	cell division cycle-associated 7-like protein		
LOC112264464	sp4 transcription factor	development of hippocampus	Zhou et al. 2009
LOC112265822	integrin beta-8	embryo implantation	Kumar et al. 2015
LOC112264472	protein FAM83H	amelogenesis	Kuga et al. 2013
si:ch211-199g17.2	uncharacterized si:ch211-199g17.2		
LOC112264474	uncharacterized LOC112264474		
LOC112264477	adhesive plaque matrix protein		
fkbp9	FKBP prolyl isomerase 9		
crtap	cartilage associated protein	bone development	Marini et al. 2007
clasp2	cytoplasmic linker associated protein 2		
LOC112265997	phosphatase and actin regulator 4A		
LOC112264671	rho guanine nucleotide exchange factor 2-like		
LOC112264672	death-associated protein kinase 2	poptosis, autophagy, inflammation	Geering 2015
LOC112264673	SH2 domain-containing protein 2A		
LOC112264674	translocon-associated protein subunit beta	endoplasmic reticulum transport	Ueda et al. 2022
LOC112266006	immunoglobulin superfamily DCC subclass member 3-like		
LOC112265906	gonadotropin-releasing hormone II receptor-like		
LOC112264898	E3 ubiquitin-protein ligase RNF5		
LOC112264899	choline transporter-like protein 4	acetylcholine synthesis	Song et al. 2013
LOC112264900	histone-lysine N-methyltransferase EHMT2	transcriptional regulation	Zhang et al. 2018
LOC112264902	zinc finger and BTB domain-containing protein 12	coagulation, inflammation	Noro et al. 2019
LOC112264904	negative elongation factor E		
LOC112264903	helicase SKI2W		
LOC112265042	NADPH--cytochrome P450 reductase		
LOC112265044	RNA-binding protein FUS	RNA processing	Baade et al. 2021
mmp28	matrix metallopeptidase 28		
LOC112266050	dedicator of cytokinesis protein 10-like		
scn3b	sodium channel, voltage-gated, type III, beta		
LOC112265064	zonadhesin	zona binding	Tardif et al. 2011
LOC112265940	uncharacterized LOC112265940		
igsf9ba	immunoglobulin superfamily, member 9Ba		
LOC112266477	gamma-aminobutyric acid receptor subunit beta-3		
LOC112266478	gamma-aminobutyric acid receptor subunit alpha-5		

LOC112266479	gamma-aminobutyric acid receptor subunit gamma-3-like		
LOC112266480	secretin receptor-like		
LOC112266999	calpain-5-like		
LOC112266998	olfactory marker protein-like		
LOC112267000	glycerophosphodiester phosphodiesterase domain-containing protein 5		
LOC112267001	serine/threonine-protein kinase PAK 1-like		
LOC112267415	aquaporin-11-like		
LOC112267002	methylosome subunit pICln		
LOC112267006	short transient receptor potential channel 4	signaling pathway	Fowler et al. 2007
aipl1	aryl hydrocarbon receptor interacting protein-like 1	visual perception	Gopalakrishna et al. 2016
abhd15a	abhydrolase domain containing 15a		
LOC112267614	inward rectifier potassium channel 13-like		
LOC112267615	EF-hand domain-containing protein D2	immune response	Wang et al. 2022
LOC112267264	ephexin-1		
LOC112267265	lysophosphatidic acid receptor 6	cellular morphology, hair growth	Zheng et al. 2020
LOC112267459	roundabout homolog 1-like		
LOC112215712	metabotropic glutamate receptor 7		
LOC112215733	proline-rich transmembrane protein 3		
LOC112215984	methylenetetrahydrofolate reductase	folic acid metabolism	Rosenblatt 2001
hck	HCK proto-oncogene, Src family tyrosine kinase		
LOC112215986	DNA endonuclease RBBP8		
gata5	GATA binding protein 5	cell metabolism, coronary artery development, vascular inflammation, oxidative stress, endothelial function	Song et al. 2020
LOC112221762	hepatocyte nuclear factor 4-alpha	lipid homeostasis	Xie et al. 2021
LOC112221764	alpha-tocopherol transfer protein-like		
LOC112221765	cAMP-dependent protein kinase inhibitor gamma		
LOC112221766	adenosine deaminase	inflammation	Skugor et al. 2008
ccn5	cellular communication network factor 5		
LOC112221770	P2Y purinoceptor 1	tissue repair, immune response	Caballero-Solares et al. 2022
LOC112221769	UDP-glucuronosyltransferase 2A2		
LOC112221771	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase	regulation of glycolysis/gluconeogenesis	Panserat et al. 2001

LOC112221772	inter-alpha-trypsin inhibitor heavy chain H6		
LOC112221774	rab GDP dissociation inhibitor alpha		
LOC112221775	V-type proton ATPase subunit S1	regulating eye growth, neuronal survival, photoreceptor morphogenesis	Nuckels et al. 2009
LOC112221404	protein pitchfork	cilia disassembly	Kinzel et al. 2010
LOC112221776	transcription initiation factor TFIID subunit 8		
LOC112221777	G0/G1 switch protein 2	adipose lipolysis	Yang et al. 2010
camk1gb	calcium/calmodulin-dependent protein kinase Igb	circadian rhythms	Tovin et al. 2012
mical1	microtubule associated monooxygenase, calponin and LIM domain containing 1	depolymerization of actin filaments	Rajan et al. 2017
src	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog		
LOC112221782	potassium voltage-gated channel subfamily G member 1		
LOC112221783	nuclear factor of activated T-cells, cytoplasmic 2	Immune response	Ji et al. 2020
LOC112223129	zinc finger protein 236		
LOC112222640	transmembrane protein 74-like		
LOC112223143	thyrotropin-releasing hormone receptor-like		
gucy2g	guanylate cyclase 2g		
sult1st6	sulfotransferase family 1, cytosolic sulfotransferase 6		
LOC112223860	WD repeat-containing protein 38		
pelo	pelota mRNA surveillance and ribosome rescue factor	regulation of mRNA processing	Machour et al. 2021
LOC112223323	progestin and adipoQ receptor family member 4-like		
cmn	calymmin		
LOC112223876	DDB1- and CUL4-associated factor 7	protein–protein interactions, cellular signaling	Misir et al. 2021
LOC112223273	potassium voltage-gated channel subfamily H member 6		
LOC112235980	chromatin modification-related protein MEAF6		
LOC112235981	four and a half LIM domains protein 3		
LOC112235982	c-Myc-binding protein		
LOC112235979	gap junction alpha-9 protein-like		
LOC112235983	akirin-1A		
LOC112235984	serine/arginine-rich splicing factor 10	mRNA splicing	Li et al. 2017
LOC112235985	B-cell receptor CD22	Immune response	Sgroi et al. 1995

LOC112235987	lysine-specific histone demethylase 1B	gene silencing, transcription factor activity regulation, cell cycle regulation	Cai et al. 2020
LOC112235988	nucleolin 2		
LOC112240005	succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial-like		
LOC112240000	proteasome subunit alpha type-2	metabolic process of carbohydrates, lipids and proteins	Guzmán-Flores et al. 2016
wdyhv1	WDYHV motif containing 1		
LOC112244702	glutamate receptor ionotropic, kainate 4		
ccdc153	coiled-coil domain containing 153		
LOC112244677	Cbl proto-oncogene, E3 ubiquitin protein ligase		
LOC112244714	cell surface glycoprotein MUC18	signal transduction	Cavallaro et al. 2011
LOC112244704	E3 ubiquitin-protein ligase RNF26-like		
c1qtnf5	C1q and TNF related 5		
LOC112244682	histone-lysine N-methyltransferase 2A-like		
LOC112244676	ubiquitin conjugation factor E4 A-like		
pdzd3a	PDZ domain containing 3a		
LOC112244689	ATP-binding cassette sub-family G member 4		
LOC112244707	porphobilinogen deaminase-like		
LOC112244681	coatomer subunit delta		
LOC112244684	latexin		
LOC112244719	inositol-trisphosphate 3-kinase C	immune response	Li et al. 2013
snrpa	small nuclear ribonucleoprotein polypeptide A		
lg13h19orf54	linkage group 13 C19orf54 homolog		
LOC112244705	protein jagged-1b		
clptm1	CLPTM1 regulator of GABA type A receptor forward trafficking		
LOC112245979	focal adhesion kinase 1		
LOC112246006	CUB and sushi domain-containing protein 3-like	regulation of dendrite development	Drury et al. 2019

Table 12. A protein-coding gene contained in the genomic region on chromosome 19 that were identified as an associated region with partial migration in the Nagara River.

Gene symbol	Gene description	Function	Reference
kcng4a	potassium voltage-gated channel, subfamily G, member 4a	development of sensory neurons	Shen et al. 2016

Figures

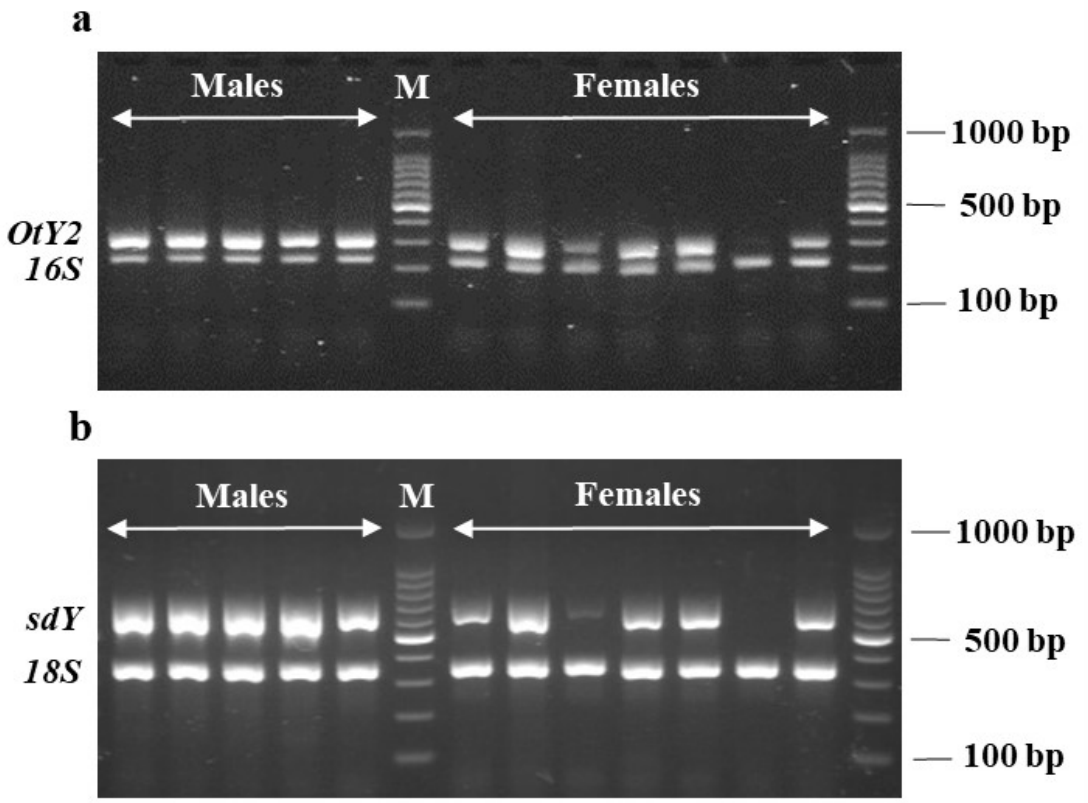
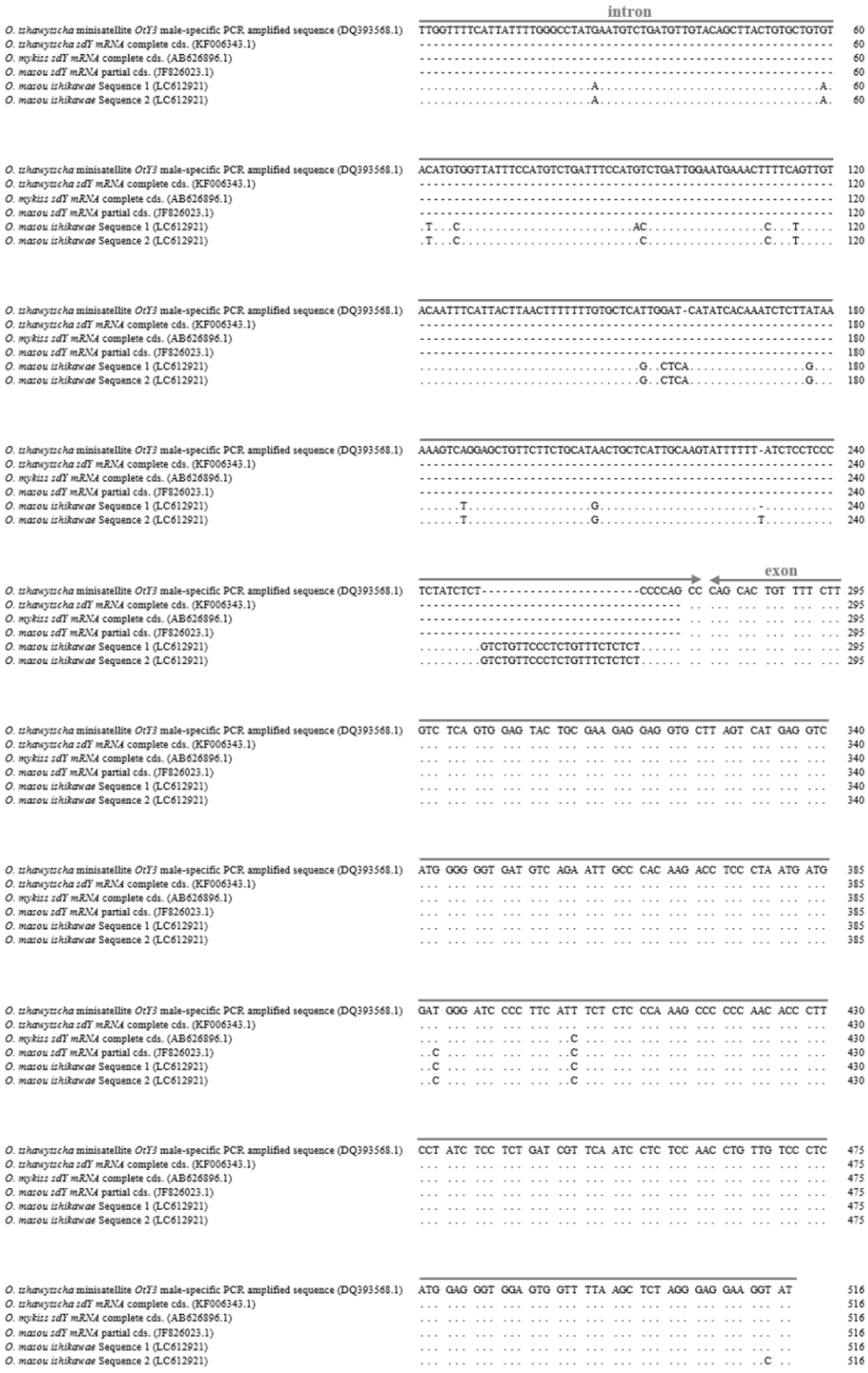


Figure 1. Electrophoresis of multiplex PCR products from male-specific markers in 1.5 % agarose gel. (a) PCR products with *OtY2m* primers and the mitochondrial *16S rRNA* primers. (b) PCR products with *sdY* primers and the nuclear *18S rRNA* primers. M, 100 bp DNA Ladder (Takara Bio Inc., Shiga, Japan).



Sequence	Number of occurrences	
	Male	Female
1	4	6
2	2	0

Figure 2. Aligned sequences of intron and exon regions of the partial *sdY* gene from red-spotted masu salmon (Sequence 1 and 2) and three *Oncorhynchus* species.

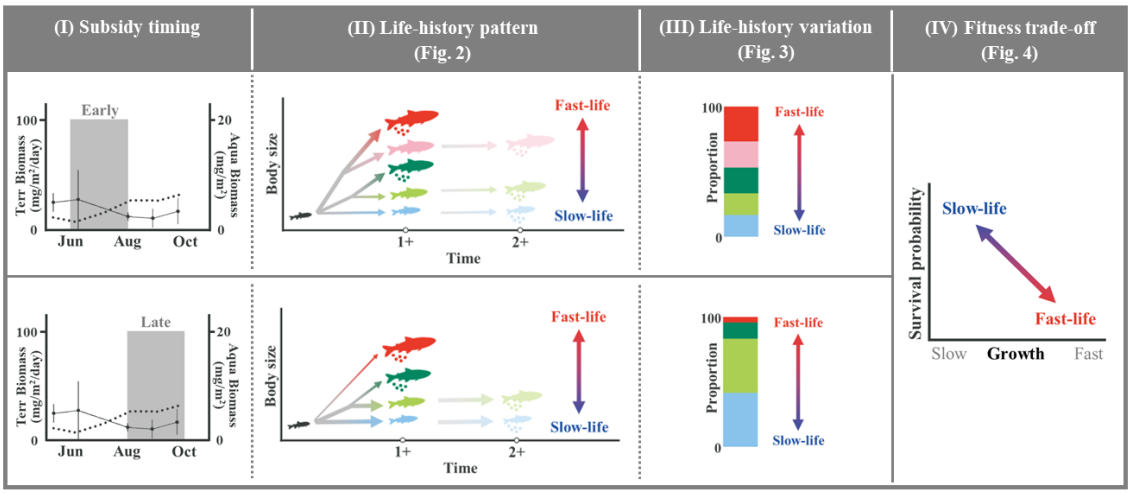


Figure 3. The hypothesized effects of subsidy timing (I) on life-history variation in the early-subsidy and late-subsidy treatments, respectively (II and III) and the growth-survival trade-off that may explain the life-history variation (IV). In the panel (I), seasonally asynchronous resource dynamics of aquatic invertebrate biomass (Aqua) and the terrestrial invertebrate subsidy (Terr) is shown; solid line represents the natural input rates of the terrestrial invertebrates in the study reach (mean ± SD); dashed line shows aquatic invertebrate biomass measured in the nearby stream (Sato et al. 2011a), which is commonly observed seasonal pattern in temperate streams. Gray squares represent the mealworm subsidy that was experimentally added as early- and late-subsidies, respectively. In the panel (II), the expected life-history patterns (growth trajectory and age at maturity) were shown; the color represents slow (blue) to fast (red) growth trajectories, while the thinness of arrows indicates the expected frequency of each life-history pattern, which is summarized in the panel (III). The life-history patterns and variation in no-subsidy control (reduced-subsidy in this study) were expected to be similar with those hypothesized in the late-subsidy along with the previous study (Sato et al. 2016).

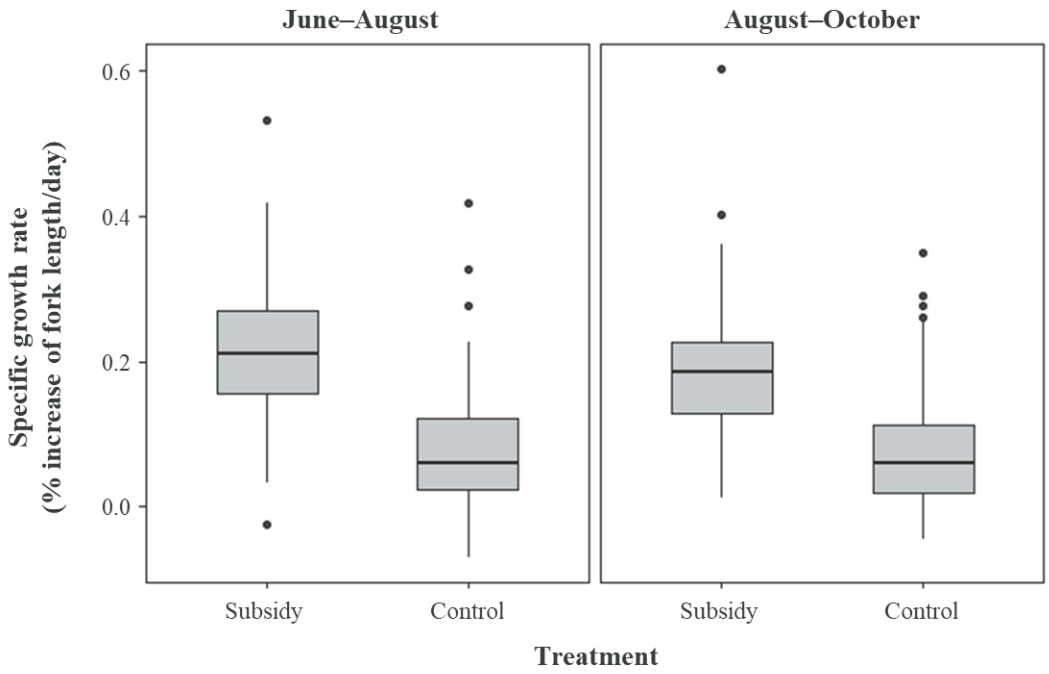


Figure 4. Boxplot of specific growth rate of individual masu salmons in terms of fork length (mm) in the subsidy and control sections in each early (June–August) and late (August–October) period.

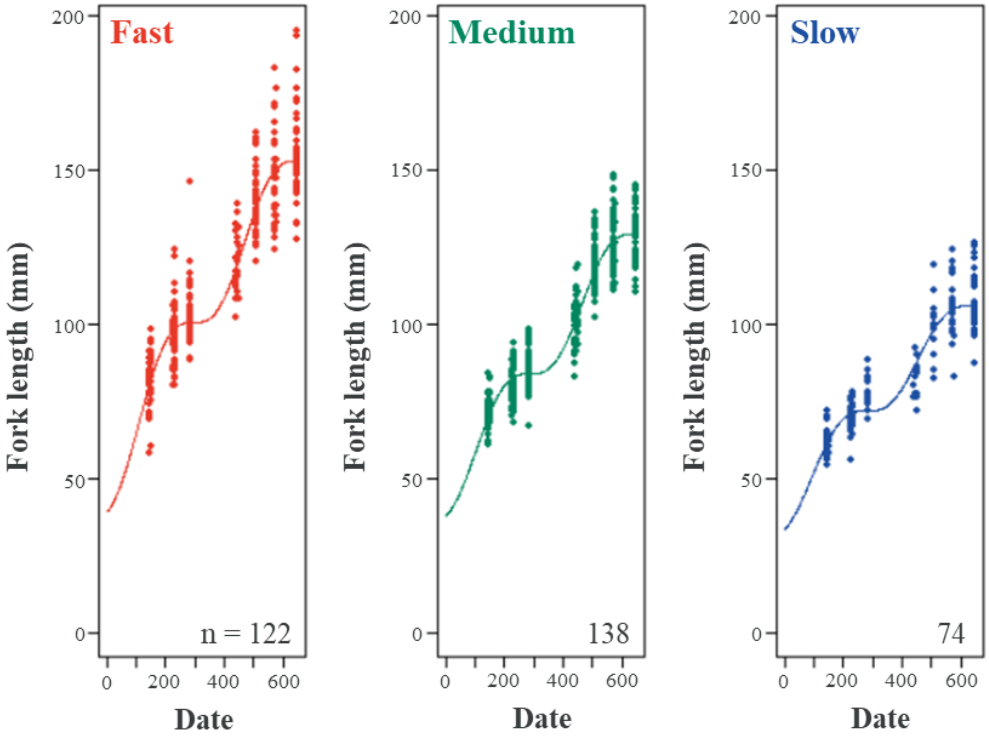


Figure 5. Estimated seasonal growth trajectories for fish belonging different growth clusters that were detected in the optimal mixture regression model. Dots in the panel represent fork lengths of individual fish at each sampling occasion.

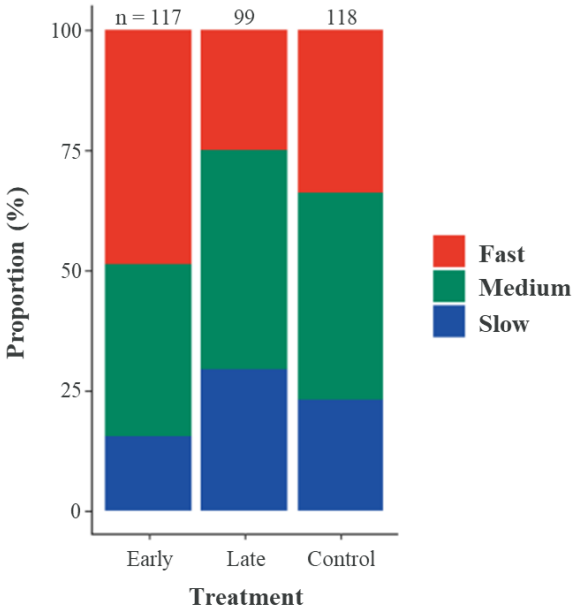


Figure 6. Proportional compositions of growth clusters for each treatment. Number of individuals is indicated above each bar.

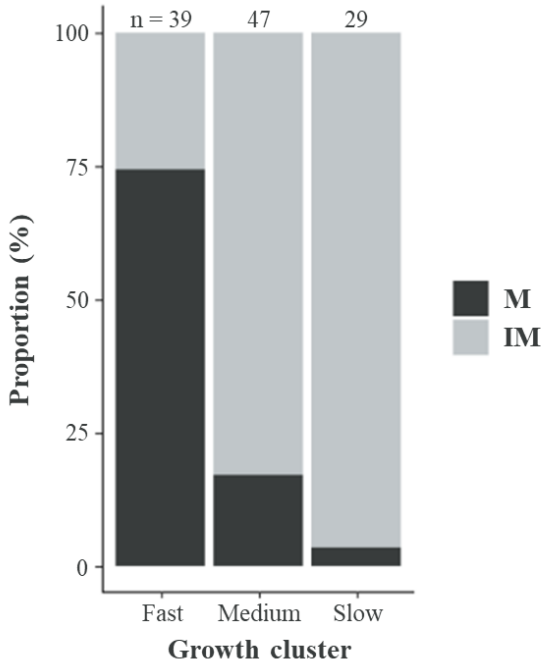


Figure 7. Proportional compositions of maturation at age-1 for each growth cluster. 'M' and 'IM' indicate the matured and immatured individuals, respectively.

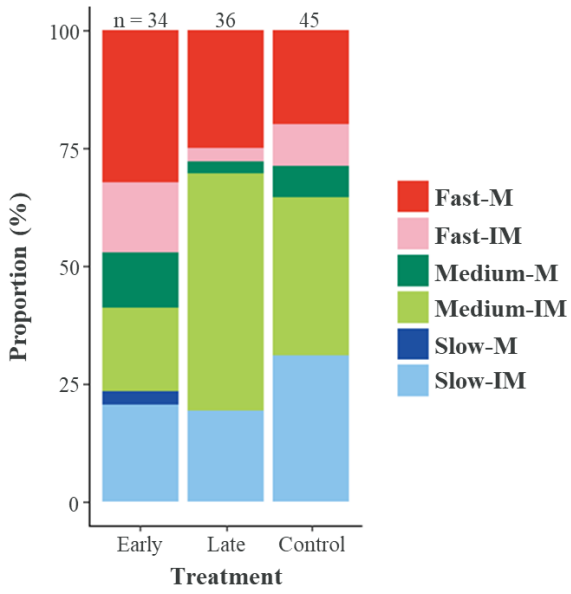


Figure 8. Proportional compositions of life-history patterns in each treatment. The life-history pattern of individual is represented by a combination of the growth cluster (first term) and maturation status at age-1 or not (second character M = matured, and IM = immatured).

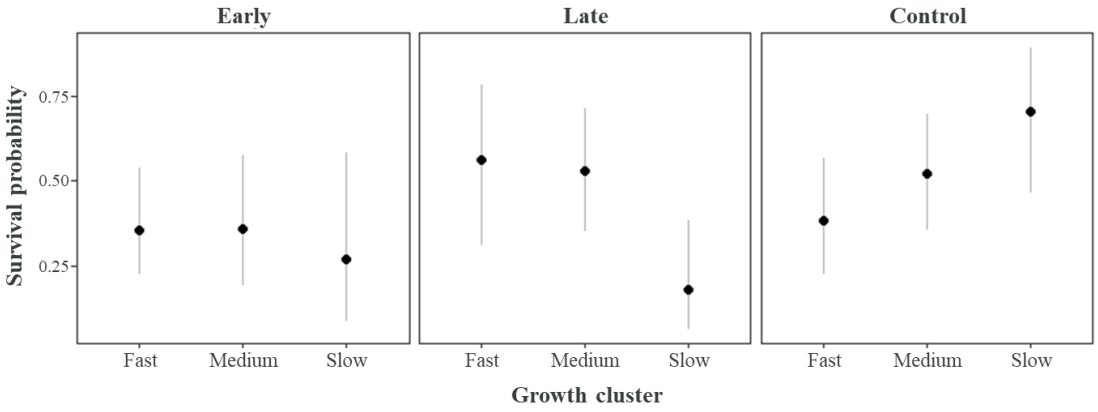


Figure 9. Association between growth clusters and survival probabilities in each treatment. Dots represent the median of estimated survival probability until reproductive season at age-1. Error bars represent 95 % credible intervals.

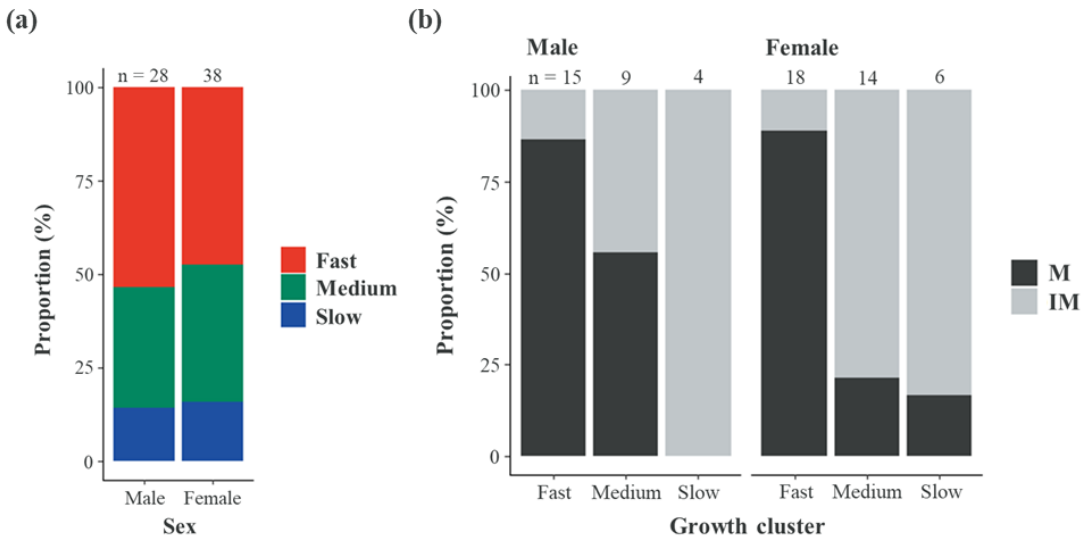


Figure 10. Proportional composition of growth clusters for each sex (a) and Proportion of maturation at age-1 for each growth cluster for each sex (b). In the panel (a), the proportional compositions did not differ between sexes (Fisher's exact test: $P = 0.94$). In the panel (b), M shows mature at age-1, while IM represents immature at age-1. The probability to maturation at age-1 was significantly associated with growth cluster ($GLMM_{binomial}$, fast vs. medium: $z = -3.62$, $P < 0.001$, fast vs. slow: $z = -3.48$, $P < 0.001$; Table 7), but not with sex ($z = -0.81$, $P = 0.42$; Table 7). In both panels, the number of individuals is shown above the bars.

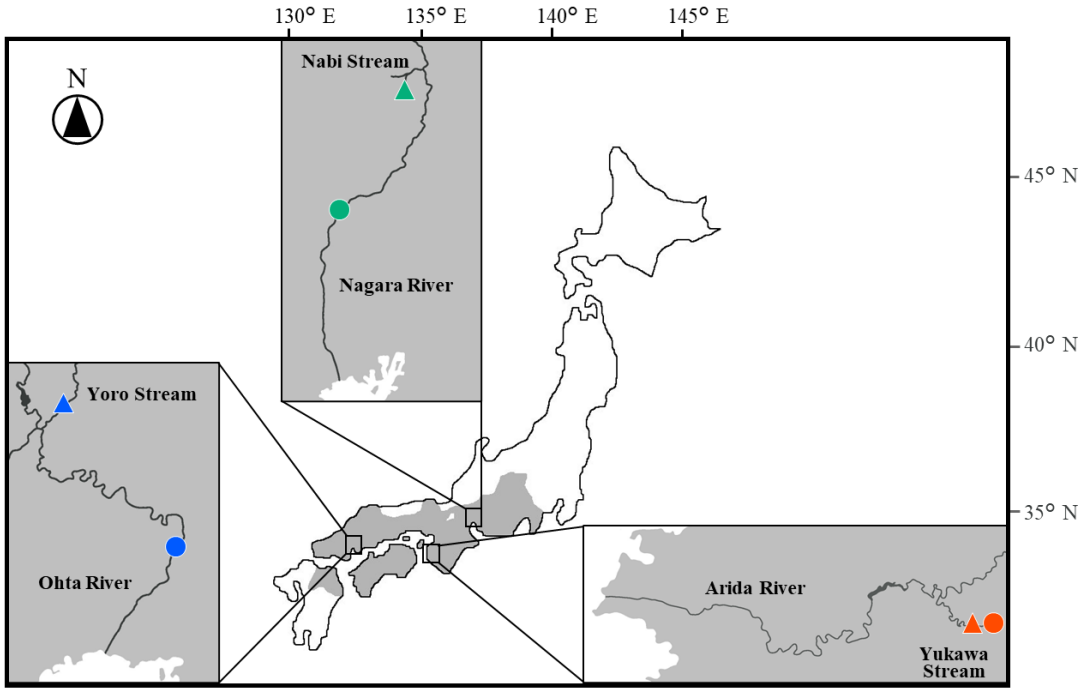


Figure 11. Map of the three sampling locations. Natural distribution range of Red-spotted masu salmon is shown in gray. Migration phenotype is represented by shape (triangle = resident, and circle = migrant).

Figure 12. Examples of two phenotypes in red-spotted masu salmon. (a) a matured female resident and (b) a smolt (migrant).

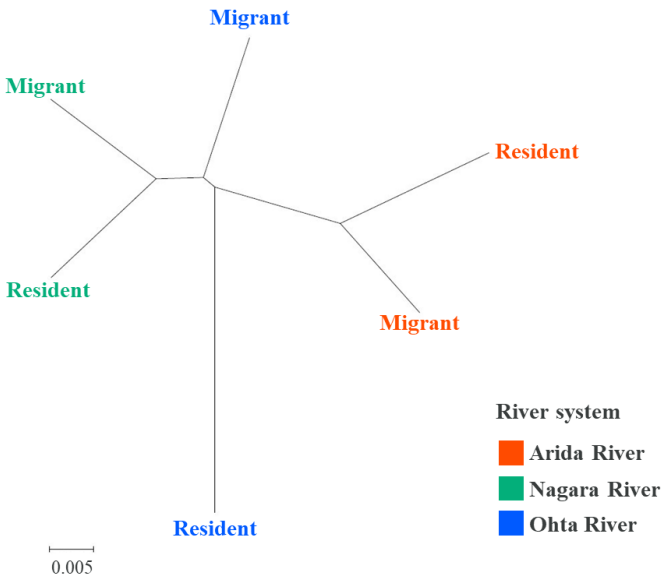


Figure 13. Genetic relationships for all pooled collections. Phylogenetic tree was constructed using the Neighbor-Joining method. The scale bar indicates F_{st}.

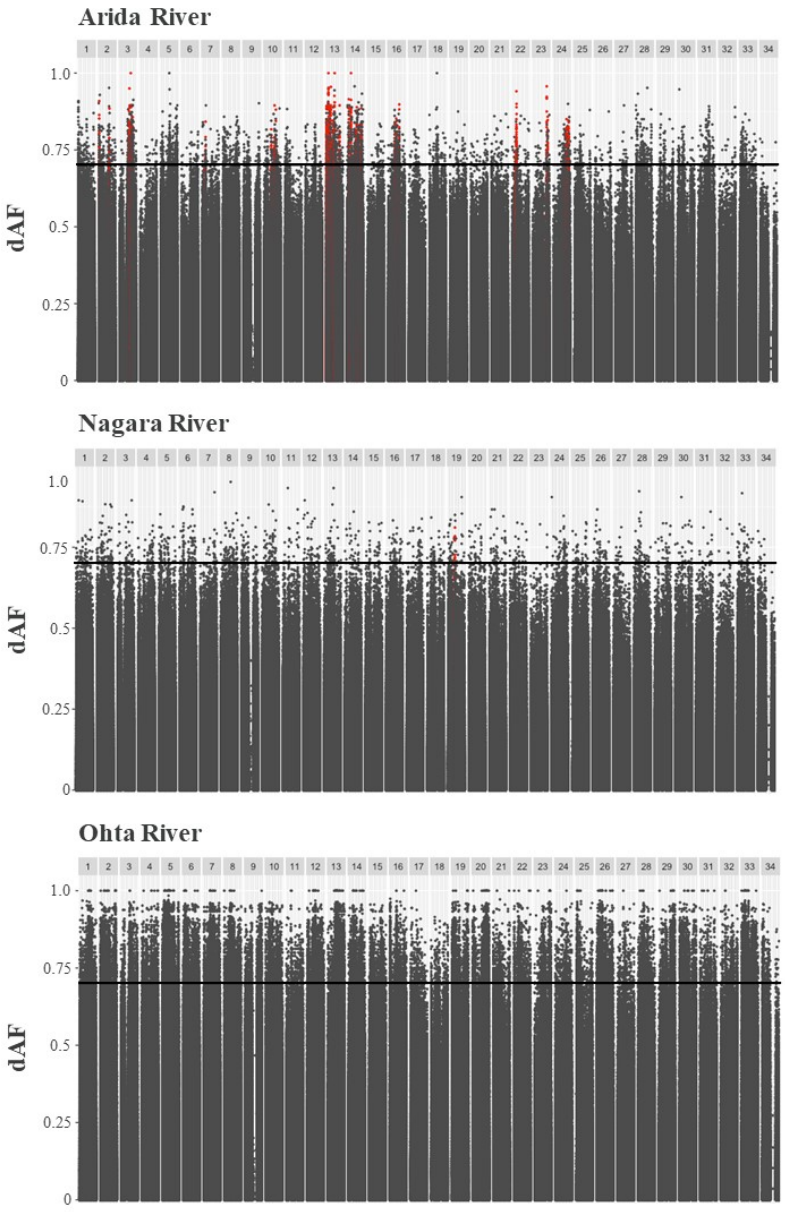


Figure 14. Differentiated genomic regions between residents and migrants across chromosomes. Plots show the allele frequency differences (dAF) for each SNP and red dots indicate the SNPs in genomic regions that include at least 10 SNPs with dAF > 0.7 in 100-kb sliding genomic windows. The *x*-axis shows the chromosomal positions (chromosome numbers are according to *O. tshawytscha* genome using genome assembly) and the y-axis shows the dAF value. The black solid lines indicate the threshold at dAF = 0.7.