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論文題目	Genetic structure and genomic analysis of population divergence of freshwater and marine species （淡水および海水魚種の遺伝的構造と集団の分化の ゲノム解析）
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## 論文内容の要旨

Understanding the factors influencing local population genetic structure and divergence is a main issue in evolutionary biology and could provide valuable information to conserve and manage the genetic diversity of species under global climate changes. Fishes are known to display great diversity of habitats, thrives in marine, estuarine and freshwater habitats and these are excellent model to study the population genetic divergence and factors contribute to it. In this thesis, I aim to explore the population genetic structure and genetic divergence of a species of freshwater and marine fishes, together with the study on temporal demographic response towards the past climatic change of closely related marine species.

In chapter 1, I have inferred the phylogeography and mtDNA genetic diversity studies of indigenous mahseer, *T. douronensis* in various river basins of Sarawak in Malaysian Borneo. *Tor douronensis* species is highly valued freshwater cyprinids in Malaysia and most widespread in Malaysian Borneo. In Sarawak, Malaysian Borneo, wild populations of *T. douronensis* have undergone declines in distribution and abundance due to significant degradation in their natural habitat from environmental activities and overfishing. To describe the genetic variation, direct sequencing of cytochrome c oxidase I (COI) gene for 134 individuals from eight rivers in Sarawak was used in this study. A total of ten haplotypes were identified with low haplotype diversity and nucleotide diversity ranged from, 0.000 to 0.291 and 0.000 to 0.014, respectively. Phylogenetic analysis revealed that *T. douronensis* mtDNA consisted of two highly divergence clades, one of which is more related to southern populations (Clade I) and the other are comprised of haplotypes from central-northern populations (Clade II). Significant level of inter-population with high pairwise  $F_{ST}$  among populations in Sarawak's river suggests little or no migration among the existing populations. The northern populations and few populations of *T. douronensis* were noted with single haplotype with no nucleotide diversity, might suggest the population could be derived from a single female parent and had undergone population bottleneck. Heterogeneity of haplotype compositions among rivers showed that contemporary geographical separation appeared to structure *T. douronensis* into discrete populations. The data raised some concerns regarding the conservation status of *T. douronensis*, which need to be addressed.

Past climatic changes in glaciation period were associated with fluctuations in global temperature and sea level temperature. These factors have been suggested as drivers of demographic change in both terrestrial and marine biota. In chapter 2, I sequenced the genome of two close species, Pacific cod, *G. macrocephalus* and Walleye pollock, *T. chalcogramma* originated from North Pacific Ocean to study temporal historical demographic during the Pleistocene epoch (11kya-2.6mya). Historical effective population size ( $N_e$ ) was traced back in time using diploid genomes of both species. By using pairwise sequentially Markovian coalescent (PSMC) analysis, variation of historical  $N_e$  is reconstruct from distribution of heterozygote sites

along the chromosomes of the genome. This finding suggests that, even though the two species share habitat and experienced similar climatic change, they showed different patterns of historical demographic during the Pleistocene epoch in regard to sea level conditions. Additionally, our finding also suggests the ecological preferences of *G. macrocephalus* and *T. chalcogramma* may have strong influences on their response to past environmental events and subsequently affected the temporal  $N_e$  during the Pleistocene period.

In chapter 3, I investigated population genetic structure and searched for genomic signatures of divergent selection in walleye Pollock (*T. chalcogramma*) in heterogeneous environments of Bering Sea and Japans' ocean. The populations differ in hydrography such as sea temperature and salinity. By utilizing single nucleotide polymorphisms (SNPs) loci associated with inter simple sequence repeat (ISSR) regions throughout the genome, the results show population genetic structure differed between local populations of Bering Sea and Japan, indicating gene flow restrictions between these spatial areas. Multiple genome scan analyses identified outlier SNPs loci that were robustly detected with very high statistical support. This study reports a suite of outlier SNPs closely located to genes, indicating potential loci subject to divergent selection in local populations of *T. chalcogramma*. These outliers SNP had strong associations with sea temperatures and salinity conditions at local populations of pollock and shows contrast spatial distribution of alleles in both populations. Hence, the present study implies population divergence in *T. chalcogramma* between Bering Sea to Japans' ocean could be possibly due to the adaptive significance in response to heterogeneity of environmental conditions.

## 論文審査結果の要旨

NADIATUL HAFIZA BINTI HASSAN 氏提出の博士論文では、魚類の集団の遺伝的構造と環境に適応的に反応する遺伝子の検出を目的とした。1 章では、マレーシアの経済的に重要な淡水魚である *Tor douronensis* は、集団が隔離されている傾向にあり、集団内での遺伝的多様性が低く、早急な保護対策が必要であることを指摘した。2 章では、スケトウダラ (*Gadus chalcogrammus*) とマダラ (*Gadus macrocephalus*) の全ゲノム配列を決定し、Pairwise Sequentially Markovian Coalescent (PSMC) により、過去 1 万年から 300 万年の有効集団サイズの変動を推定した。その結果、過去の海水面変動の激しい時期に、スケトウダラの個体数は、マダラに比べて比較的安定しており、マダラの方が、環境変動に対して感受性が高いと推定された。3 章では、日本期間階およびベーリング海のマダラの個体群を用いて、ゲノムワイドな 160 の SNP を用いて解析した。 $F_{ST}$  ベースの検出法である Lositan と BayeScan および環境との相関をみる Bayenv および BayeScenv を用いて、自然選択を受けていると推定された 16 の SNP を検出した。これらの SNP を含んだ遺伝的構造は、ベーリング海と日本近海の個体群では、明確な違いが観察されたが、16 の SNP を除くと違いは減少したことから、選択に働いている遺伝子が集団間の違いを形成していると考えられた。また、4 つの手法で共通して検出された SNP は 3 つであり、その一つは、ZXDC 遺伝子上流域に位置し、この遺伝子は免疫 (MHC の活性) に関する遺伝子であると推定された。この遺伝子は、特に、塩分および海水温と相関を示した。これらの研究は、今後温暖化に伴う魚類の進化的反応および個体数の変化を予測する上で非常に重要な結果を提供している。これらの研究は、NADIATUL HAFIZA BINTI HASSAN 氏が主導で行った研究で、NADIATUL HAFIZA BINTI HASSAN 氏は研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、NADIATUL HAFIZA BINTI HASSAN 提出の論文は、博士（生命科学）の博士論文として合格と認める。