

**Origin, diversity, and evolutionary implications of unisexual vertebrates:
comparative study on gynogenetic and hybridogenetic fishes**

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Background

The paradox of sex is a central question in evolutionary biology. Asexual species are rare and predicted to be short-lived owing to the lack of recombination, which results in the loss of genetic diversity and restricts adaptive potential. Among unisexual vertebrates, there are various forms of clonal reproduction (parthenogenesis, gynogenesis, hybridogenesis) that are frequently associated with polyploidy. Gynogenesis and hybridogenesis depend on sperm from a related sexual species to initiate embryogenesis. In gynogenesis, sperm is used only for activation of cell division without genetic contribution. On the other hand, hybridogenesis uses sperm for fertilization despite the exclusion of the paternal genome during gametogenesis, resulting in the clonal transmission of the remaining maternal genome. Recent phylogenetic studies have suggested that gynogenesis and hybridogenesis are often older than theoretical estimation, showing high genetic variability. To understand the evolutionary potential and significance of asexual organisms, it is necessary to investigate the origin, extent of genetic diversity, and mechanisms facilitating their genetic diversity, which also leads to deepen the understanding of the problems of sex and recombination.

Because of the potentially frequent occurrences of sexual–asexual conversion with ploidy change, the two cyprinid groups, gynogenetic *Carassius* complex and hybridogenetic *Squalius alburnoides* complex, are promising models for investigating the evolution of asexuality in vertebrates. Although these unisexual fishes show significant genetic variability suggesting underlying diversification mechanisms, these mechanisms and phylogenetic entity remain unclear because of the lack of genome-wide analyses.

The purpose of this thesis was to elucidate the origin and diversification mechanisms of *Carassius* and *S. alburnoides* complexes through the integrative analysis of phylogeny, molecular evolution, and allele expression based on a number of loci. The similarity and unique features of their evolutionary processes are comparatively discussed, raising new questions regarding the origin and evolutionary implications of unisexual vertebrates.

Material and Methods

In Chapter 2, to cost-effectively determine the ploidy of the *Carassius* fish, which is difficult to discriminate morphologically, a microsatellite multiplex panel was developed, and its accuracy was confirmed by flow cytometry. The clonal diversity of the triploid gynogenetic *Carassius* from Lake Biwa was evaluated using the markers.

In Chapter 3, to elucidate the genetic relationships and differences between the population structure of sexual and asexual *Carassius*, mitochondrial DNA gene sequences and microsatellites were used to assess approximately 1,000 specimens collected from a wide range of the Japanese archipelago and adjacent areas. To reveal the genetic composition of gynogenetic *Carassius*, admixture analysis based on dozens of nuclear markers and genome-wide allele expression analysis using transcriptome sequences were conducted.

In Chapter 4, the deposited transcriptome sequences of hybrid *S. alburnoides* and related Leuciscinae (Cyprinidae) fishes were used to investigate the origin of *S. alburnoides*, a European hybridogenetic cyprinid, and evaluate the role of presumed compensation mechanisms against mutational load. Using over 4000 aligned genes, the following analyses were conducted: gene topology analysis for the detection of gene flow, molecular evolution analysis for accumulation of synonymous and nonsynonymous mutations, and allele expression analysis to test the buffering effect of paternal host alleles on mutation accumulation in the inherited maternal genome.

Results and Discussion

The newly developed method for the molecular determination of ploidy showed good

agreement (97.3%) with flow cytometry, and the characterization of a wild population in Lake Biwa showed high clonal diversity (Chapter 2). This cost-effective ploidy determination scheme was extensively used for successive study on *Carassius* fishes.

Integrative analysis of a number of nuclear loci for diploid and triploid *Carassius* demonstrated that the Japanese triploid *Carassius* is a hybrid of Japanese and Eurasian lineages (Chapter 3). High genetic diversity among gynogenetic *Carassius* was inferred to have been facilitated via directional gene flow from conspecific sexual diploid and increased establishment of immigrants. This is the first evidence of occasional sex with interploidy gene flow in natural populations of gynogenetic vertebrates.

Gene tree analysis among *S. alburnoides* and related species detected no significant vestiges of gene flow, suggesting accurate paternal exclusion in hybridogenesis (Chapter 4). The inherited maternal genome in *S. alburnoides* showed less codon bias than any other Leuciscinae fishes examined, whereas the ratio of nonsynonymous to synonymous mutations was compatible with related sexual endangered species. Finally, allele expression analysis demonstrated biased use of paternal host alleles. These results support the scenario that transient vigorous host alleles mask recessive mutations in the inherited maternal genome, contributing to the long-term persistence of this unisexual fish.

Conclusion

This thesis examined the origin and diversification mechanisms in two cyprinid gynogenetic and hybridogenetic fish groups with frequent occurrences of sexual–asexual conversion. Hybrid origin and accurate homologous synapsis suggested by *S. alburnoides* emphasize the modifications of meiotic program by hybridization. Both unisexual fishes possessed antagonistic mechanisms against mutational load, though their mechanisms and subsequent genome evolution patterns differed. The high genetic variability of the Japanese gynogenetic *Carassius* and *S. alburnoides* should be facilitated by hybridization and triploidization as other unisexual vertebrates, though hybridization and ploidy change also bring adverse effects such as genetic incompatibility and gene dosage imbalance. Exploring how unisexual vertebrates manage these problems deepens our understanding of adaptability of unisexual organisms, as well as the flexibility and complexity of genome evolution and gene regulation systems in vertebrates.

要旨

無性生殖種は組換えの欠如による変動環境への適応能の低下と有害変異の蓄積によって、進化的に短命であると予測されている。しかしながら、近年、無性生殖の一形式である雌性発生・雑種発生種では進化的に長命な場合があることが明らかとなってきた。無性生殖をする脊椎動物の中でも、コイ科魚類のフナ *Carassius* 属（雌性発生）と *Squalius alburnoides* 種群（雑種発生）は、頻繁に有性型から無性型が生じたことが示唆される稀有な例であり、無性生殖種の進化プロセスを研究する良いモデルである。これらの種群の無性型は、高い遺伝的多様性を示し、何らかの多様性の創出・維持機構の存在が示唆される。しかし、そのような機構や無性生殖種の系統的な実体は明らかとなっていない。本論文では、両者の起源と多様化プロセスを解明するために系統・分子進化・アレル発現に関する解析を行った。そして、それらの結果の比較を通じて脊椎動物における無性生殖の起源と進化可能性、進化的意義について議論した。

まず、*Carassius* 属 (*C. buergeri* 種群) の無性型の起源と多様性の創出・維持機構の解明を試みた。*Carassius* 属の無性型 3 倍体 (3n) は有性型 (2n) の精子を発生の刺激に利用するが、その精子は遺伝的に子孫に全く貢献しない雌性発生を行う。これらの集団遺伝・系統解析に先立ち、種や倍数性の判別が極めて困難な本属魚類の効率的な識別法として、核複数遺伝子座のマイクロサテライト (STR) を用いた倍数性判定手法を開発し、また野外集団における高いクローン多様性を明らかにした (第 2 章)。そして、この手法を日本列島を中心とする広域の野外集団に適用し、mtDNA・STR 分析により有性および無性型の集団構造を調べ、さらに多数核遺伝子座の分子系統・アレル発現解析を行った (第 3 章)。系統およびアレル発現解析の結果、日本列島の 3n のゲノム組成は日本と大陸系統の交雑様を示した。一方、2n と 3n は多様な mtDNA ハプロタイプを共有していたが、2n は明瞭な地理的集団構造を示す一方で、3n は地理的構造が不明瞭で、大半の水系で複数のクローン系統が共存した。無性型の高い遺伝的多様性は、稀な有性生殖に起因する 2n から 3n への遺伝子流動と高い移動分散性によってもたらされていることが示唆される。

次に、ヨーロッパ産のコイ科 *Squalius alburnoides* 種群の起源とゲノム進化パターンを解明した (第 4 章)。*S. alburnoides* は雑種発生種 (2 倍体および 3 倍体) であり、つまり他種である父親のゲノム (P ゲノム) を捨て、母親のゲノム (A ゲノム) のみからなる卵を形成し、それが精子 (P ゲノム) と受精する。本種は

進化的に長命であるが、それをもたらす機構として、有性宿主種からの遺伝子流動、減数分裂の存在、あるいはPゲノムによる遺伝ゲノム系統(Aゲノム)における有害変異の非顕在化が考えられる。これらの仮説を、近縁種を含む登録RNA-seqデータを用いて系統・分子進化・アレル発現解析を行い、検証した。その結果、AゲノムにPゲノムからの遺伝子流動の痕跡は見出されず、一方、Aゲノムにおいて有害変異の蓄積を示唆するコドンバイアスの低下とアミノ酸変異の蓄積傾向がみられ、後者は絶滅寸前の近縁有性種と同程度だった。またA・Pいずれか一方の発現が顕著に多い遺伝子は、その大半がPゲノムに偏っていた。以上の結果は、本種には配偶子形成時に父性Pゲノムを排除する厳密な機構が存在し、減数分裂によって排除されない劣性有害変異などによる問題を、有性宿主由来のアレルを利用して潜在化させていることを示唆する。

以上をもとに、総合考察(第5章)では、脊椎動物における無性生殖種の進化プロセスの共通性と相違点を整理し、無性生殖の起源と種間交雑の関係性、および無性生殖種の進化過程と異質倍数化との関連性について議論した。