Physiological and ecological studies on life history evolution of amphidromous goby fishes

Yumeki Oto

Introduction

Repeated colonisation of freshwater areas by marine organisms is one of the major events that has led to diversification of species and their life histories. Multiple marine-originated lineages have diversified amphidromous species, which hatch in freshwater areas, spend their larval period in seawater, and then return to freshwater as juveniles. Since the landlocking of amphidromous species has triggered species radiation in some fish families, it is an important event for understanding the origin of the remarkable biodiversity in freshwater ecosystems. However, our understanding on the evolutionary pattern of life history and physiological adaptation during freshwater colonisation via amphidromy is still limited (Chapter 1). To deepen our understanding, this thesis aimed to clarify the pattern of habitat shifts at the early evolutionary stage of amphidromy in a fish group, and then reveal what physiological traits enable animals to colonise freshwater or restrain their colonisation.

Materials and Methods

The marine-originated genus *Gymnogobius* (Gobiidae) includes at least three amphidromous and four freshwater species in addition to eight marine and estuarine species, and the diversity of this group makes it an attractive system for studying the process of freshwater colonisation. I first examined the habitat use of an amphidromous species, *Gymnogobius petschiliensis*, which highly depends on brackish water habitats (i.e. ancestral habitats). Subsequently, I conducted laboratory experiments and gene expression analysis to reveal the physiological factors that enable or restrict the freshwater colonisation of this species and its congeners, with a specific focus on each stage of life history.

In Chapter 2, I conducted field surveys on the distribution pattern of *G. petschiliensis* in two rivers in central Japan, to compare brackish water dependence of the adult habitat with that of the spawning ground.

To reveal physiological traits that enable adult *G. petschiliensis* to inhabit freshwater environments, I compared the physiological costs of the adult fish in various salinity conditions in the laboratory (Chapter 3). Additionally, I examined the salinity preference of juvenile *G. petschiliensis* returning to freshwater areas (Chapter 4). The behaviours of juveniles were observed in experimental tanks where fish could move between sections filled with water at different salinities.

To elucidate the key physiological factors for the landlocking of amphidromous species, I compared freshwater tolerance of larval fish and sought to elucidate the underlying molecular mechanisms of the tolerance among three amphidromous *Gymnogobius* species that differed in dependency on freshwater environments (Chapter 5). First, I compared the survival rates of freshwater- and seawater-challenged larvae among the three species. Second, I compared whole-body transcriptomes of freshwater- and seawater-challenged larvae for each species using RNA-Seq analysis.

Results and Discussion

In Chapter 2, field surveys conducted in the two rivers revealed that the adult density of *G. petschiliensis* was higher in freshwater than in brackish water areas during the non-spawning season.

However, the adult density in brackish water areas increased during the spawning season, and egg clutches were concentrated in brackish water areas. In contrast, both adult habitat and spawning grounds of a typical amphidromous congener (*G. urotaenia*) were restricted to freshwater areas. The concentration of spawning ground in brackish water areas supports strong evolutionary conservatism of reproductive physiology in amphidromous *Gymnogobius* species.

In Chapter 3, aquarium experiments under different salinity conditions revealed that the energetic cost of adult *G. petschiliensis* in freshwater was higher compared to that of adult *G. petschiliensis* in the other salinity conditions. Thus, the optimal salinity for adults should be higher than the osmotic pressure of body fluid (1/4–1/3 seawater), which suggests that living in freshwater was not physiologically optimum. In Chapter 4, juvenile fish of *G. petschiliensis* significantly preferred 2/3 seawater to freshwater. Preference between 2/3 seawater and seawater was almost the same. Thus, this species may migrate upstream from the sea to freshwater areas against its preference for saline waters. The results in Chapters 3 and 4 suggest that adults and juveniles of *G. petschiliensis* enter and inhabit freshwater habitats (physiologically suboptimal) by utilising their flexible osmoregulatory ability. High energetic cost in freshwater may be compensated by ecological benefits in freshwater habitats, such as low predatory and competitive pressures.

In Chapter 5, laboratory experiments clarified that larval survivability in freshwater conditions was highest in *G. urotaenia* among three related species. Differential gene expression analysis showed that genes encoding Aquaporin-3 (responsible for water discharge) and SLC13 (responsible for salt uptake) were more greatly upregulated in freshwater-challenged *G. urotaenia* larvae compared to those of the other two species. Thus, enhanced expressions of those genes may be important for larval adaptation to freshwater and landlocking in *Gymnogobius*.

Conclusion

This study provides three important insights into the evolutionary processes and mechanisms of amphidromy: (1) The spawning ground of amphidromous species may be more phylogenetically constrained than their adult habitat; (2) Flexible physiological traits of adult and juvenile fish would enable them to enter suboptimal freshwater environments in the early stage of freshwater colonisation; (3) The species' ability to form landlocked populations is associated with larval freshwater tolerance, which might be determined by expression levels of genes encoding osmoregulatory transporters, such as Aquaporin-3 and SLC13. To further understand the evolutionary mechanisms underlying freshwater colonisation, it is essential to elucidate which ecological factors promote habitat shifts and which physiological traits restrict larval freshwater colonisation.