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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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Name

学位論文題目：
Title of Dissertation　A study on population genetic structure of *Rhynchocypris lagowskii* based on mitochondrial DNA cytochrome *b* analysis
(ミトコンドリアDNAシトクロム*b*解析によるアブラハヤの遺伝的集団構造に関する研究)

学位論文要約：
Dissertation Summary

In biogeography studies there are usually common use freshwater fishes. Freshwater resources and their ecosystems are the main crisis in present time. Freshwater ecosystem and fish has become one of the most threatened on earth due to multitude of stressors, over harvest, alien invasive species, and climate change including urbanization and associated habitat alteration (Cooke et al., 2013). Four land bridges connect the Japanese archipelago at Sakhalin, the Kuriles, the Korean peninsula, and the Ryukyu Islands to the Eurasian continent that might have affect on the genetic structures and distributions of many species (Hotta, 1974). *R. lagowskii* (Japanese downstream fatminnow) is a small cyprinid fish that is endemic to Japan. There is no comprehensive study on population genetic diversity of *R. lagowskii*. To understand the genetic structure of *R. lagowskii* species it is necessary to compare with other freshwater fish about the history of genetic divergence in Japan. And so we can know the contribution of the Japanese Islands by doing it. In this study we examine the genetic divergence of *R. lagowskii* by utilizing mtDNA sequence in specimens collected from various localities in Japan.

In this study we used those materials and methods: 1. Study area and sample collections, 2. Mitochondrial DNA extraction, 3. Polymerase chain reaction amplification, 4. Agarose gel electrophoresis, 5. Sequence method, 6. Sequence analysis.

Chapter-1: Genetic population structure of the Japanese downstream minnow (*Rhynchocypris lagowskii*) based on mitochondrial DNA sequence

Result: Phylogenetic analyses on *R. lagowskii*

We constructed phylogenetic tree based on the nucleotide sequence obtained from cyt *b* region of mitochondrial DNA. The sequence of 460 sites of the cyt *b* region was determined for 221 specimens of *R. lagowskii* with outgroup of *R oxycephalus* species. According to these variable sites, a total of 66 haplotypes were obtained from the species of *R. lagowskii*. Haplotype diversities (*h*) ranged from 0.0000 ± 0.0000 to 1.0000 ± 0.1265 and nucleotide diversities (π) ranged from 0.0000 ± 0.0000 to 0.017826 ± 0.011657. In neighbor-joining tree showed three deeply diverged groups (Group 1 to 3). The neighbor-joining (NJ) tree of mtDNA haplotypes also showed similar topologies, and consistently revealed three deeply diverged groups (Group 1 to 3) of *R. lagowskii*. To see their gene genealogy, we constructed a phylogenetic network based on mtDNA cyt *b* sequences (Fig.1). The most parsimonious network of mtDNA haplotype estimated using the TCS algorithm (Templeton et al. 1992). Total 66 haplotype were occurs in 29 localities like a NJ tree. Three geographical groups were found in this network. Haplotype 1-36 belong to group 1, haplotype 37-40 belong to group 2 and haplotype 41-66 belong to group 3. Specifically, the populations belong to group 2 or group 3 showed remarkable differentiation from the populations belong to group 1.

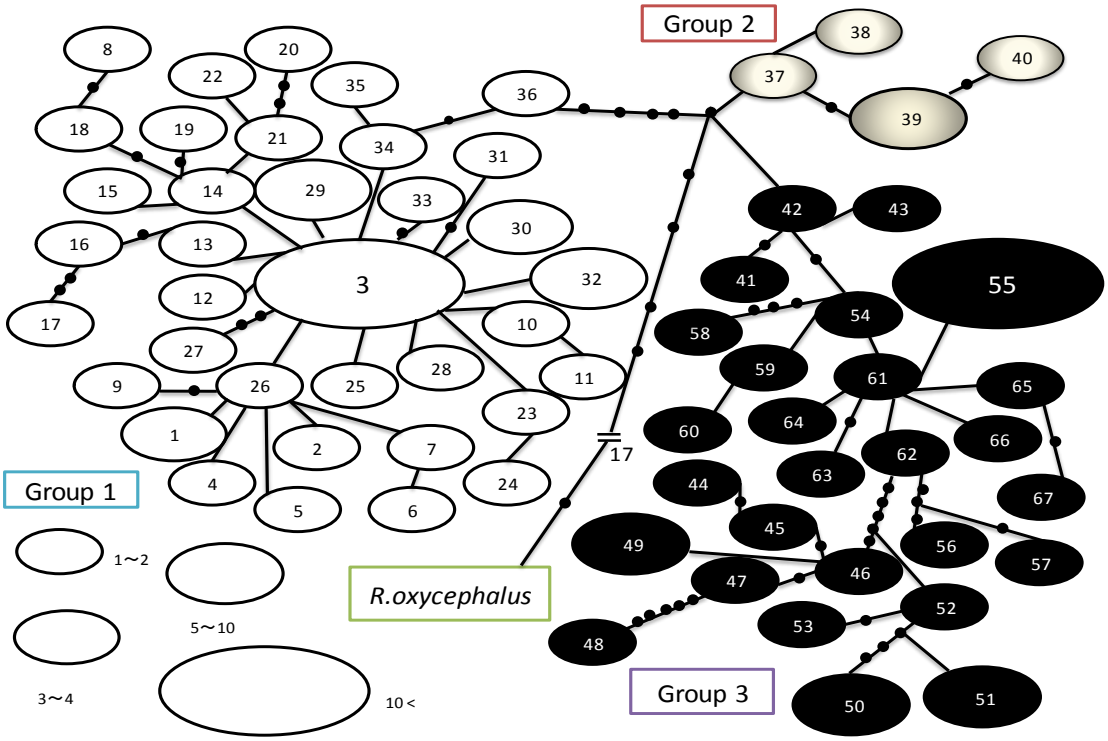

Fig. 1. The Haplotype network for *Rhynchocypris lagowskii*. The small black-filled circles are missing haplotypes needed to connect observed haplotype. The size of the circle is proportional to the number of individuals.

Genetic diversity within populations

To compare genetic diversity between all populations we calculated the *F*st value. The *F*st value was recorded 0.86220 between group 1 and group 2, 0.61342 between group 2 and group 3, 0.79380 between group 1 and group 3. The *F*st value between the Sea of Japan and Pacific Ocean was 0.23969. The global *F*st based on mtDNA was 0.86220 ($P< 0.001$). No significant differentiation is found these samples ($P< 0.05$). We've added examination about the genetic diversity between the Sea of Japan and Pacific Ocean. The Pacific Ocean area's genetic diversity was 0.9295±0.0100, while the Japan Sea area's one was 0.7838 ±0.0339. The genetic diversity was 0.7735±0.0299 in group 1, 0.6182±0.1643 in group 2 and 0.8720±0.0282 in group 3. The nucleotide diversity was 0.20183±0.0103600 in the Pacific Ocean and 0.008505 ± 0.004747 in the Sea of Japan. On the other hand, the nucleotide diversity was 0.003518 ± 0.002315 in the group 1, 0.002688 ± 0.002075 in the group 2 and 0.012231 ± 0.006596 in the group 3.

We made a map which showed the mixed rate of three groups in each localities. In this map the blue part in the pie-chart showed the mixed rate of group 1, red pie-chart showed the mixed rate of group 2 and yellow pie-chart showed the mixed rate of group 3. If all was group 1, the pie-chart became all the blue; if all was group 2, the pie-chart became all the red and if all was group 3, the pie-chart became all the yellow. The samples of Asamizu River (15) and Iwai River (16) belonged to the 100% group 3, but was distributed over the domain where the group 1 inhabited. Mixed rate of group 1 was 50% at Oshikiri River in the Mogami River system, 10% at Maruko River in the Omono River system and 40% at Nezugaseki River in the Nezugaseki River system. Group 2 was 20% at Hakui River in the Hakui River system and group 3 was 28% at Yamada River in the Same River system and 12% at Toki River in the Ara River system (Fig.2). The mixed sample of group 1 and 3 appeared at some spots in the same river system regardless of geographical distribution. This result suggested a

creation thing of two possibility for the cause of this, invaded individuals or relicts. From past records, it was suggested that it was move likely to be invaded from group 3 domain. It might be cause of Japanese river make huge network for their irrigation. It may mating with other population be more frequently or maintain lineage of other threatened species or to loss of genetic unpredictability by genetic float in each local wild population.

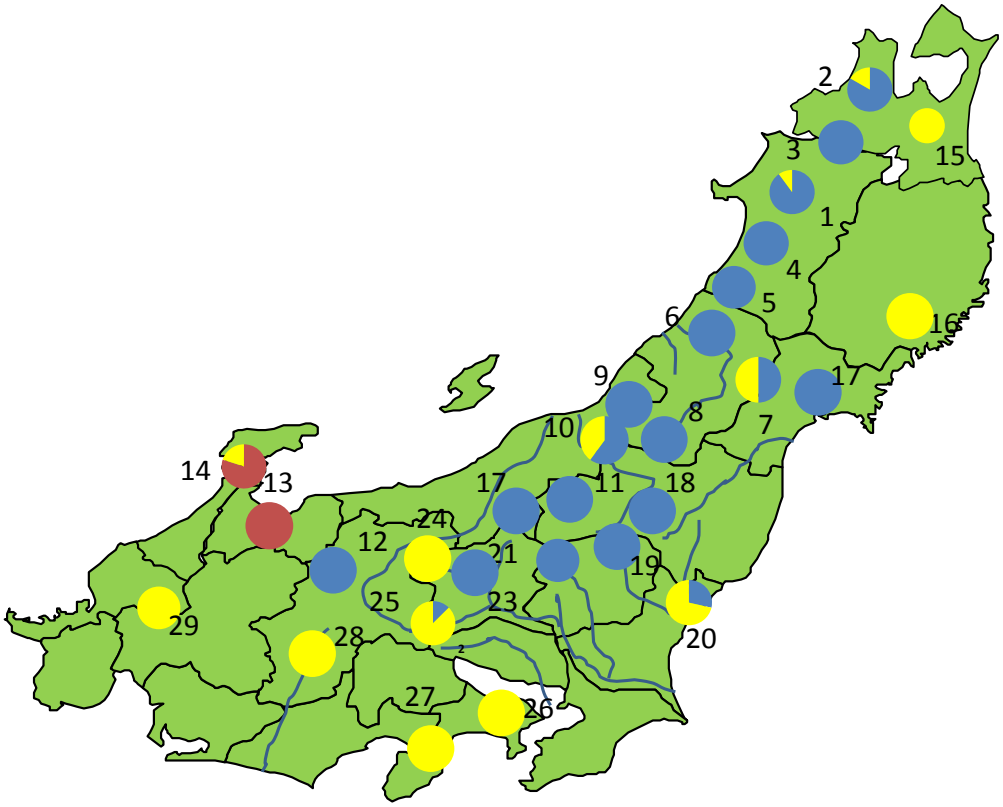

Fig. 2. The mixed rate of three groups in each localities of *Rhynchocypris lagowskii*. Blue pie-chart represent the group 1, red pie-chart represent the group 2 and yellow pie-chart represent the group 3. Sampling localities of *Rhynchocypris lagowskii* examined: 1- Maruko River; 2- Hira River; 3- Kosaka River; 4- Yunosawa River; 5- Mi River; 6- Tachiyazawa River; 7- Oshikiri River; 8- Ootaru River; 9- Kakuda River; 10- Nezugaseki River; 11- Daimonn River; 12- Chikuma River; 13- Kamisyou River; 14- Hakui River; 15- Asamizu River; 16- Iwai River; 17- Nishitanaka River; 18- Harase River; 19- Kumato River; 20- Yamada River; 21- Oshi River; 22- Kuro River; 23- Yamada River(A); 24- Karasu River; 25- Toki River; 26- Kushi River; 27- Ooba River; 28- Miya River; 29- Amano River.

Chapter-2: Appearance of the non-indigenous mtDNA haplotypes of *Rhynchocypris lagowskii* in Tohoku, Japan

In this chapter we were searched for the distribution of indigenous and non-indigenous and invaded *R. lagowskii* populations in Tohoku region or not. Concerning this fact, the present investigation focused the origin of the Iwate populations and checks the distribution of indigenous and non-indigenous and invaded fish populations by using mtDNA haplotype.

Result: Diversity of mtDNA sequences

The nucleotide sequence was established for the 429 base pairs (bp) of the mitochondrial DNA cyt *b* region were sequenced each sample of *R. lagowskii*. We found total fourty-seven haplotype from eighteen localities. Variable nucleotide positions found in the 429 bp fragment for the *R. lagowskii* species. We constructed phylogenetic tree based on the nucleotide sequence obtained from cyt *b* region. The sequence of 429 bp of the cyt *b* region was determined for 138 specimens of *R. lagowskii* we examined as an outgroup with *R. oxycephalus*. According to these variable sites, a total three group of haplotypes were obtained from the species of *R. lagowskii* like chapter 1. Haplotype diversities (h) ranged from 0.2000 ± 0.1541 to 1.0000 ± 0.2722 and nucleotide diversities (π) ranged from 0.000000 ± 0.000000 to 0.017730 ± 0.011063 (Table 1). We found very high nucleotide diversity in Nyu River (0.017730 ± 0.011063), Yazawa River (0.015400 ± 0.009413) and Hira River (0.012340 ± 0.008305) that notified the high polymorphism. The group 2 collected from Kamishou River all samples and Nyu River one sample are found in this group.

Table 1. Halpotype diversity and nucleotide diversity of *Rhynchocypris lagowskii*

1	River name	Halpotype Diversiy	Nucleotide Diversity
2	Aizawa River	0.2500 ± 0.1802	0.000532 ± 0.000754
3	Tachiyazawa River	0.2000 ± 0.1541	0.000851 ± 0.000969
4	Nojiri River	0.6667 ± 0.1598	0.001621 ± 0.001534
5	Nyu River	0.8000 ± 0.1721	0.017730 ± 0.011063
6	Yoshino River	0.5000 ± 0.2652	0.002128 ± 0.002108
7	Suna River	1.0000 ± 0.2722	0.007092 ± 0.006165
8	Oguro River	0.9524 ± 0.0955	0.005471 ± 0.003810
9	Ootaru River	1.0000 ± 0.0764	0.006890 ± 0.004619
10	Kakuda River	0.7333 ± 0.1552	0.001844 ± 0.001722
11	Yazawa River	0.9524 ± 0.0955	0.015400 ± 0.009413
12	Fujisawa River	0.8571 ± 0.1083	0.003040 ± 0.002350
13	Hira River	1.0000 ± 0.1265	0.012340 ± 0.008305
14	Asamizu River	0.3333 ± 0.2152	0.002128 ± 0.001903
15	Shakuri River	0.4286 ± 0.1687	0.001820 ± 0.027900

We found some population mixed group 1 and group 2 from the Mogami River system and Aka River system. The mixed rate of group 3 haplotypes to group 1 haplotypes were 50% at Oshikiri River, 17% at Nyu River in the Mogami River system, 43% at Yazawa River in the Aka River system, 40% at Nezugaseki River in the Nezugaseki River system and 17% at Hira River in the Iwaki River system. The correlation between haplotype and nucleotide diversities was calculated in each localities (Table 1). The localities mixed two or three groups were higher in the polymorplism than other one. We found a clean relation between the localities. Some population showed remarkeble position with other group area. Thus the overall position of this localities support the hypothesis that the effect of the loss of genetic unpredictability by genetic float in each local wild population.

(注) 要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
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