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研究課題名（英文）Parallel evolution of complex adaptations through introgression: the study case of pelvic fin brooding in Sulawesi medaka

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研究成果の概要（和文）：メスの骨盤鰭型育雛は非育雛に比べ、骨盤鰭が長く、腹部の凹みが深く、育雛時間が長いことがわかった。QTL解析の結果、骨盤鰭の抱卵は、物理的にリンクしていない複数の遺伝子座によって制御されていることが明らかになった。全ゲノム比較解析の結果、混血は見られなかった。さらに、中央スラウェシからOryzias属の新種1種を発見した。系統解析の結果、この新種は他のOryzias属の腹鰭育雛と同じクレードに属することが判明した。また、*O. eversi*の純系ファミリーを作成し、すべてのF1個体のddRADシーケンスから、特定のSNPが性分化に完全に関連していることを明らかにした。

研究成果の学術的意義や社会的意義

本研究では、メダカの腹鰭飼育をモデル系として、複雑な適応のゲノム構造を調べ、複雑な形質が種の系統を越えて水平移動しているかどうかを評価した。近い将来、本研究で明らかになった遺伝子座をもとに、骨盤鰭繁殖に関連する個々の遺伝子をノックアウトしたり、非繁殖種を用いてトランスジェニック個体を作製することが可能になると考えられる。このような遺伝子工学的アプローチは、複雑な形質の進化に関する仮説を実験的に検証するユニークな機会を提供するものである。このように、本研究の成果は、確かな経験的証拠と進化仮説を立て検証するためのモデルシステムを提供することで、複雑な適応の進化の分野に影響を与えることが期待される。

研究成果の概要（英文）：Our analyzes revealed that females of pelvic-fin brooders have longer pelvic fins and a deeper abdominal concavity, and that they can carry eggs for longer than non-brooding species. QTL analyses revealed that pelvic-fin brooding is controlled by multiple and unlinked loci. Genome wide comparative analyses detected no signatures of admixture between the two lineages of pelvic-fin brooders. A previously unknown species of pelvic-fin brooder *Oryzias* was discovered from central Sulawesi. Phylogenetic analyses revealed that the new species belongs to the same clades as other pelvic fin brooders in genus *Oryzias*. A pure strain of *O. eversi* was rear. All F1 individuals were ddRAD sequenced. A single SNP was found to be perfectly associated, heterozygote for all males and homozygotes for all females.

研究分野：Biological sciences

キーワード：Complex traits QTLs *Oryzias* Multiple loci Pelvic-fin brooding Evolution

1. 研究開始当初の背景

Complex adaptations require the synergic and functional coordination of multiple morphological, behavioral, and physiological components. For example, echolocation in bats and some birds can only function when both the emitter device of ultrasonic waves and the receiver organ of its echoes has evolved, and ovoviviparity/viviparity in some groups of fishes requires both the ability for females to hold eggs inside the body and copulative organs in males. Therefore, complex adaptations are expected not to evolve frequently given that multiple and highly specific mutations are necessary and that intermediary evolutionary stages represent little or no benefit in the fitness of the organisms. Thus, the theory of natural selection cannot account for the rapid evolution of complex adaptations¹. Nevertheless, several complex adaptations have been found to have evolved repeatedly; for example, the repeated evolution of ovoviviparity/viviparity in the phylogeny of fishes^{2-3*}. Such events of parallel evolution of complex adaptations are one of the biggest enigmas in evolutionary biology¹

Recent advances in sequencing technologies have opened new possibilities to our understanding of this enigma. Especially, hybridization has been drawing much attention as a relevant mechanism in parallel evolution, because it can aid adaptation by transferring beneficial traits between species^{4*-6*}. For instance, it has been demonstrated that genes associated with the protective color-pattern on wings of *Heliconius* butterflies are extensively exchanged across sympatric species^{7*}, that pesticide resistance in the western European house mouse originated from hybridization with Algerian mouse^{8*}, and that the color perception of cichlid fishes in Lake Victoria was primarily determined by a past admixture between two ancestral lineages^{9*}. I think that similarly adaptive introgression could be the missing piece to explain the repeated evolution of complex adaptations.

This study will focus on the parallel evolution of “pelvic-fin brooding” in family Adrianichthyidae, known as “medaka” in Japan. Most medaka species deposit eggs on plants or other material without further care and are capable of producing a new egg clutch every day^{10*-11*}. However, it is known that in some species, females carry eggs with their pelvic fins until hatch^{12*-14*}. This pelvic-fin brooding is considered to be a complex adaptation, because it requires the simultaneous evolution of (a) oddly elongated pelvic fins to hold the egg clutch in place, (b) a pronounced abdominal concavity where the clutch is kept and (c) a synchronous egg/gonad maturation cycle where a new egg clutch is not generated until the previous one has hatch (Fig. 1)^{12*-15*}. I am currently mapping loci separately for each of these components of pelvic-fin brooding using F₂ hybrids between a pelvic-fin brooder and a non-pelvic-fin brooder.

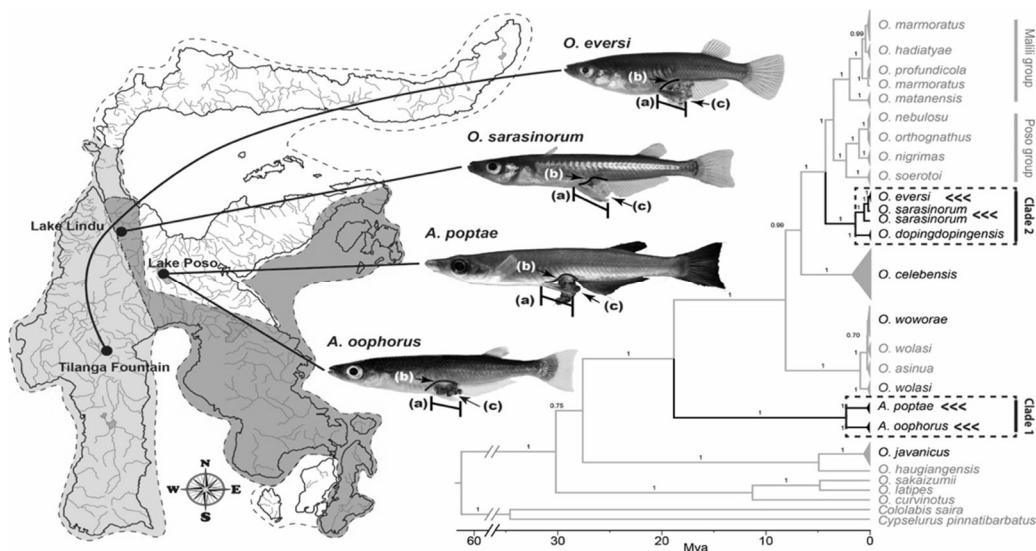


Figure 1. Phylogenetic reconstruction showing the repeated evolution of pelvic-fin brooding (<<<) in family Adrianichthyidae (Clade 1 and Clade 2). Pictures of medaka showing (a) elongated pelvic-fins, (b) abdominal concavity, (c) synchronous egg/gonad maturation cycle, characteristics common to all pelvic-fin brooding species. Map of Sulawesi showing the type localities for the species were pelvic-fin brooding is present, along with a basic delimitation of the tectonic composition of the island in dash lines (- - -); grey= Asia plate, dark= Australia plate, white= Ophiolite Belt. Modified from Mokodongan & Yamahira (2015).

Recent phylogenetic studies revealed that pelvic-fin brooding has evolved in two distinct lineages of medaka species endemic to Sulawesi Island, an island in Malay Archipelago^{16*}(Fig. 1). Several lines of available evidence have led us to think that the parallel evolution of pelvic-fin brooding might have originated from introgression between these two phylogenetically distant clades, rather than through independent evolution. First, there are striking morphological similarities between these distant clades, to the extent that some of the species were taxonomically classified into a single independent genus

(*Xenopoecilus*, currently not valid)^{12*, 17*}. Second, our preliminary analyses have demonstrated extensive past admixtures in the medaka phylogeny, especially in the Sulawesi lineage containing pelvic-fin brooder species (Fig. 2). Given that tectonic movements are very active in this small island^{18*} (Fig. 1), it is highly possible that these currently allopatric clades had a chance to be admixed in the past. **Thus, the key scientific question of this study is as follows: “Does the parallel evolution of pelvic-fin brooding in medaka reflects introgression due to historical hybridization between two distinct clades?”**

2 . 研究の目的

The aim of this research is first to reconstruct population trees among all medaka species in Sulawesi Island using genome-wide single nuclear polymorphisms (SNPs) and examine possible historical admixture events between the two lineages of pelvic-fin brooders. Second, succeeding the ongoing mapping project, I will perform comprehensive whole-genome comparative analyses between pelvic-fin brooders and non-brooders and verify if the genes responsible for pelvic-fin brooding are homologous across all brooder species.

This research will be not only a case study which dissects the genomic architecture of a complex adaptation but also will be the first study which demonstrates that complex traits can be transferred horizontally between phylogenetically distant species. Generally, it is assumed that the evolution of complex adaptations is very slow in nature because it depends on the fixation of multiple, highly-specific mutations, where intermediate stages seemingly provide little or no benefit. If our hypothesis on adaptive introgression of genes for pelvic-fin brooding is correct, our results will be a major breakthrough in the understanding of the parallel evolution of complex adaptations.

Additionally, given that medaka is a well-known model organism in the field of vertebrates' genomics, in the near future it will be possible for us to individually knock-out genes associated to pelvic-fin brooding or to create transgenic individuals using non-brooder species. Such genetic engineering approaches will give us a unique opportunity to experimentally test hypotheses on the evolution of pelvic-fin brooding, for instance, how this trait has passed over fitness valleys until reaching its present functional stage. Thus, this study will lead the world for years to come in the field of the evolution of complex adaptations by providing solid empirical bases and a model system to formulate and test evolutionary hypotheses.

3 . 研究の方法

Characterization of traits associated with pelvic-fin brooding – The length of pelvic-fin, extend of the abdominal concavity and days carrying eggs, were quantified across 17 species of medaka endemic to Sulawesi, four pelvic-fin brooders and 13 non-brooders. The pelvic-fin length of each female was measured and standardized by the standard length (SL). The extent of the abdominal concavity was expressed as the triangular area between the insertion points of the pelvic and anal fins, standardized by the square of SL, then log-transformed. The number of days for which females carried eggs was also quantified. Females were checked twice daily for the presence of eggs in the abdomen. When a female was carrying eggs, it was isolated from the male until the eggs were dropped or until larvae hatched. This observation was repeated three times for each species using different pairs, with some exceptions¹⁹.

A species tree family adrianchthyids in Sulawesi was estimated. We used the concatenated sequences of orthologous genes obtained from whole-genome sequencing data generated by Ansai et al. (2021)²⁰, to construct a maximum-likelihood tree with *O. javanicus* and *O. dancena* as outgroups. We tested whether each of pelvic-fin length, the extent of the abdominal concavity, and the number of days carrying eggs differed between pelvic-fin brooders and non-brooders when taking phylogenetic relationships among species into consideration, using phylogenetic generalized least squares (PGLS)²¹.

QTL mapping of pelvic-fin brooding – Interspecies crosses were prepared using one female *O. everisi* (pelvic-fin brooder) and one male *O. dopingdopingensis* (non-brooder) (G_0). Five pairs of one F_1 female and one F_1 male were made. The length of pelvic-fin, the extent of the abdominal concavity, and the number of days carrying eggs were recorder for all F_1 females. The same measurements were recorded for all F_2 females offspring of these F_1 pairs as before. Parents (G_0) and F_2 individuals were euthanized and tissue samples were dissected and preserved in 99% ethanol at -80°C .

Genomic DNA was extracted from preserved tissues. Genomic data were generated using double-digested restriction site-associated DNA sequencing (ddRAD-seq), following Peterson et al. (2012)²² with minor modifications. Raw reads were curated, and mapped to the genome assembly of *O. celebensis* (OryCel 1.0)²⁰ and used for single nucleotide polymorphisms (SNPs) calling. These SNPs were used to construct a linkage map including 139 F_2 individuals and 737 markers. Association mapping was then performed separately for pelvic-fin length, the extent of the abdominal concavity, and the number of days carrying eggs, using multiple QTL modeling (MQM) and single QTL analyses. The position of the associated loci was estimated using 95% Bayesian credibility interval¹⁹.

Tests of admixture between two distant clades of pelvic-fin brooders – Short read sequences of the whole genome of twenty adrianichthyid species/populations endemic to Sulawesi were retrieved from the DDBJ-DRA. Reads were trimmed and mapped to the reference genome assembly of *O. celebensis*²⁰. Bases at variant sites were called and a vcf file containing all variant and invariant sites was generated. A sub-dataset including only four pelvic-fin brooders, *O. dopingdopingensis*, and four non-brooding species was generated, and all SNPs in biallelic sites in within chromosome contigs were extracted. To test for admixture between the two clades of pelvic-fin brooders, f_4 statistics were computed using the qpDstat function with f_4 mode in the ADMIXTOOLS²³.

To identify potentially introgressed regions between the two clades of pelvic-fin brooders, we performed genome-wide scans using SAGUARO²⁴. The analysis was performed while distinguishing chromosomes based on the *O. celebensis* assembly. The percentage of genomic regions assigned to each unique topology was calculated. Additionally, the significant loci detected in the QTL analyses above were examined to assess the possibility of introgression in these regions between the two clades of pelvic-fin brooders¹⁹.

4 . 研究成果

Traits associated with pelvic-fin brooding – Phylogeny analyses revealed six major lineages within Sulawesi, each supported by 100% bootstrap (Figure 2). The two *Adrianichthys* species with pelvic fin-brooding (*A. oophorus* and *A. poptae*) formed a clade 1, while the two *Oryzias* species with pelvic-fin brooding (*O. everisi* and *O. sarasinorum*) belonged to Clade 5. Demonstrating that pelvic-fin brooding is polyphyletic and likely evolved independently in two distinct lineages.

Females of the pelvic-fin brooders had significantly longer pelvic fins than non-brooders (Figure 2a) (PGLS, estimate = 0.071 ± 0.016 SE, $t = 4.547$, $p < 0.001$). Similarly, females of the pelvic-fin brooders also had significantly deeper abdominal concavity than non-brooder species (Figure 2b) (PGLS, estimate = 0.439 ± 0.166 SE, $t = 2.638$, $p = 0.020$). Females of the pelvic-fin brooders carried eggs for 12 to 23 days after fertilization, while all non-brooder species drooped eggs within a day after fertilization (Figure 2c) (PGLS, estimate = 14.246 ± 1.154 SE, $t = 12.350$, $p < 0.001$).

Genetic architecture controlling pelvic-fin brooding – The pelvic-fin lengths of F₁ hybrid females were close to those of *O. everisi* females. Abdominal concavity and the number of days for egg carrying of F₁ females tended to be intermediate between the parental species, suggesting that these traits may differ in the genetic basis. Phenotypes of the F₂ hybrids varied greatly, covering the phenotypic trait values of the parental species. No correlation was found between the length of pelvic-fins or the extent of concavity and the number of days carrying egg in F₂ females. In contrast, the correlation between pelvic-fin lengths and concavity was significant ($r = 0.338$, $p = 0.002$).

MQM analyses using the F₂ females revealed that pelvic-fin lengths, the extent of abdominal concavity, and the number of days for egg carrying were mapped at different loci on different chromosomes (Figure 3). For the length of pelvic-fin, we identified a single highly significant peak of LOD score on chr 24 (Qfin1:chr24). This major locus explained 39% of the observed phenotypic variance. This was concordant with the results from single QTL analysis.

MQM analysis of abdominal concavity revealed three QTL on different chromosomes (Figure 3b): QTL on chr 17_19 (Qcon1:chr17_19), QTL on chr 6_23 (Qcon2:chr6_23), and QTL on chr 13 (Qcon3:chr13). The effect of each locus was relatively small with Qcon1:chr17_19, Qcon2:chr6_23, and Qcon3:chr13 explaining only 3%, 9%, and 4% of the phenotypic variance, respectively. No significant locus was detected by single QTL analysis. These data indicate that this trait is highly polygenic.

For the number of days carrying eggs, two significant loci were identified by MQM analyses (Figure 3c): QTL on chr 8 (Qegg1:chr8) and QTL on chr 15 (Qegg2:chr15). They explained 13% and 12% of the observed variance, respectively. Single QTL analysis also detected these two loci, although their effects were not significant.

No evidence for admixture between pelvic-fin brooders was found – The f_4 -statistics were positive in all topologies, except when *O. dopingdopingensis* was treated as P₂, indicating no admixture between *Adrianichthys* and pelvic-fin brooding *Oryzias*. The negative f_4 -statistics when P₂ = *O. dopingdopingensis* were therefore considered to reflect not admixture between *Adrianichthys* and brooding *Oryzias* but admixture within *Oryzias* species. The Saguaro analysis found no topologies supporting admixture between *Adrianichthys* and brooding *Oryzias*. Topological variations were mainly due admixture within *Oryzias*, supporting the finding from the f_4 -statistics.

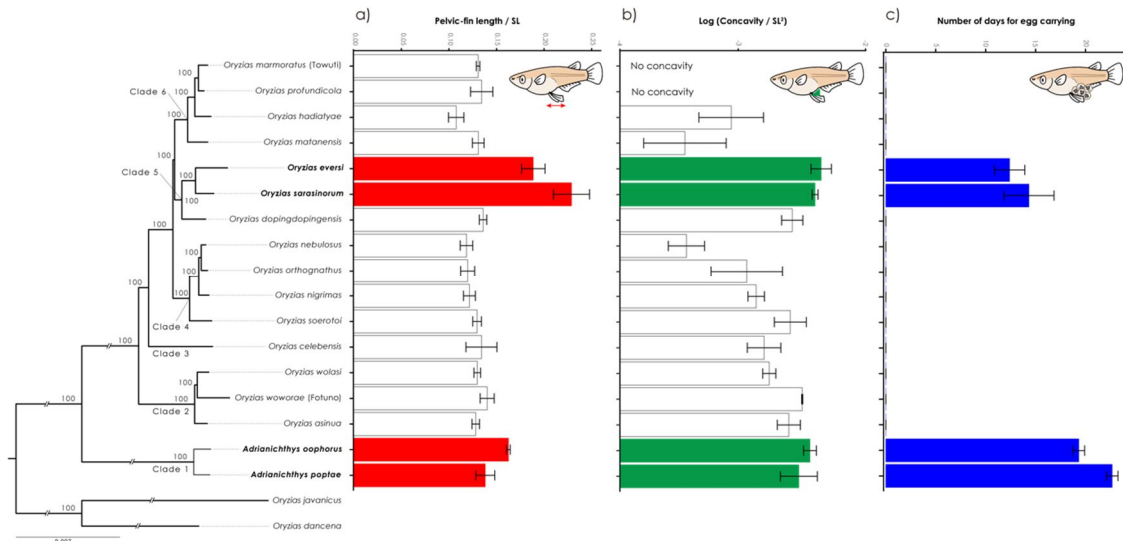


Figure 2. Maximum-likelihood phylogenetic analyses of Sulawesi adrianichthyids base on 10,174 single-copy orthologous genes and three characters associated with pelvic-fin brooding. Species in bold, and with colored bars represent pelvic-fin brooders.

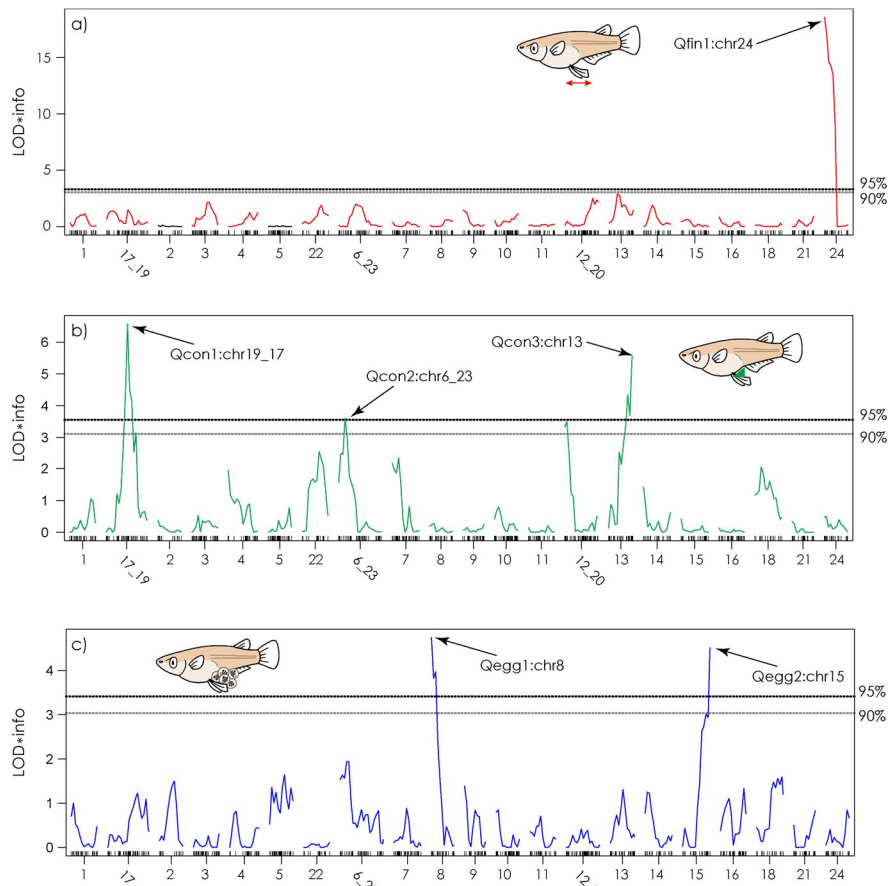


Figure 3. LOD score profiles, multiplied by information index, for (a) pelvic-fin length, (b) the extent of the abdominal concavity, and (c) the number of days eggs were carried estimated from MQM analysis. Significance levels (90% and 95%) are shown by dotted lines.

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5. 主な発表論文等

〔雑誌論文〕 計3件（うち査読付論文 3件/うち国際共著 3件/うちオープンアクセス 3件）

1. 著者名 Montenegro Javier, Fujimoto Shingo, Ansai Satoshi, Nagano Atsushi J., Sato Masahiro, Maeda Yusuke, Tanaka Rieko, Masengi Kawilarang W. A., Kimura Ryosuke, Kitano Jun, Yamahira Kazunori	4. 巻 0
2. 論文標題 Genetic basis for the evolution of pelvic fin brooding, a new mode of reproduction, in a Sulawesian fish	5. 発行年 2022年
3. 雑誌名 Molecular Ecology	6. 最初と最後の頁 1-14
掲載論文のDOI (デジタルオブジェクト識別子) 10.1111/mec.16555	査読の有無 有
オープンアクセス オープンアクセスとしている(また、その予定である)	国際共著 該当する

1. 著者名 Ansai Satoshi, Montenegro Javier, Masengi Kawilarang W. A., Nagano Atsushi J., Yamahira Kazunori, Kitano Jun	4. 巻 35
2. 論文標題 Diversity of sex chromosomes in Sulawesian medaka fishes	5. 発行年 2022年
3. 雑誌名 Journal of Evolutionary Biology	6. 最初と最後の頁 1751 ~ 1764
掲載論文のDOI (デジタルオブジェクト識別子) 10.1111/jeb.14076	査読の有無 有
オープンアクセス オープンアクセスとしている(また、その予定である)	国際共著 該当する

1. 著者名 Abdul Gani, Novian Suhendra, Fabian Herder, Julia Schwarzer, Javier Montenegro, Muh. Herjayanto, Daniel F. Mokodongan	4. 巻 71
2. 論文標題 A new endemic species of pelvic-brooding ricefish (Beloniformes: Adrianichthyidae: Oryzias) from Lake Kalimpa 'a, Sulawesi, Indonesia	5. 発行年 2022年
3. 雑誌名 Bonn zoological Bulletin	6. 最初と最後の頁 77-85
掲載論文のDOI (デジタルオブジェクト識別子) 10.20363/BZB-2021.71.1.001	査読の有無 有
オープンアクセス オープンアクセスとしている(また、その予定である)	国際共著 該当する

〔学会発表〕 計1件（うち招待講演 0件/うち国際学会 0件）

1. 発表者名 Javier Montenegro
2. 発表標題 Convergent or introgression? repeated evolution of reproductive modes in medaka fishes
3. 学会等名 The Ecological Society of Japan
4. 発表年 2020年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

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7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関