Origins of two hemiclonal hybrids among three *Hexogrammos* species (Teleostei: Hexagrammidae): genetic diversification through host switching

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Abstract
Two natural, hemiclonal hybrid strains were discovered in three *Hexogrammos* species. The natural hybrids, all of which were females that produced haploid eggs containing only the *Hexagrammos octogrammus* genome (maternal ancestor; hereafter *Hoc*), generated F₁ hybrid-type offspring by fertilization with haploid sperm of *Hexagrammos agrammus* or *Hexagrammos otakii* (paternal species; *Hag* and *Hot*, respectively). This study was performed to clarify the extent of diversification between the two hybrids and the maternal ancestor. Genealogical analysis using mtDNA revealed that all 38 *Hoc/Hot* hybrids formed a branch (Branch I) with 18 of the 33 *Hoc/Hag* hybrids. No haplotype sharing was observed with the maternal ancestor. Further, microsatellite DNA analysis suggested that the members of Branch I shared the same hemiclonal genome set. The results suggested that *Hoc/Hot* hybrids originated by anomalous hybridization, or “host switching,” between *Hoc/Hag* and *Hot*, and not from interspecific hybridization between *Hoc* and *Hot*. The remaining 9 of 11 *Hoc/Hag* haplotypes and all of the 27 *Hoc* haplotypes were mixed within the genealogical tree, as if they had originated from multiple mutations. However, *Hoc/Hag* could also mate with *Hoc*. Although offspring from this host switch (Backcross-*Hoc*) have the same genome as normal *Hoc*, a part of their genome retains genetic factors capable of producing hemiclones. Consequently, when a descendant of a BC-*Hoc* hybrid mates with *Hag* males, a new hemiclone lineage will arise. Multiple haplotype revival through host switching from a single mutation in hybrids is another possible hypothesis for the observed mixing of *Hoc/Hag* haplotypes within the mtDNA genealogical tree.

Keywords
hybridogenesis, diversification of hybrids, host switching, maternal inheritance, Improving longevity through host switching backcross
1 | INTRODUCTION

Although most eukaryotes have retained sexual reproduction with recombination as a reproductive strategy, some have developed unisexual modes of reproduction, such as clonal reproduction (parthenogenesis and gynogenesis) and hemiclonal reproduction (hybridogenesis), which involve hybridization between different species (Dawley, 1989; Hubbs & Hubbs, 1932; Lampert & Schartl, 2008). In clonal modes of unisexual reproduction, females produce unreduced diploid or triploid eggs by different cytogenetic mechanisms that develop normally without any biological or genetic contribution from males, and no male offspring are produced (Dawley, 1989).

Unlike sexual reproduction, in which there is the added cost of producing of males, unisexual reproducing organisms do not incur these additional costs during reproduction (Maynard Smith, 1978). Consequently, unisexual taxa are considered to be at an advantage in terms of their ability to colonize new habitats and outcompete organisms that employ sexual reproduction (Avise, 2008). However, unlike sexually reproducing organisms, unisexual taxa are more likely to accumulate deleterious mutations (Kondrashov, 1988; Rice & Friberg, 2009). Consequently, the long-term survival of unisexual taxa that lack novel genetic adaptations to perturbations in the environment or to attacks by parasites is relatively limited (Bengtsson, 2009; Neiman & Koskella, 2009), implying that they are potentially evolutionary dead ends that are at greater risk of extinction (Bell, 1982; Bengtsson, 2009; Maynard Smith, 1986). In response to these limitations, several mechanisms have been identified that mitigate against the severe genetic disadvantages associated with a unisexual mode of reproduction (Loewe & Lamatsch, 2008; Scharl, Wilde, Schlupp, & Parzefall, 1995). For example, in the Amazon molly (Poecilia formosa), small parts of the paternal genome (microchromosomes) can remain in the oocyte during gynogenesis (Lamatsch, Nanda, Schlupp, Epplen, & Schmid, 2004; Scharl et al., 1995). In addition, polyplody has been observed in both clonal and hemiclonal species, including the tompinniax (Poeciliopsis monacha-lucida) (Cimino & Schultz, 1970; Schultz, 1969; Vrijenhoek, Dawley, Cole, & Bogart, 1989), Pelophylax water frog complex (Graf & Pelaz, 1989), stick insects (Scali, 2009), Iberian minnow (Carmona, Sanjur, Doadrio, Machordom, & Vrijenhoek, 1997), Australian carp gudgeon (Hypseleotris hybrid) (Schmidt, Bond, Adams, & Hughes, 2011), and greenling (Hexagrammos hybrid) (Kimura-Kawaguchi et al., 2014). Male hybridogenesis in which the maternal genome is discarded has also been reported in the Pelophylax water frog complex (Lehtonen, Schmidt, Heubel, & Kokko, 2013) and the Australian carp gudgeon (Schmidt et al., 2011).

In hybridogenesis, although females produce genetically identical haploid eggs without any genetic recombination, genetic variation is maintained by renewal of the paternal genome every generation. In this way, hybridogenesis compensates for the costs associated with sexual reproduction while retaining some of the benefits of clonal reproduction (Vrijenhoek, 1994). These advantages, despite involving a unisexual mode of reproduction, should enable hemiclonal animal lineages to remain viable for longer than clonal lineages.

Hybridogenesis, gynogenesis, and parthenogenesis are all considered to have originated from hybridization between different species (Lamatsch & Stöck, 2009; Vrijenhoek, Angus, & Schultz, 1977). Speciation in two geographically separated populations can occur when a contiguous population is separated by a vicariant event of some kind. Under such conditions, genetic differences gradually arise between the separated populations, often resulting in what is referred to as allopatric speciation (Coyne & Orr, 2004). In such cases, if secondary contact occurs before premating reproductive isolation has fully developed, natural hybrids will appear (Barton & Hewitt, 1989).

Although most hybrids typically have low fitness and low reproductive viability due to the inherent incompatibility of the genomes from different species, in some instances, hybrids may be able to survive by employing unisexual reproduction without the recombination of genomes (Ellstrand et al., 2010). For example, hemiclonal reproduction has recently been reported in two Hexagrammos hybrid strains (Kimura-Kawaguchi et al., 2014). The natural hybrids produce haploid eggs containing only the Hexagrammos octogrammus genome (maternal ancestor) and generate F1 hybrid-type offspring by fertilization with the haploid sperm of either Hexagrammos agrarius or Hexagrammos otakii (paternal species); in this way, the genome set of the natural hybrids is composed of a hemiclonally transmitted maternal genome and a recombined paternal genome. Similarly, because the second generations of a backcross between natural hybrids and paternal species reproduce by hybridogenesis in the same way as the maternal generation of the natural hybrids, hemiclonal reproduction is maternally inherited over successive generations by backcrossing.

loach hybrids have existed for approximately 300,000 years (Janko et al., 2005).
with paternal species. In addition, Kimura-Kawaguchi et al. (2014) also found that artificial F₁ hybrids produced by crossing pure species generated recombinant gametes, suggesting that although the artificial F₁ hybrids have the same genome composition as hemiclonal hybrids, hemiclonal hybrids do not always result from a hybridization event. In addition, hemiclonal hybrids exhibit genetic differences (mutations) that do not occur in wild-type parental species.

Maternal inheritance markers can be used to clarify when hybridization occurred. Genealogical relationships among parental Hexagrammid species were estimated using polymorphic mitochondrial and nuclear DNA markers (Crow, Kanamoto, & Bernardi, 2004). Hexagrammos agrammus and H. otakii (paternal species) are the most closely related taxa in this genus. The common ancestor of H. agrammus and H. otakii (paternal species) underwent allopatric divergence from H. octogrammus (maternal ancestor) approximately 2.2–3.6 million years ago, and H. otakii and H. agrammus underwent sympatric divergence from the common ancestor approximately 1.2–2.0 million years ago. Secondary contact between the maternal and paternal species probably occurred after sympatric speciation during the Pleistocene (Brykov & Podlesnykh, 2001; Shinohara, 1994). Hexagrammos hybridization is the only known hybridogenetic system in marine fishes inhabiting the North Pacific Ocean. The potentially low extinction potential of these Hexagrammos hybrids is considered to be due to the diversity of habitats, and the longevity, structure, and fluctuation in populations of these species would likely differ from (hemi)clonal organisms distributed in more restricted environments, such as rivers and ponds. The present study was conducted to clarify the origin and diversification of two Hexagrammos hybrids and the maternal parent species (H. octogrammus) using maternal inheritance markers.

2 MATERIALS AND METHODS

2.1 Fish sampling and species identification

For genealogical analysis of the two natural, hemiclonal, hybrid strains, H. octogrammus/H. agrammus (Hoc/Hag) and H. octogrammus/H. otakii (Hoc/Hot), and the maternal ancestor H. octogrammus (Hoc), fishes were captured using gill nets and traps on a coastal reef off Usujiri, Japan, from 2004 to 2010 (Fig. 1). Specimens were identified based on diagnostic external morphological diagnostic characteristics, such as the number of lateral lines, flap pairs, and the caudal fin shape, following Nakabo (2000) and Shinohara (1994), as described previously (Kimura-Kawaguchi et al., 2014). A total of 40 Hoc, 31 Hoc/Hag, and 38 Hoc/Hot specimens were used in the present study. Muscle or fin tissue samples were collected from the fish and preserved in 99% ethanol at −10°C until genetic analysis. The paternal species, H. agrammus and H. otakii, and the closely related Hexagrammos decagrammus, Hexagrammos lagocephalus, Hexagrammos stelleri, Pleurogrammus azonus, and Pleurogrammus monopterygius, all of which are held in the collection at the Usujiri Fisheries Station, were included in the genealogical analysis.

In addition, to estimate the allele frequencies for the paternal species of the two natural hybrid strains using microsatellites, 33 H. agrammus and 34 H. otakii were captured using hand nets while SCUBA diving on a coastal reef off Usujiri, Japan, from 2010 to 2013. Tissues from these specimens were preserved in 99% ethanol and stored at −10°C until genetic analysis.

2.2 Polymerase chain reaction conditions and mitochondrial DNA sequencing

Total genomic DNA was extracted using a Quick Gene DNA tissue kit S (Fujifilm, Japan) according to the manufacturer’s instructions and stored in a refrigerator at 4°C until use.

Three regions of the mitochondrial genome (i.e., cytochrome b [cyt b], 12S-16S rRNA, and cytochrome oxidase [CO] I) of the mitochondrial DNA of Hoc/Hag, Hoc/Hot, Hoc, and the outgroup species were sequenced (Table 1). The first two regions and the third region were amplified using the primer sets of Kimura, Yanagimoto, and Munehara (2007) and Ward, Zemlak, Innes, Last, and Hebert (2005), respectively. Polymerase chain reactions (PCRs) were performed in 50 μl volumes containing 25 μl Emerald Amp™ PCR Master Mix (Takara Bio Inc., Japan), 22 μl sterile distilled water, 0.5 μl of each 5 μmol/l primer, and 2 μl of template DNA (50–100 ng). The PCR profiles for the three regions consisted of an initial denaturation step at 94°C for 2 min, followed by 30–40 cycles of denaturation at 94°C for 30 s, annealing at 55°C, and extension at 72°C for 30 s, with a final extension step of 72°C for 7 min. After the final extension step, samples were stored at 4°C. Amplification was performed using a Takara PCR Thermal Cycler Dice (Takara Bio Inc.), and PCR products were purified using a NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel GmbH & Co. KG, Germany). PCR products were sequenced with an autosequencer (3130 Genetic Analyzer, Applied Biosystems, CA) by Macrogen Japan Corporation using the same PCR primers.

2.3 Sequence analysis

Specimen sequences were aligned using the Clustal W computer program (Higgins, Thompson, & Gibson, 1994). Genealogical analysis among haplotypes was performed using MEGA software (version 6.06; Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The nucleotide substitution model for each gene was selected using Kakusan4 (Tanabe, 2011), and sequence data were also subjected to a maximum-likelihood (ML) analysis. Phylogenetic relationships between each partition were inferred by the ML method using RAxML (version 7.2.8; Stamatakis, 2006). Nucleotide divergences were computed using the Kimura 2-parameter model (Kimura, 1980), and a genealogical tree was constructed using the neighbor-joining method (Saitou & Nei, 1987). The robustness of the topology nodes was assessed using the bootstrap method with 1000 replications (Felsenstein, 1985). Unrooted statistical parsimony haplotype networks were created to connect mitochondrial DNA (mtDNA) haplotypes using TCS 1.21 (Clement, Posada, & Cradell, 2000).
Allelic analysis using microsatellite DNA

In hybridogenesis, nuclear DNA inherited from the maternal ancestor is maternally inherited in the same way as mtDNA, which means that microsatellite marker is also well suited for genealogical analysis in hemi-clone organisms. To examine the sharing of alleles among the natural hybrids (Hoc/Hag and Hoc/Hot) and Hoc, three highly polymorphic microsatellite loci (hexoc 6, hexoc 14, and hexoc 21) were examined (Table 1, Kimura-Kawaguchi et al., 2014). The methods used for the amplification of microsatellite DNA and genotyping of PCR products were the same as those employed in a previous study (Kimura-Kawaguchi et al., 2014).

RESULTS

3.1 Genealogical analysis of Hexagrammos octogrammus and the two natural hybrid strains using mtDNA

Nucleotide sequences were obtained for a total of 2,498 base pairs (bp), 994 bp of the Cyt b region, 918 bp of the 12S-16S rRNA region, and 586 bp of the COI region. A total of 35 haplotypes (including subhaplotypes defined as the same arrangement of nucleotides except for a synonymous substitution) were identified in sequences.
from 109 individuals: 27 haplotypes from 40 Hoc individuals, 11 haplotypes from 31 Hag/Hag individuals, and two haplotypes from 38 Hoc/Hot individuals (Table 2). The identified haplotypes had 63 polymorphic sites. Sequences of each haplotype and of each outgroup were deposited in GenBank under the accession numbers listed in Table S1.

The maximum-likelihood analysis showed that the two natural hybrid strains (i.e., Hoc/Hag and Hoc/Hot) formed a cluster within Hoc—separately from both Hot and Hag—implying that both hybrid strains had Hoc as the maternal species (Fig. 1). Within the Hoc cluster, the Hoc/Hag and Hoc haplotypes were mixed with the other samples in the genealogical tree, with Hoc/Hag exhibiting multiple hemiclones as if they had originated independently from separate mutations. Conversely, all of the Hoc/Hot haplotypes belonged to a single branch (Branch I) within the clade consisting of a combination and closely related Hoc, Hag/Hag, and Hoc/Hot haplotypes.

A total of 32 haplotypes were included in the haplotype network for Hoc, Heg/Hag, and Hoc/Hot individuals (Fig. 2). Two of the haplotypes (hap 3 and hap 15) contained two and three subhaplotypes (subhap 3-1 and 3-2, subhap 15-1, 15-2, and 15-3), which could be distinguished from each other by a non-synonymous substitution. From 1 to 20 mutational steps were found among the 11 Hoc/Hag haplotypes. Three of the haplotypes that Hoc/Hag shared with Hoc were connected by four and seven mutational steps. All 38 of the Hoc/Hot hybrids and 18 Hoc/Hag hybrids were clustered in Branch I, and 37 of the Hoc/Hot hybrids and 17 of the Hoc/Hag hybrids shared hap 1. A total of 56 (70.8%) of the 79 hybrids used in the present study were grouped in one cluster, and there were no haplotypes that shared the Hoc paternal ancestor. The extent of genetic divergence in Branch I was very low, with only one substitution in 2,498 bp detected in 12S-16S rRNA. Conversely, the minimum number of mutational sites between Hoc and Hoc/Hot (hap 1 and subhap 15-2) was one substitution in 12S-16S rRNA and two substitutions in COI. Thus, the results showed that the mtDNA of Hoc/Hot was more similar to Hoc/Hag than it was to the maternal ancestor, Hoc.

### 3.2 Sharing of alleles among the two natural hybrid strains and *Hexagrammos octagranum*

The characteristics of the microsatellite loci used for genotyping were shown in Table 3. In the parental species (Hag, Hot, and Hoc), all three loci had sufficiently high heterozygosities and low Hardy–Weinberg equilibrium deviation probabilities, which meant that the microsatellite loci were well suited for genetic analysis and that there were marked differences in the size of alleles among parental species (Fig. 3). Conversely, in the two natural hybrids, the observed heterozygosities approached to 1, except for hexoc 6 in Hoc/Hag, and the probability of deviation from Hardy–Weinberg equilibrium assessed by a chi-squared test was high (Table 3). This finding was illustrated by the natural hybrids that possessed a hemiclonal genome set inherited from the maternal ancestor (Hoc) and a different genome set inherited from the paternal species (Hot or Hag).

Interestingly, 37 of the 38 Hoc/Hot hybrids shared the same alleles at hexoc 6 (116 bp) and hexoc 21 (148 bp), and all 18 of the Hoc/Hag hybrids in Branch I also shared these allele sets (Table 4). In 55 hybrids, hexoc 14 was either 122 bp or 124 bp in length; the one exception in Branch I, ID399, shared alleles at hexoc 14 and hexoc 21, but the alleles at hexoc 6 were unique. Because the 116-bp allele at hexoc 6 in 37 Hoc/Hot hybrids was smaller than the size range observed in *H. otakii* (Fig. 3, Table 3), the shared allele in Branch I was likely hemiclonally inherited from the maternal ancestor, Hoc. The 148-bp allele at hexoc 21 in all 56 hybrids in Branch I was larger than the size ranges observed in both Hot and Hag, implying that the common allele was also hemiclonally inherited from Hoc. The 122-bp or 124-bp alleles at hexoc 14 in 18 Hoc/Hag hybrids in Branch I were larger than the size range observed in Hag, implying that these alleles also appeared to be hemiclonally inherited from Hoc. The allele frequencies of the alleles (116 bp at hexoc 6, 122 bp or 124 bp at hexoc 14, and 148 bp at hexoc 21) that were shared among the hybrids were all less than 10% in Hoc (Table 3). In addition, the specific allele observed at hexoc 6 in ID399 probably varied from homologous alleles, as microsatellite DNA occasionally mutates during generation changes (Guichoux et al., 2011). Supposing that the 122-bp and 124-bp alleles at hexoc 14 were homologous and that they slipped during several generation changes, then the common homologous allele set at the three loci would occur at a frequency of less than 0.04%. Such a low rate suggested that all of the individuals in Branch I, that is, 18 Hoc/Hag and 38 Hoc/Hot, originated from the same hybridogen.

Regarding the other 13 Hoc/Hag hybrids, three individuals (ID233, ID782, and ID888; all hap 22) shared the same alleles at the three loci examined in this study; judging from the size ranges of the alleles in Hag and Hoc, the alleles were considered to have been hemiclonally inherited from Hoc. Similarly, two individuals (ID875 and ID877; both hap 23) shared the same maternal alleles.

### Table 1: PCR primer sequences used in the present study

| Locus (accession no.) | Sequence 5′—3′ (upper, forward; lower, reverse) |
|-----------------------|-----------------------------------------------|
| cytochrome b (AF087409, 087410, 087412) | ATGGCAAGGCTACAGAAAA TCTAAAGGCTGTGGTTCT |
| 12-16S rRNA (AF084629, 084631) | CGGGAACTACAGAAAA GAGCCTGAGAAGA |
| COI (DQ107581–DQ108334) | TCAACAAACCAAAAGACATGGCAGCAGTAGCTTGGGTGCTGGCAAGATCA |
| hexoc 6 (AB690324) | GGTATCTTCTTCTGCTCTAG AAAGTTTGTGCTCTACAGGACTGAG |
| hexoc 14 (AB690329) | CGGGTAGTCGAAGCATGAG TTTTGACTTGTTGGTTCTT |
| hexoc 21 (AB690332) | CACATTCTACAAACAGCTTG AGTTATGACATGAGCTGAAGA |
These five individuals shared the same allele at hexoc 6 and hexoc 21, even though both haplotypes had a different allele at hexoc 14. Only 1 bp of the 2,498 bp of mtDNA analyzed was found to differ between hap 22 and hap 23 (Table 2), implying that there was a high possibility that hap 22 and hap 23 originated from the same hybridogen.

Although ID278 (hap 7) and ID217 (hap 3-2) shared alleles at every locus, the 112-bp allele that was common to hexoc 21 was inherited from Hag, judging from the size range of the alleles in both parental species (Fig. 3, Table 4). Of the remaining 7 Hoc/Hag hybrids, none shared any alleles at the three loci with the other hybrids.

4 | DISCUSSION

4.1 | Hoc/Hat born from host switch

Because both hybridogenetic hybrids (Hoc/Hat and Hoc/Hag) had Hoc mtDNA haplotypes, H. octogrammus (Hoc) is considered to be the maternal ancestor of these hybrids (Crow et al., 2007; Kimura et al., 2007). Although morphological (Shinohara, 1994) and molecular studies (Crow et al., 2004) have demonstrated that H. agrammus (Hag) and H. otakii (Hat) are the closest relatives (sister species), hybrids between these two species have rarely ever been observed in areas where these species are sympatrically distributed (Crow et al., 2007; Kimura & Munehara, 2010; Kimura & Munehara, 2011). Conversely, natural hybrids (Hoc/Hat and Hoc/Hag) between distant species have been shown to propagate by hemiagonal reproduction, with hybridization occurring after secondary contact (Kimura-Kawaguchi et al., 2014). Because hybrids typically have low fitness and survivability, parental species typically avoid hybridization by reinforcing species recognition, as failure to do so would result in these species interbreeding and forming a single species (Coyne & Orr, 2004; Ellstrand et al., 2010). Hemiclonal reproduction is one mechanism that allows hybrids to survive while avoiding genetic recombination (Burt & Trivers, 2006). Because all of the hemiclonal hybrids are fertile females capable of breeding with males of the paternal species, the two natural hybrid populations can be considered to be independent of Hoc, Hat, and Hag (Kimura-Kawaguchi et al., 2014). The hybrids produce diploid eggs containing only the Hoc genome (maternal ancestor), as the paternal genome is discarded and F1 hybrid-type offspring are generated by fertilization with haploid sperm from either Hag or Hoc (paternal ancestor); the entire paternal genome is displaced at every generation change. When a Hoc/Hat hybrid mates with a Hat male, the entire paternal genome of the descendants will change from Hag to Hoc. The genome of the descendants will therefore constitute the hemiclonal Hoc genome and a normal Hat genome, to produce the Hoc/Hat hybrids.

Genealogical analysis using mtDNA revealed that Hoc/Hat hybrids formed a cluster with Hoc/Hag (Branch 1), which did not contain any Hoc individuals (Fig. 1). This branch (Branch I) was supported by high bootstrap values. In the microsatellite DNA analyses, the individuals in Branch I shared a common allele set consisting of three loci, indicating that the Hoc/Hat hybrids inherited an identical hemiclonal genome set from Hoc/Hag. The low levels of diversity observed in the mtDNA and microsatellite DNA analyses showed that Hoc/Hat hybrids originated by anomalous hybridization events between Hoc/Hag and Hat. Although it was previously considered that the occurrence of numerous hybrids was the result of rampant hybridization between Hoc and Hat (Crow et al., 2007), Hoc/Hat hybrids are unlikely to have appeared due to interspecific hybridization. Changes in the species of the sperm donor among hybrids employing (hemi)clonal reproduction are referred to as “host switching” (Choleva, Apostolou, Rab, & Janko, 2008). Host switching has been reported in other unisexual fish lineages (e.g., Squalius hybrids: Alves, Coelho, Collins-Pereira, & Dowling, 1997; Poecilia hybrids: Niemitz, Kreutzfeldt, Schartl, Pazefall, & Schlupp, 2002; Schlupp, Parzefall, & Schartl, 2002; Cobitis hybrids: Janko et al., 2005; Poeciliopsis hybrids: Cunha, Coelho, Carmona, & Doadrio, 2004; Mateos & Vrijenhoek, 2002, 2005; Sousa-Santos, Collins-Pereira, & Almada, 2006) and amphibiens (e.g., Ambystoma hybrids: Hedges, Bogart, & Maxon, 1992; Spolsky, Phillips, & Uzzell, 1992; Pelophylax: Arano, Llorente, Herrero, Sanchiz, 1995).

Why do hybrids change the species of the sperm donor? Host switching may arise when the primary hybrids require a sperm donor after the extinction of the parental species. However, Hexagrammos hybrids are widespread in the North Pacific Ocean, and both parental species (Hag and Hat) coexist. Thus, while pre-reproductive isolation between the paternal species of this genus is likely to have occurred due to subtle differences in habitat preference and parental care (Kimura & Munehara, 2010, 2011), breeding season and site preference are known to overlap among Hag, Hat, and Hoc (Munehara, Takenaka, & Takenaka, 2000). For example, Hoc and Hag inhabit shallow seaweed beds, while Hat inhabits deeper reefs and sandy bottomed environments where seaweeds are scarce (Kimura & Munehara, 2010, 2011). Hexagrammos species employ breeding territories and the polyandrous females visit the multiple males' territories where they spawn and produce adhesive egg masses that are then guarded by the males (Munehara, Kanamoto, & Miura, 2000; Munehara, Takenaka, & Takenaka, 2000). Females show a preference for large males that are good guardians (Kvarnemo & Simmons, 2013; Maan & Seehausen, 2011). Because Hat males with territories have larger bodies and larger egg masses than Hag males (Munehara, Kanamoto, et al., 2000), Hoc/Hag hybrids may prefer to mate with Hat males. While reproductive isolation is considered to be effective for maintaining species, some anomaly must have allowed Hoc/Hag hybrids to achieve host switching at some point in the evolutionary history of these species.

In Branch I, hap 1 was the most dominant haplotype and the difference between hap 1 and hap 2 was only one mutational step in 2,498 bp (Fig. 2). In addition, an identical mutation in hexoc 14 was found in both hap 1 and hap 2 of Hoc/Hag hybrids. These findings strongly suggest that host switching first occurred as hap 1 of Hoc/Hag became more widespread, and then hap 1 Hoc/Hat increased in number. Given that the direction of mate choice was from Hag to Hat by Hoc/Hag, and that Hoc/Hat only occurred in Branch I, the reverse host
### Table 2

Variable nucleotide sites in 2,498 bp of 35 haplotypes, including three subhaplotypes in the three mtDNA regions assayed in *Hoc*, *Hoc/Hag*, and *Hoc/Hot*

| Nucleotide position | Cyt b                  |
|---------------------|------------------------|
| 1 2 2 2 2 2 3 3 4 4 | 5 5 5 5 5 5 6 6 7 7 7 7 7 8 8 9 9 9 9 |
| 8 2 4 4 7 8 3 4 0 5 3 4 5 6 7 9 6 8 2 3 5 6 7 8 9 0 3 3 4 |

| Haplotypes | 6 2 4 9 3 5 7 8 8 6 4 0 5 7 6 7 9 1 6 5 6 2 7 2 7 0 0 6 2 |
|------------|---------------------------------------------------------------|
| hap1       | A C C C A T C A T C G A A T C G A T G A A C C C T T G C T       |
| hap2       | . . . . . . G . . . . . A . . . . . T                               |
| hap3-1     | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap3-2     | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap4       | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap5       | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap6       | G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap7       | T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap8       | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap9       | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap10      | T . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap11      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap12      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap13      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap14      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap15-1    | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap15-2    | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap15-3    | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap16      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap17      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap18      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap19      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap20      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap21      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap22      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap23      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap24      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap25      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap26      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap27      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap28      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap29      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap30      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap31      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |
| hap32      | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . |

Dots indicate nucleotide identity with the haplotype 1 sequence.

Switch probably did not occur. *Hoc/Hag* and *Hoc/Hot* shared both hap 1 and hap 2. It is currently not clear whether host switching occurred in hap 2 again or not.

Thus, mtDNA and microsatellite DNA are considered to have mutated during generation changes. Assuming that the molecular clock of mtDNA in *Hexagrammos* is 1.5–2.5% per million years (Crow et al.,
Munehara et al. (2004; Meyer, Kocher, Basasibwaki, & Wilson, 1990), host switching likely first occurred 17,000–27,000 years ago. Assuming a mutation rate for microsatellite DNA of $10^{-3}$–$10^{-4}$ per single frame shift slippage (Guichoux et al., 2011), the first host switch occurred approximately 2,000–20,000 years ago (assuming a generation period of 2 years).
There is a strong possibility that Hoc/Hag hybrids changed sperm donors to both Hot and Hoc, although the evidence is somewhat inconclusive. We consider that the genome of Hoc/Hag hybrids is constituted by both Hoc and Hag genomes, and this mode of host switching (i.e., Hoc/Hag crossing with Hoc instead of Hag) may be more likely than Hoc/Hag hybrids mating with Hot. When a Hoc/Hag hybrid mates with a male of the maternal species, Hoc, the offspring (backcrossed Hoc; BC-Hoc) become Hoc (Fig. 4). BC-Hoc has the same morphological characteristics as normal Hoc, but the two Hoc genomes differ somewhat with respect to the genetic material they contain. We reported previously that natural Hoc/Hag hybrids produced haploid eggs containing only the maternal genome, whereas artificial F₁ hybrids (i.e., crosses between Hoc and Hag) produced haploid eggs containing a recombinant genome (Kimura-Kawaguchi et al., 2014). The artificial F₁ hybrids had the same genome composition as the natural hybrids, but the reproductive system differed between the two hybrids; that is, the Hoc genome of the natural hybrids carried genetic factors that facilitated hybridogenesis, which were not present in the normal Hoc genome. Although this mechanism has not yet been resolved at a cytological level, we found that BC-Hoc individuals produced recombinant gametes (Kimura-Kawaguchi et al., 2014; in preparation). In other words, when Hexagrammos species have homogeneous genomes, meiosis occurs normally in germ cells.
without any genome conflicts. Clonal or hemiclonal reproduction is thus one way in which the low survivability resulting from genome heterogeneity can be avoided in hybridizing organisms (Burt & Trivers, 2006; Jones & Pašakinskienė, 2005). However, hybridogenesis is very rare, occurring only when specific limitations imposed by genetic compatibility have been removed in conjunction with as yet unknown genetic factors.

BC-Hoc individuals can be discriminated from normal Hoc individuals, as the backcrosses possess genetic factors that are capable of inducing hybridogenesis. When fertile BC-Hoc males mated with normal Hoc females, the progeny inherited the mtDNA haplotype of the normal Hoc. Consequently, a Hoc individual possessing specific genetic factors capable of inducing hybridogenesis (carrier) is considered to have altered the mtDNA haplotype. Moreover, when a carrier mates with a Hag male, a new hemiclone lineage will arise. Such hemiclone revival through host switching can increase the diversity of Hoc/Hag haplotypes, even if the mutation facilitating hybridogenesis may have occurred only once. Multiple haplotype revival through host switching from a single mutation in hybrids is another possible hypothesis for the observed mixing of Hoc/Hag haplotypes within the mtDNA genealogical tree.

High levels of mtDNA diversity were also found in *P. monachalucida* (Quattro, Avise, & Vrijenhoek, 1991, 1992). In the *Poeciliopsis* complex, involvement of host switching through a third species (*P. viriosa*) appeared to generate new hemiclonal lineages (Mateos & Vrijenhoek, 2002). However, Schultz (1973) demonstrated that it was very difficult to reproduce such a clonal reproductive lineage by artificial hybridization between parental species. The intact genome of the maternal species is transferred into haploid eggs and the genome of the paternal species is eliminated. However, this means that at least two extraordinary steps must occur during oogenesis: elimination of the paternal genome and duplication of the maternal genome (Ogielska, 1994, 2009; Tunner & Heppich-Tunner, 1991; Vinogradov, Borkin, Gunther, & Rosanov, 1990). Some of the genetic factors required for inducing hybridogenesis may be located at different loci and distributed on different chromosomes during recombination in BC-Hoc. It is thus likely that only when a Hoc genome bearing the correct set of genetic factors hybridizes with a Hag genome, a new hemiclone lineage can possibly arise.

### 4.3 Improving longevity through host switching in hemiclones

In organisms that employ unisexual reproduction, individuals can produce offspring without any genetic contribution from males, and no male offspring are produced. As a result, once they arise, unisexual species are considered to be at an advantage when colonizing new habitats or when competing with sexually reproducing...
| Species name | ID of specimens | Genotypes of mtDNA | Haplotypes of mtDNA | Genotypes of mtDNA | Haplotypes of mtDNA | Genotypes of mtDNA | Haplotypes of mtDNA | Genotypes of mtDNA | Haplotypes of mtDNA |
|--------------|-----------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|
| Hoc          | 21 17           | 112 116 118 128    | 146 162 146 162     | 110 116 82 124     | 104 148 104 148     | 129 104 148 104 148 | 130 104 148 104 148 | 136 104 148 104 148 | 130 104 148 104 148 |
| 22 15-2      | 124 138 122 136 | 160 164            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 23 6         | 104 128 114 128 | 162 172            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 24 13        | 98 108 120 138  | 136 168            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 26 24        | 94 96 118 122    | 152 160            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 27 15-2      | 132 142 118 132  | 158 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 31 15-1      | 134 146 114 118  | 144 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 32 28        | 98 98 118 122    | 144 144            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 33 29        | 110 134 114 126  | 168 176            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 38 30        | 112 120 118 134  | 150 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 39 11        | 94 96 118 122    | 150 160            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 270 31       | 134 146 128 128  | 146 150            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 271 9        | 138 144 112 134  | 142 148            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 272 3-2      | 110 140 112 134  | 134 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 273 5        | 112 138 128 136  | 144 164            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 276 3-2      | 92 106 114 118   | 142 148            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 280 5        | 102 112 112 114  | 138 160            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 282 18       | 98 112 122 134   | 154 186            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 283 5        | 108 114 128 134  | 170 174            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 284 15-3     | 92 116 112 114   | 132 150            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 285 21       | 116 122 122 128  | 150 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 286 14       | 94 94 118 128    | 144 144            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 288 15-2     | 106 124 114 134  | 158 162            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 289 5        | 106 112 116 116  | 148 156            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 290 10       | 92 110 114 128   | 150 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 291 19       | 92 94 118 130    | 158 158            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 293 24       | 108 154 116 122  | 152 160            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 294 5        | 102 130 88 130   | 156 162            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |
| 296 32       | 94 102 114 138   | 160 160            |                     | 116 116 88 116 112 | 144 136 144 136     |                     |                     |                     |                     |

(Continues)
This theory is illustrated by the rapid expansion of Branch I in which hap1 was dominant (17 of 31 Hoc/Hag and 37 of 38 Hoc/Hot). Within the context of the long-term survival of a population or species, unisexual species must mitigate the risks posed by the accumulation of deleterious mutations (Kondrashov, 1988; Welch & Meselson 2000). In hybridogenesis, the genome derived from the paternal species is renewed every generation and genetic variation is maintained; in this respect, it is different from gynogenesis in which an entire genome set is inherited by offspring. In addition, gametes are produced through recombination in sexually reproducing organisms, but not in hemiclonal systems when homologous genomes are combined. This is another advantage of hybridogenesis. Deleterious mutations that have accumulated in a hemiclone can be dispersed by recombination in carriers. Such purging of deleterious mutations is possible when hybridogens coexist with maternal species. This episodic host switching ensures that the longevity of the hemiclone lineage is improved by increasing genetic variability, provided that the maternal species continues to inhabit the hybrid zone or occurs in adjacent habitats. When did the genetic factors inducing hybridogenesis come into existence? The paternal species Hot and Hag diverged sympatrically approximately 1.2–2.0 million years ago (Crow, Munehara, & Bernardi, 2010). The mutations producing these genetic factors may possibly have arisen before speciation.

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DATA ACCESSIBILITY

Accession numbers (DDBJ) of the mtDNA sequences and the genotypes of microsatellite DNA data for specimens used in this study are shown in Table S1 and Table 4, respectively. Morphological data for specimens are provided in the supporting information for Kimura-Kawaguchi et al. (2014). Additional DDBJ numbers are presented in the supporting information for Kimura-Kawaguchi et al. (2014). All other data are available from the authors on request.

CONFLICT OF INTEREST

None declared.

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FIGURE 4 Results for hybridizations occurring among three Hexagrammos species and two hemiclonal hybrids. Uppercase letters superimposed on fish represent the genomes of each species, with asterisks indicating that the genome possesses the genetic factor responsible for inducing hybridogenesis. (A and B) Represent a normal backcross of hemiclonal hybrids. (C) Represents hybridization between Hoc and Hag. The $F_1$ offspring (Hoc × Hag) produce gametes that have undergone recombination, but the descendants of the $F_1$ offspring will disappear because genetic introgression among the parental species via two hybrid populations does not occur (Kimura-Kawaguchi et al., 2014). (D) Represents host switching, which generated Hoc/Hot. The rectangle contains hybridization events that have not yet been observed. When a Hoc/Hag hybrid mates with a male of the maternal species, the offspring (backcrossed Hoc) become Hoc (carriers). After several generations, if these carriers mate with Hag, the offspring may produce a new hybridogenetic strain.
Ellstrand, N. C., Briggs, D., Kaus, A., Lubinsky, P., McDade, L. A., Preston, K., Prince, L. M., Regan, H. M., Rorive, V., Ryder, O. A., & Schiereneck, K. A. (2010). Got hybridization? A multidisciplinary approach for informing science policy. BioScience, 60, 384–388.

Felsenstein, J. (1985). Confidence-limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783–791.

Graf, J. D. & Pelaz, M. P. (1989). Evolutionary genetics of the Rana esculenta complex. In R. M. Dawley & J. P. Bogart (Eds.), Evolution and ecology of unisexual vertebrates (pp. 289–301). New York, NY: New York State Museum.

Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Lepoivre, C., Malusa, T., Revardel, E., Salin, F., & Petit, R. J. (2011). Current trends in microsatellite genotyping. Molecular Ecology Resources, 11, 591–611.

Hedges, S. B., Bogart, J. P., & Maxon, L. R. (1992). Ancestry of unisexual salamanders. Nature, 356, 708–710.

Higgins, D., Thompson, J., & Gibson, T. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22, 4673–4680.

Hubbs, C. L., & Hubbs, L. C. (1932). Apparent parthenogenesis in nature, in a form of fish of hybrid origin. Science, 76, 628–630.

Itono, M., Morishima, K., Fujimoto, T., Bando, E., Yamaha, E., & Arai, K. (2006). Premeiotic endomitosis produces diploid eggs in the natural clone loach, Misgurnus anguillicaudatus (Teleostei: Cobitidae). Journal of Experimental Zoology Part A: Comparative Experimental Biology, 305, 513–523.

Janko, K., Bohlen, J., Lamatsch, D., Flajšhans, M., Epplen, J. T., Ráb, P., Kotlik, P., & Šlechtová, V. (2007). The gynogenetic reproduction of diploid and triploid hybrid spined loaches (Cobitis: Teleostei), and their ability to establish successful clonal lineages on the evolution of polyploidy in asexual vertebrates. Genetica, 131, 185–194.

Janko, K., Culling, M. A., Ráb, P., & Kotlik, P. (2005). Ice age cloning – comparison of the Quaternary evolutionary histories of sexual and clonal forms of spiny loaches (Cobitis: Teleostei) using the analysis of mitochondrial DNA variation. Molecular Ecology, 14, 2991–3004.

Jones, N., & Pašakinskienė, I. (2005). Genome conflict in the Gramineae. New Phytologist, 165, 391–409.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16, 111–120.

Kimura, R. M., & Munehara, H. (2010). The disruption of habitat isolation among three Hexagrammos species by artificial habitat alternations that create mosaic-habitat. Ecological Research, 25, 41–50.

Kimura, R. M., & Munehara, H. (2011). Spawning substrata are important for breeding habitat selection but do not determine premating reproductive isolation in three sympatric Hexagrammos species. Journal of Fish Biology, 78, 112–126.

Kimura, M., Yanagimoto, T., & Munehara, H. (2007). Maternal identification of hybrid eggs in Hexagrammos spp. by means of multiplex amplified product length polymorphism of mitochondrial DNA. Aquatic Biology, 1, 187–194.

Kimura-Kawaguchi, M., Horita, M., Abe, S., Arai, K., Kawata, M., & Munehara, H. (2014). Identification of hemi-clonal reproduction in three species of Hexagrammos marine reef fishes. Journal of Fish Biology, 85, 189–209.

Kondrashov, A. S. (1988). Deleterious mutations and the evolution of sexual reproduction. Nature, 336, 435–440.

Kvarnemo, C., & Simmons, L. W. (2013). Polyandry as a mediator of sexual selection before and after mating. Philosophical Transactions of the Royal Society, 368, 2012–2042.

Lamatsch, D. K., Nanda, I., Schlupp, I., Epplen, J. T., & Schmid, M. (2004). Distribution and stability of supernumerary microchromosomes on natural populations of the Amazon molly, Poecilia formosa. Cytogenet Genome Resources, 106, 189–194.

Loewe, L., & Lamatsch, D. K. (2008). Quantifying the threat if extinction from Muller’s ratchet in the diploid Amazon molly (Poecilia formosa). BMC Evolutionary Biology, 8, 88.

Lynch, M., & Gabriel, W. (1990). Mutation load and the survival of small populations. Evolution, 44, 1725–1737.

Maan, M. E., & Seehausen, O. (2011). Ecology, sexual selection and speciation. Ecology Letters, 14, 591–602.

Matsch, D. K., & Stöck, M. (2009). The evolutionary biology of parthenogenesis. In I. Schön, K. Martens & P. V. Dijk (Eds.), Lost sex the evolutionary biology of parthenogenesis (pp. 399–432). Dordrecht Heidelberg London, UK and New York, NY: Springer.

Meyer, M. M., Salzburger, W., & Schartl, M. (2006). Hybrid origin of sword-tail species (Teleostei: Xiphophorus clemenciae) driven by sexual selection. Molecular Ecology, 15, 721–730.

Munehara, H., Kanamoto, Z., & Miura, T. (2000). Spawning behavior and the evolution of polyploidy in the fish genus Poeciliopsis. Journal of Heredity, 91, 1–8.

Maynard Smith, J. (1978). The evolution of sex. Cambridge, UK: Cambridge University Press.

Maynard Smith, J. (1986). Contemplating life without sex. Nature, 324, 300–301.

Maynard Smith, J. (1992). Age and the unequal lineages. Nature, 356, 661–662.

Meyer, A., Kocher, T. D., Basasibwaki, B., & Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial-DNA sequences. Nature, 347, 550–553.

Meyers, M. M., Salzburger, W., & Schartl, M. (2006). Hybrid origin of sword-tail species (Teleostei: Xiphophorus clemenciae) driven by sexual selection. Molecular Biology, 15, 721–730.

Munehara, H., Kanamoto, Z., & Miura, T. (2000). Spawning behavior and interspecific breeding in three Japanese greenlings (Hexagrammidae). Ichthyological Research, 47, 287–292.

Niemeitz, A., Kreutzfeldt, R., Schartl, M., Pazefall, J., & Schlupp, I. (2002). Male reproductive isolation in three sympatric Hexagrammos species by artificial habitat alternations that create mosaic-habitat. Ecological Research, 25, 41–50.

Ohno, S. (1970). Evolution by gene duplication. New York, NY: Springer Verlag.

Quattro, J. M., Avise, J. C., & Vrijenhoek, R. C. (1991). Molecular evidence for multiple origins of hybridogenetic fish clones (Poeciliidae: Poeciliopsis). Genetica, 127, 391–398.

Quattro, J. M., Avise, J. C., & Vrijenhoek, R. C. (1992). An ancient clonal lineage in the fish genus Poeciliopsis (Atheriniformes: Poeciliidae). Proceedings of the National Academy of Sciences the United States of America, 89, 348–352.

Rice, W. R., & Friberg, U. (2009). A graphical approach to lineage selection between. In I. Schön, K. Martens & P. V. Dijk (Eds.), Lost sex the evolutionary biology of parthenogenesis (pp. 399–432). Dordrecht Heidelberg London, UK and New York, NY: Springer.
the evolutionary biology of parthenogenesis (pp. 75–98). Dordrecht Heidelberg London, UK and New York, NY: Springer.
Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4, 406–425.
Scali, V. (2009). Metasexual stick insects: Model pathways to losing sex and bringing it back. In I. Schöhn, K. Martens & P. V. Dijk (Eds.), Lost sex the evolutionary biology of parthenogenesis (pp. 317–346). Dordrecht Heidelberg London, UK and New York, NY: Springer.
Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J. T., Schmid, M., & Parzefall, J. (1995). Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. Nature, 373, 68–71.
Schartl, M., Wilde, B., Schlupp, I., & Parzefall, J. (1995). Evolutionary origin of a parthenoform, the Amazon molly Poecilia formosa, on the basis of a molecular genealogy. Evolution, 49, 827–835.
Schlupp, I., Parzefall, J., & Schartl, M. (2002). Biogeography of the Amazon molly, Poecilia formosa. Journal of Biogeography, 29, 1–6.
Schmidt, D. J., Bond, N. R., Adams, M., & Hughes, J. M. (2011). Cytonuclear evidence for hybridogenetic reproduction in natural populations of the Australian carp gudgeon (Hypseleotris: Eleotridae). Molecular Ecology, 20, 3367–3380.
Schultz, R. J. (1969). Hybridization, unisexualy, and polyploidy in the teleost Poeciliopsis (Poeciliidae) and other vertebrates. American Naturalist, 103, 605–619.
Schultz, R. J. (1973). Unisexual fish: Laboratory synthesis of a "species". Science, 179, 180–181.
Shinohara, G. (1994). Comparative morphology and phylogeny of the suborder Hexagrammoidei and related taxa (Pisces: Scorpaeniformes). Memoirs of the Faculty of Fisheries Hokkaido University, 41, 1–97.
Sousa-Santos, C., Collares-Pereira, M. J., & Almada, V. C. (2006). Evidence of extensive mitochondrial introgression with nearly complete substitution of the typical Squalius pyrenaicus-like mtDNA of the Squalius alburnoides complex (Cyprinidae) in an independent Iberian drainage. Journal of Fish Biology, 68(B), 292–301.
Spolsky, C., Phillips, C. A., & Uzzell, T. (1992). Gynogenetic reproduction in hybrid mole salamanders (genus Ambystoma). Evolution, 46, 1935–1944.
Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22, 2688–2690.
Stöck, M., Lampert, K. P., Müller, D., Schlupp, I., & Schartl, M. (2010). Monophylectic origin of multiple clonal lineages in an asexual fish (Poecilia formosa). Molecular Ecology, 19, 5204–5215.
Tanabe, A. S. (2011). Kakusan4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. Molecular Ecology Resources, 11, 914–921.
Tunner, H. G., & Heppich-Tunner, S. (1991). Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. Naturwissenschaften, 78, 32–34.
Uzzell, T. M., & Berger, L. (1975). Electrophoretic phenotypes of Rana lessonae, Rana ridibunda, and their hybridogenetic associate Rana esculenta. Proceedings of the National Academy of Sciences the United States of America, 127, 13–24.
Vinogradov, A. E., Borkin, L. I., Gunther, R., & Rosanov, J. M. (1990). Genome elimination in diploid and triploid Rana esculentus males: Cytological evidence from DNA flow cytometry. Genome, 33, 619–627.
Vollf, J. N. (2005). Genome evolution and biodiversity in teleost fish. Heredity, 94, 280–294.
Vrijenhoek, R. C. (1994). Unisexual fish: Model systems for studying ecology and evolution. Annual Review of Ecology and Systematics, 25, 71–96.
Vrijenhoek, R. C., Angus, R. A., & Schultz, R. J. (1977). Variation and heterozygosity in sexually vs. clonally reproducing populations of Poeciliopsis. Evolution, 31, 767–781.
Vrijenhoek, R. C., Dawley, R. M., Cole, C. J., & Bogart, J. B. (1989). A list of the known unisexual vertebrates. In R. M. Dawley & J. B. Bogart (Eds.), Evolution and ecology of unisexual vertebrates (pp. 19–23). Albany, NY and New York, NY: New York State Museum.
Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia’s fish species. Philosophical Transactions of the Royal Society, 360, 1847–1857.
Welch, D. M., & Meselson, M. (2000). Evidence for the evolution of Bdelloid rotifers without sexual reproduction or genetic exchange. Science, 288, 1211–1215.

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