Visual-based male mate preference for conspecific females in mutually ornamented fish: possible importance of species recognition hypothesis

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Abstract

Because sexual selection typically acts on males, evolution of conspicuous ornamentation in females has been understudied. Genetic correlation between sexes and sexual or social selection on females have been proposed to explain female ornamentation, but they cannot fully explain observed patterns in nature such as female ornamentation in non-territorial, promiscuous species. The species recognition hypothesis, which postulates that ornamentation is adaptive because it prevents maladaptive hybridization, might plausibly explain female ornamentation. We examined the possibility of this hypothesis in two sympatric, non-territorial, promiscuous fish species. Both sexes of *Tribolodon hakonensis* and *T. sachalinensis* display species-specific conspicuous coloration in the breeding season. We conducted visual-based mate choice experiments using *T. hakonensis* males and compared their association times between conspecific and heterospecific females. Males spent more time near conspecifics, indicating that *T. hakonensis* males used visual cues to recognize conspecifics. Because the females presented to the males did not differ in body size, shape and behavior between the two species, the male preference for conspecifics was probably based on the females' nuptial coloration. These results suggest that female ornamentation may evolve or be maintained by not only sexual or social selection within a species but also interspecies interactions (e.g. hybridization).

Keywords male mate choice ・ sexual monomorphism ・ sexual isolation ・ breeding coloration ・ Far Eastern daces

Introduction

Elaborate ornamentation has been well documented in male animals to have evolved through mate choice or intraspecific competition (i.e. sexual selection Darwin 1871; Andersson 1994). In many species across taxa, females also display elaborate ornamentation (Kraaijeveld et al. 2007). The emphasis on sexual selection in males has led to the notion that female ornamentation is non-adaptive; rather, it is merely a by-product of male ornamentation due to genetic correlations between the sexes (Lande 1980). This genetic correlation hypothesis requires male ornamentation, possibly evolved through sexual selection. However, a comparative study across >3,000 birds showed that the elaboration of female coloration positively correlates with male coloration, but not with male sexual selection which was quantified by social polygyny, male-biased sexual size dimorphism and lack of paternal care. Rather, male sexual selection leads to sexual dichromatism with bright colored males and dull colored females (Dale et al. 2015). Hence, male sexual selection alone may not explain the wide prevalence of female ornamentation. Alternatively, recent research on female ornamentation has stressed the importance of sexual selection in females (Kraaijeveld et al. 2007; Clutton-Brock 2007) as well as of badges of status, irrespective of sex, in reducing antagonistic social interactions (i.e. social selection Tobias et al. 2012). Both sexual selection and social selection, which are associated with intraspecific interactions, may be valid explanations for female ornamentation, but they cannot fully explain some patterns observed in the wild. For example, the comparative study above also showed that the elaboration of female coloration is weakly associated with female sexual and social selection (cooperative breeding was used as an indicator of social selection: Dale et al. 2015). In fishes, both males and females of non-territorial, promiscuous species often exhibit ornamentation (Lachner 1952; Sakai 1995; Sabaj et al. 2000), even though sexual selection and social interactions are expected to be weak in such non-territorial, promiscuous species. Thus, researchers should pay more attention to possible factors other than intraspecific interaction as an alternative driver of evolution of elaborate female ornamentation (or mutual ornamentation: both males and females have ornamentation).

More than 100 years ago, Wallace (Wallace 1889) proposed that conspicuous species-specific ornaments reduce harmful interspecific interactions such as hybridization and aggression by ensuring correct species recognition. This hypothesis has logical appeal for female/mutual ornamentation because selection against hybridization can act on sexual signals not only in males but also in females (Takakura et al. 2015). In other words, selection against hybridization can favor male mate preference for conspecific females over heterospecifics based on female ornamentation. It has been widely recognized that male mate preference is weaker than females mate preference, because the sexual difference in investment on offspring usually leads to a male-biased sex ratio. This intensifies the male-male competition and reduces the opportunity for male mate choice (Darwin 1871; Edward and Chapman 2011). Male mate preference in comparing conspecific females rarely evolves in response to indirect selection resulting from the increasing quality of offspring: the cost of intense male-male competition mitigates the indirect benefit of choosing better females (Härdling and Kokko 2005; Servedio and Lande 2006). Still, the direct selection, which results from increasing reproductive success and/or survival of the focal males, is sometimes strong enough to drive the evolution of male mate preference in comparing conspecifics (Servedio and Lande 2006). For example, males frequently prefer high-fertility females in various taxa (fish, Sargent et al. 1986; birds, Monaghan et al. 1996; amphibians, Liao and Lu 2009). In mate choice comparing con- and heterospecific females, indirect selection may also be strong enough to shape male mate preference, because hybrid fitness is often considerably lower than that of pure crosses (Servedio 2007). Empirical studies have provided increasing support for the male species recognition (e.g. Seehausen et al. 1999; Svensson et al. 2007; Zoppoth et al. 2013; Moran et al. 2017; Roberts and Mendelson 2017). These studies, however, have focused mainly on non-ornamented species including species that rely on odors in mate choice (Roelofs and Comeau 1969; Shine et al. 2002), or male-ornamented species (Knight and Turner 1999; Wong et al. 2005; Espinedo et al. 2010; West and Kodric-Brown 2015; Moran et al. 2017; Roberts and Mendelson 2017). In limited studies on female or mutually ornamented species, chemical or tactile communications are not experimentally excluded (Wiernasz 1995; Seehausen et al. 1999; Deering and Scriber 2002; Jiggins et al. 2004; Zoppoth et al. 2013), which is insufficient to show the importance of visual cues in male mate choice. Hence, the species recognition hypothesis (Wallace 1889) has rarely been formally tested in the context of female ornamentation (but see Svensson et al. 2007; Pierotti et al. 2008).

Far Eastern daces (genus *Tribolodon*, family Cyprinidae) are an excellent study system in which to examine whether the species recognition hypothesis can explain female ornamentation. The Far Eastern dace species *T. hakonensis* and *T. sachalinensis* (hereafter *Th* and *Ts*, respectively) are morphologically similar, but both sexes exhibit species-specific conspicuous coloration only in their breeding season (i.e. mutual ornamentation). *Th* has three orange and two black horizontal bands on its head and along its flanks and orange fins, whereas *Ts* has red cheeks and fins (figure 1) (Nakamura 1969; Atsumi et al. 2018). Further, both species are non-territorial and they spawn in schools (Ito 1975; Sakai 1995). These behaviors may preclude the current major hypotheses proposed to explain female ornamentation (i.e. social selection, sexual selection in females, and the genetic correlation between sexes combined with sexual selection in males). Instead, the breeding ecology of *Th* and *Ts* suggests that the species recognition hypothesis is more likely to explain female ornamentation in these species. Although the breeding season and habitat of these two species overlap (Gritsenko 1982; Sakai 1995) and hatching rate of F1 hybrids is high (Atsumi et al. 2018), hybrid swarms have rarely been observed in the wild (Hanzawa et al. 1984; Sakai and Hamada 1985; Sakai et al. 2007). These findings suggest that these species discriminate between conspecifics and heterospecifics and thereby avoid hybridization. The aim of this study was to investigate the role of female nuptial coloration in sexually isolating these two Far Eastern dace species. We examined whether (i) visible female traits enhanced *Th* male visual-based species recognition in a two-choice experiment, and (ii) visible female traits other than coloration (i.e. behavior and body size and shape) differ between the species. If *Th* males showed a preference for conspecifics based on visual cues and females did not differ behaviorally or morphologically between species, then female ornamentation (nuptial coloration) should be a key trait by which *Th* males discriminate between conspecific and heterospecific females.

Methods

(a) Study species

Far Eastern daces, *Th* (*Tribolodon hakonensis*) and *Ts* (*T. sachalinensis*) are deeply diverged (10–20 Mya: Imoto et al. 2013) but hybridizing cyprinid fishes (Sakai and Hamada 1985; Sakai et al. 2004). The two species are sympatry in the Northern Japanese archipelago and Sakhalin: *Th* widely ranges from Kyushu Island (Southern Japan) to Sakhalin Island and around the Sea of Japan and; *Ts* distributes from Northern Honshu Island (Northern Japan) to Sakhalin (Sakai et al. 2004; Watanabe et al. 2018). They typically mature at age four and their lifespan is over 10 years (Sakai 1995, but see Atsumi and Koizumi

2017a). During the non-breeding season, the two species distribute across rivers and estuaries at a low density (Gritsenko 1982; Gavrenkov et al. 2004; Moriyama et al. 2008; Katano et al. 2010), and are hardly distinguishable in coloration (grey to silver color) and morphology (Nakamura 1969; Gavrenkov and Ivankov 1981; Ivankov et al. 2016). Throughout their life-history, they do not show territoriality and aggressiveness (Katano et al. 2010; Ogawa and Katano 2016).

From Spring to Early-Summer, matured fish aggregate to the middle reaches of rivers and form dense spawning schools (Ito 1975; Gritsenko 1982; Sakai 1995). Since two species are iteroparous and continue growing after maturation, spawning schools consist of individuals vary in size (10–30 cm in fork length, FL, in *Th* and 7–23 cm FL in *Ts*: Ito 1975; Sakai 1995). Importantly, these species often form mixed-species spawning schools because they greatly overlap in breeding season and habitat (Gritsenko 1982; Sakai 1995). Spatiotemporal overlap in spawning allows hybridization between the two species but hybridization is limited in the wild. Hybrids are rarely found in Southern Hokkaido island (Northern Japan) (F1 hybrids, 0–4.8 %; later generation hybrids, 0–1 %: Sakai and Hamada 1985) but never been found in Northern Honshu Island (Hanzawa et al. 1984; Sakai et al. 2007) and Sakhalin (Polyakova et al. 2015). Prezygotic isolation between the two species may be maintained by temporal and chemical isolation: two species somewhat differ in reproductive timing (*Th*, May to June; *Ts*, June to July: Ito 1975; Sakai 1995); males of *Ts*, at least, can discriminate species by male urine and odor of egg (Sakai and Yoshii 1990). Another candidate for the prezygotic isolation mechanism is that species recognition based on nuptial coloration. During their breeding season, matured males and females of Far Eastern daces show species-specific nuptial coloration without morphological change (Nakamura 1969; Gritsenko 1982; Sakai 1995). Their nuptial coloration differs in the presence/absence of orange and black horizontal band along their flanks (figure 1: Nakamura 1969; Sviridov et al. 2002, 2003). The color pattern does not differ across sexes and body size (Sviridov et al. 2002, 2003). The mechanism of postzygotic isolation is unknown. Given that the hatching rate of F1 hybrids is high (Atsumi et al. 2018), fitness reduction of hybrids may occur in later life stages or in reproduction.

(b) Sample collection and rearing conditions

Fish used in the mate choice experiment were collected from Kotoni-Hassamu River in central Hokkaido, northern Japan (see electronic supplementary material, figure S1). We collected daces by using throw nets during their breeding season, from 18 May to 23 June 2017.

The collected fish were separated according to sex and species and kept in segmented reaches of an artificial channel next to the Horonai River in the Tomakomai experimental forest of Hokkaido University (see electronic supplementary material, figure S1) at 13–16 ˚C, the temperature range at which they spawn (Gritsenko 1982), under natural lighting conditions. Sex was determined by squeezing the abdomen, forcing the release of gametes from the gonoduct, and species were identified by their nuptial coloration. Individuals were marked for identification by clipping their fins in unique combinations. For one to seven weeks before the experiment, all collected fish were fed on pellet food once a day. All fish kept breeding coloration during the study period.

(c) Mate choice experiments

We conducted the male mate choice experiment using *Th* males, not *Ts* males because of the small sample size of *Ts* males. An acrylic aquarium (250 cm × 100 cm × 100 cm deep, filled with fresh water to 30 cm depth) located in an outdoor facility was used as the experimental arena for the male mate choice experiment (figure 2). At each end of the arena, we placed a small glass aquarium (60 cm × 30 cm × 36 cm deep, water depth 30 cm). The bottom surfaces of both the arena and the aquaria were covered by gravel. A two-choice experiment was conducted in which one *Th* male was placed in the arena (outside the small aquaria), a *Th* female was placed in one of the two small aquaria (i.e., stimulus fish compartment), and a *Ts* female was placed in the other. Con- and heterospecific females were alternately presented in the left and right aquarium. This experimental setting allowed the male fish to use visual and acoustic information, but not chemical cues, to recognize the species of the females. We note, as sound-producing organs (e.g. drumming muscle surrounding swim bladder and spines on the fins: Amorim 2006) are not found in Far Eastern daces (KA personal observation), acoustic communication is less likely to occur. To evaluate male choice, we defined three resident zones: one adjacent to each stimulus fish compartment (‘‘association zones’’, within 50 cm of each end of the arena) and a middle ‘‘neutral’’ zone (more than 50 cm from either end) (figure 2). The focal male and stimulus females were put into the aquaria and allowed to acclimate for 2 min before the trial was started. As male behavior did not differ qualitatively between the early and late phase of the experiment (KA personal observations), we consider that acclimation time was enough. During each trial, we made video recordings of the behaviors of the male and females in the two association zones. Using the video recordings, we measured the male association time with each stimulus female for a total of 18 min. It was not possible to record data blind because our study involved focal animals. In 7 of 43 trials, the subject male did not visit either female during the 18-min experimental period. We considered that those individuals were not sexually active, or that they did not recognize either stimulus as female, and excluded those trials from the analysis.

Body size of subject males and stimulus females was measured as fork length (FL). Trials of 37 males (215.7 ± 34.3 mm, FL ± SD) were included in the analysis. Body size (FL) of the stimulus females (five *Th* and four *Ts*) used in these trials was not different between the species (*Th*, 192.2 ± 11.8 mm; *Ts*, 183.4 ± 7.3 mm; $P > 0.2$, *t*-test). In each mate choice trial, the difference in body sizes between the stimulus females was within 7%. The size difference between female pairs, as well as male body size, were considered in the statistical analysis.

Trials were conducted from 12 July to 17 July 2017 during daytime hours (i.e. 10:00–18:10). Water temperature was 16 ± 1 ˚C and light conditions were controlled by a roof over the outdoor facility (to reduce sunlight) and a 100 W fluorescent lamp. After each day's trials, the experimental arena and stimulus compartments were emptied, dried, and refilled with well water to reduce the potential effects of odors and chemicals from previous trials. Stimulus females were used multiple times and the possible effect of stimulus female pairs was accommodated for the analysis.

(d) Stimulus female behavior

Neither of these Far Eastern dace species show characteristic mating behaviors such as dancing (Ito 1975; Gritsenko 1982), nor did we observe such behaviors during our experiment (KA personal observation). Stimulus female behavior was evaluated from the video recordings by measuring horizontal position (0, far side from subject male; 1, middle; 2, near side), vertical position (0, bottom; 1, middle; 2, water surface), body angle with respect to the subject male (0, away; 1, vertical; 2, towards), and swimming activity (0, no swimming; 1, slowly swimming using fins only; 2, actively swimming by using fins and body undulation). Measurements were made at 1-min intervals during each 18-min mate choice trial. Thus, the four behavioral measurements were recorded 19 times in each trial.

(e) Stimulus female morphology

The body shape of the stimulus females was analyzed by using a landmark-based geometric morphometrics analysis to quantify the overall shape of the body in lateral view. The shape was captured by 11 digitized landmarks generated using TpsDig 2.19 software (Rohlf 2013), selected to capture major body regions (figure 1C), from fish images obtained from the video clips. As the landmarks were made based on moving specimens, landmarks were set on cranial morphology and fin insertions that are relatively stable while fish swims.

(f) Statistical analysis: male mate preference

As a measure of mate preference for conspecifics, we calculated the strength of the preference (SOP, e.g. (Tobler et al. 2008; Roberts and Mendelson 2017)) as follows:

$$\mathrm{SOP} = \frac{(\text{association time with conspecifics}) - (\text{association time with heterospecifics})}{(\text{association time with conspecifics}) + (\text{association time with heterospecifics})}$$

SOP is positive when the association time with conspecifics is longer, and is interpreted to indicate a preference for conspecifics.

To examine whether male Far Eastern daces prefer conspecific females on the basis of visual stimuli while controlling the effect of subject male body size and stimulus female pairs, a linear mixed model with normal errors (LMM) was fitted. The explanatory variables were (i) body size of subject males, (ii) body size difference between the stimulus females, represented as the ratio of conspecific body size to heterospecific body size, (iii) position of conspecific females (i.e. side effect) and (iv) their interaction. Stimulus female pair ID was included as a categorical random effect. All possible combinations of models using these predictor variables were compared using the BIC. In the best model, the statistical significance of each parameter was computed via the Satterthwaite approximation**.** We also evaluated the importance of those parameters by model averaging. All analyses were performed in

R 3.4.3 (R Development Core Team 2017). We used following R packages: the lme4 package to construct LMMs; the MuMIn package to select LMMs and model averaging; and the lmerTest package to evaluate the significance of parameters within LMM,

(g) Statistical analysis: female behavior

We conducted a PCA based on a correlation matrix to investigate variations in female behavior in the pooled data of the two species. The first two PCs successfully explained nearly 80% of total variance (PC1, 47.5%; PC2, 30.6%: table S1). We used the PC1 and PC2 values in the subsequent analysis.

To evaluate interspecies differences in behavior, we fitted an LMM to each behavioral PC value. The possible effect of female individuals and trials were accommodated as categorical random effects. The significance of each parameter was computed via the Satterthwaite approximation (Satterthwaite 1946)**.** We used the lme4 and the lmerTest packages of R.

(h) Statistical analysis: female morphology

Landmark coordinates were aligned by a generalized Procrustes analysis, which centers, scales, and rotates landmark configurations to minimize the least-squares distance between shapes (Zelditch et al. 2012). The interspecies difference in body shape was evaluated by Procrustes ANOVA which assesses shape variation and covariation for a set of landmark coordinates aligned by Procrustes analysis (Klingenberg and McIntyre 1998). The geometric morphometrics analyses were conducted with MorphoJ 1.06d software (Klingenberg 2011).

Results

The total association time varied across trials (62.8 ± 31.1 % of total trial time). Males of *TH* preferred conspecific females on the basis of the visual stimulus: in the best model, SOP was significantly positive (0.41, $P = 0.014$, figure 3). The best model ranked by the BIC did not include the male body size, the female size difference, or their interaction (delta BIC > 3.8; table 1). The averaged model also showed no significant effect of the body size and gave similar estimates of SOP (0.40, $P < 0.05$; appendix S1 and table S2 in electronic supplementary material). On average, regardless of stimuli female pairs, association time with conspecifics was 11.7 ± 23.0 times (±SD), and 254.8 ± 423.7 s, longer during all 18-min mate choice trials.

We found no interspecies differences in stimulus female behavior and morphology. The LMMs fitted to the PC1–2 axes of behavioral data showed no significant behavioral difference between species (PC1, $P > 0.1$; PC2, $P > 0.8$: table S3 in electronic supplementary material). In addition, Procrustes ANOVA indicated no interspecies difference in body shape ($F = 1.29$, $P > 0.2$).

Discussion

Although the species recognition hypothesis (Wallace 1889) can plausibly explain the mechanism driving evolution or maintenance of ornamentation in females as well as males, it has rarely been demonstrated previously (Svensson et al. 2007; Pierotti et al. 2008). The results of our experiment, conducted with two Far Eastern dace species in which the females display nuptial ornamentation, provide the support of the ability of this hypothesis to explain female ornamentation. The mate choice trials allowed the males to use visual information, but not chemical information, to recognize females. In these trials, *Th* males stayed near conspecific females 11.7 times longer with than they stayed near heterospecific females, indicating that male *Th* can discriminate two Far Eastern dace species visually. Potential visual signals or cues for male choice include body size, morphology, coloration, and behavior, or combinations of these. By using females of similar size as stimuli in the experiments, we precluded a possible body size effect on mate choice. Accordingly, LMM model selection and averaging inferred no effect of female body size. Furthermore, the results of our behavioral and morphometry analyses indicated that female behavior and morphology, at least as captured by our metrics, did not differ between the species. Thus, our experimental results suggest that female nuptial coloration is a signal that enables males to discriminate conspecific females from heterospecific ones. The species recognition hypothesis (Wallace 1889), which was originally proposed to explain the function of male ornamentation as a barrier against hybridization, may therefore also be valid for explaining female ornamentation.

In species with pair mating, it is well known that male mate choice based on female traits is important in maintaining species boundaries (Roelofs and Comeau 1969; von Schilcher and Dow 1977; Espinedo et al. 2010). In this study, we found that male mate preference exists even in species with promiscuous mating behavior. Because of such a mating system in Far Eastern daces, male mate choice may not function during actual spawning activity. Rather, male mate choice may play a role before spawning begins. For reproduction, individuals of these two species first aggregate into spawning schools. Thus, individuals may use the species-specific nuptial coloration to ensure their aggregation with conspecifics. Although the spawning habitats of these two species overlap, their spawning schools are largely segregated by species (Ito 1975). Visual cues may allow such fine-scale spatial segregation because they are fast and highly directional compared to chemical cues (Partan and Marler 2005; Gillam 2011). This spatial segregation means that individuals mate exclusively with conspecifics, even if their mate choices are random within the schools (Ito 1975). Thus, a visual preference for conspecifics might be a mechanism that prevents hybridization and allows these two Far Eastern dace species to coexist. Intraspecific spawning aggregations are taxonomically widely observed, especially in fishes (Domeier and Colin 1997; Anderson et al. 2016). It may be relatively common that mutual ornamentation ensures such aggregation and thereby reduces hybridization.

Our results may also partly explain the mechanism responsible for the observed geographic variation of nuptial coloration in *Th* across Japan. *Th* shows greater variation in nuptial coloration patterns in regions where it is allopatric with its hybridizing species (i.e. *Ts* and *T. brandtii*) than in regions where it is sympatric with those species (Atsumi and Koizumi 2017b). If the nuptial coloration of *Th* contributes to sexual isolation as suggested by the present study, selection against hybridization should favor species-specific color patterns in sympatric regions, thereby reducing the variation in nuptial coloration of *Th* in those regions. In allopatric regions, the absence of selection against hybridization might allow diversification of nuptial coloration. The geographic variation in coloration patterns might therefore reflect the absence or presence of interspecific interactions.

The evolution of female ornamentation has long been puzzling in evolutionary biology (Darwin 1871; Kraaijeveld et al. 2007; Tobias et al. 2012), and several explanations focusing on sexual and social selection have been proposed (Kraaijeveld et al. 2007; Tobias et al. 2012). Although the alternative hypotheses of the ornament function, i.e., sexual and social selection, were not experimentally excluded in this study, these processes may be weak in Far Eastern daces. Social selection should be precluded because Far Eastern daces are neither territorial nor aggressive throughout their life (Katano et al. 2010; Ogawa and Katano 2016). Generally, sexual selection can act in the dense species-specific spawning school through various processes such as alternative mating tactics (Suzuki et al. 2008), lek (Windle and Rose 2007) and scramble intrasexual competition (Byrne and Roberts 2004). However, sexual selection may be weak in Far Eastern daces because they are promiscuous and hardly aggressive (Ito 1975; Gritsenko 1982; Sakai 1995; Shirotori et al. 2006), which reduces opportunities for mate choice and intrasexual competition. Further, though the quantitative study is still awaited, the nuptial coloration seems not to follow the general patterns under sexual selection: the coloration does not vary with sexes and body size (Nakamura 1969; Sviridov et al. 2002, 2003). As only matured fish exhibit nuptial coloration (Nakamura 1969; Sakai 1995), nuptial coloration should show their maturity. Hence, species recognition, together with maturity recognition, may be an important function of the female nuptial coloration in these two Far Eastern dace species. Given that social interaction among Far Eastern daces is weak throughout their life-history (Katano et al. 2010; Ogawa and Katano 2016), the major benefit of species recognition is possibly to avoid hybridization, rather than to avoid harmful social competition. In general, distinguishing between conspecifics and heterospecifics benefits individuals, regardless of their sex, in a variety of taxa because it prevents harmful interspecific interactions (e.g., hybridization, interference competition, and predation) (Couzin and Krause 2003; Okamoto and Grether 2013). Therefore, species recognition where female ornamentation is used as a signal trait may be ubiquitous in nature.

The species recognition hypothesis, and sexual and social selection hypotheses are not mutually exclusive, because ornamentation can convey multiple signals, including about species, quality as a mate, and social status, for species recognition, sexual selection, and social selection, respectively (Hill 2015). Further, these mechanisms may act interactively. For example, a small difference between species initially selected to prevent hybridization (i.e., species recognition) might later be enhanced by sexual selection (Mendelson and Shaw 2012). Or mutual ornamentation resulting from sexual or social selection might also function as an effective barrier to interspecific hybridization. Both theories and empirical studies have supported that disruptive or divergent sexual selection diverges male sexual trait and female preference thereby causing sympatric or allopatric speciation (Higashi et al. 1999; Seehausen et al. 1999; Maan and Seehausen 2011; Selz et al. 2016; Servedio 2016). Similarly, female ornamentation may diverge through disruptive/divergent sexual selection on females, or the combination of disruptive/divergent sexual selection on males and genetic correlation between sexes. Such interactive processes may drive the evolution of female ornament in Far Eastern daces because the selection against hybridization alone is probably not strong enough considering the high hatching rate of F1 hybrids (Atsumi et al. 2018). Considering the sexual or social selection and species recognition together, as well as genetic correlation, would further our understanding of the evolution and maintenance of female ornamentation.

Ethics approval

Formal approval for the experiment was granted by The Institutional Animal Care and Use Committee of Hokkaido University (approval no. 28-2). Sampling was approved by the local government of Hokkaido Prefecture.

Consent for publication

Not applicable

Availability of data and material

All data used in this study and codes used in analyses are deposited in appendix.

Competing interests

The authors declare no competing interests.

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Authors' contributions

KA designed the study and performed the experiments. OK contributed to the study design. KA, OK, and IK prepared the manuscript.

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Figure and table captions

Table 1 Results of model selection on LMMs examining possible effect of male body size and female body size on preference for conspecific females

Figure 1 Nuptial coloration in females of **A** *Tribolodon hakonensis* (*Th*) and **B** *T. sachalinensis* (*Ts*) with color bars. Arrows indicate the positions of red/orange bands. **C** Positions of the 22 landmarks used in the geometric morphometrics analysis

Figure 2 Diagram of the mate choice experimental set-up

Figure 3 Visual-based male mate choice experimental results for each stimulus female pair (circles) and its overall mean estimated by the best linear mixed model determined by BIC model selection (triangle). For the results of each female pair, the black circles on the left side show strength of preference (SOP) of individual males. The bars indicate SD