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

Authors: Keisuke Atsumi[1], Osamu Kishida[2] and Itsuro Koizumi[1,3]

Affiliations:

[1]Graduate School of Environmental Science, Hokkaido University, N10W5 Sapporo, Hokkaido 060-0810, Japan

[2]Tomakomai Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Takaoka, Tomakomai, Hokkaido 053-0035, Japan

[3]Faculty of Environmental Earth Science, Hokkaido University, N10W5 Sapporo, Hokkaido 060-0810, Japan

Corresponding author: K. Atsumi

Tel.: +81 11 706 2620; fax: +81 11 706 4864; email: srk_fukafuka@eis.hokudai.ac.jp

ORCID ID: https://orcid.org/0000-0002-8206-4977

E-mail address:

Keisuke Atsumi: srk_fukafuka@eis.hokudai.ac.jp

Osamu Kishida: kishida@fsc.hokudai.ac.jp

Itsuro Koizumi: itsuro@ees.hokudai.ac.jp

Running head: Female nuptial coloration can reduce hybridization

Abstract

Because sexual selection typically acts on males, evolution of conspicuous ornamentation in females has been a mystery. 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. However, the species recognition hypothesis, which postulates that ornamentation is adaptive because it prevents maladaptive hybridization, might plausibly explain female ornamentation. We tested 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 stayed near conspecifics longer, 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 (Collection 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 negatively correlates with male sexual selection (Dale et al. 2015). Hence, male sexual selection does not explain the wide prevalence of female ornamentation. Alternatively, recent research on female ornamentation has stressed the importance of sexual selection in females (Collection 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 (Dale et al. 2015). In fishes, both males and females of non-territorial, promiscuous species (e.g. cyprinids such as *Notropis lutipinnis* and *Nocomis leptocephalus*) often exhibit ornamentation, 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 AR. 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). Similarly, some studies have shown that female traits ensure male species recognition, thereby reducing hybridization. These studies, however, have focused only on non-ornamented species, including species that rely on odours (Roelofs and Comeau 1969) in mate choice, or male-ornamented species (Espinedo et al. 2010; West and Kodric-Brown 2015; Roberts and Mendelson 2017). Hence, the species recognition hypothesis (Wallace AR. 1889) has not been explicitly examined in the context of female ornamentation.

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 should 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, O 1982; Sakai 1995) and survival of F1 hybrids between them 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 visually 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.

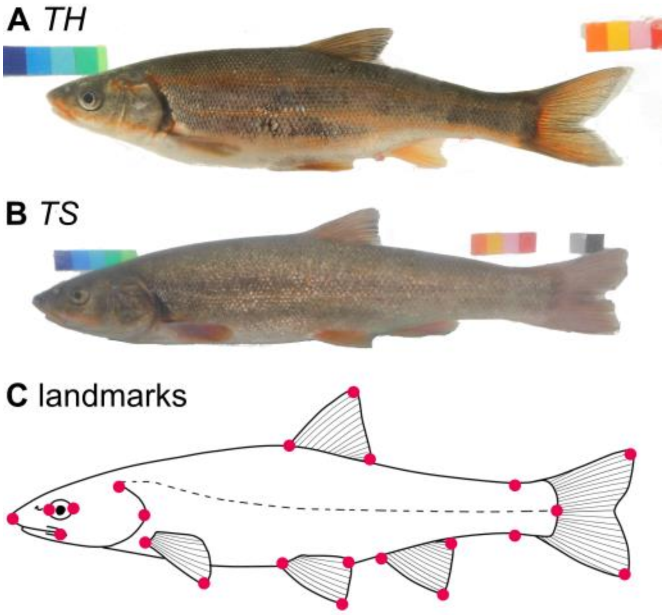

Figure 1 Nuptial coloration in females of **A** *Tribolodon hakonensis* (*Th*) and **B** *T. sachalinensis* (*Ts*) with colour bars. **C** Positions of the 22 landmarks used in the geometric morphometrics analysis

Methods

(a) 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, O 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.

(b) Mate choice experiments

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. This experimental setting allowed the male fish to use visual information, but not chemical cues, to recognize the species of the females. 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. 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 odours and chemicals from previous trials. Stimulus females were used multiple times and the possible effect of stimulus female pairs were accommodated for the analysis.

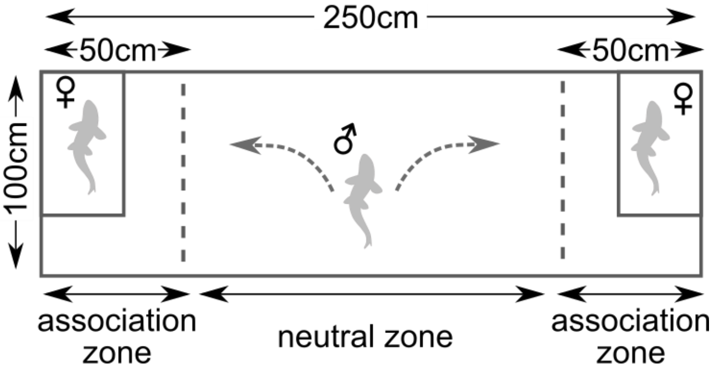

Figure 2 Diagram of the mate choice experimental set-up

(c) Stimulus female behavior

Neither of these Far Eastern dace species show characteristic mating behaviors such as dancing (Ito 1975; Gritsenko, O 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.

(d) Stimulus female morphology

The body shape of the stimulus females was analysed by using a landmark-based geometric morphometrics analysis to quantify the overall shape of the body in lateral view. The shape was captured by 22 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.

(e) 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, and (iii) 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,

(f) 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 behavioral 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.

(g) Statistical analysis: female morphology

Landmark coordinates were aligned by a generalized Procrustes analysis, which centres, 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

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 > 5.8; table 1). The averaged model also showed no significant effect of the body size and gave similar estimates of SOP (0.42, $P < 0.01$; appendix S1 and table S2 in electronic supplementary material). On average, regardless of stimuli female pairs, association time with conspecifics was 11.7 times, and 254.8 s, longer during all 18-min mate choice trials.

We found no interspecies differences in stimulus female behavior and morphology. The LMMs adopted to behavioral PC1–2 showed no significant 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 ($R^2 = 0.015$, $P < 0.7$).

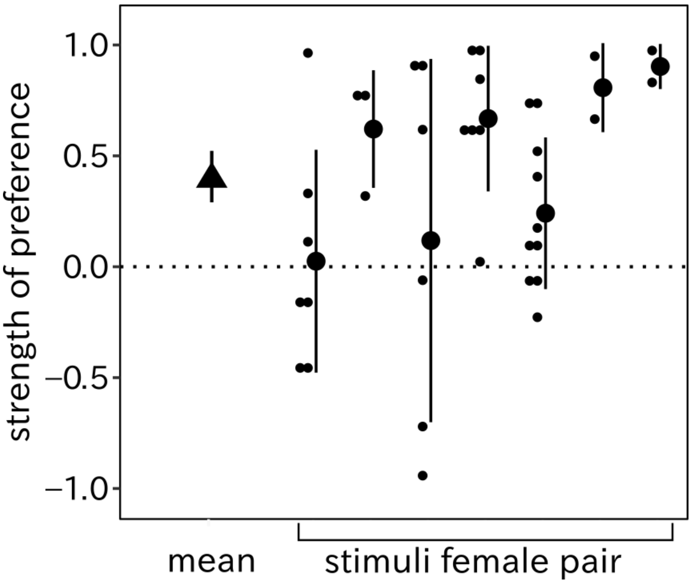

Figure 3 Visual-based male mate choice experimental results for each stimulus female pair (circles) and its overall mean estimated by the best linear mixed model determined by BIC model selection (triangle). For the results of each female pair, the black circles on the left side show strength of preference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

	explanatory variables					model selection			
	Intercept	Body size of subject males	[1]Stimulus female size difference	Interaction	df	log Likelihood	BIC	Delta BIC	weight
Models ranked by Bayesian Information Criteria (BIC)	0.416				3	-28.59	68	0	0.916
	0.420		0.034		4	-29.72	73.9	5.89	0.048
	0.419	-0.020			4	-30.07	74.6	6.59	0.034
	0.423	-0.028	0.040		5	-31.16	80.4	12.38	0.002
	0.408	0.005	0.019	0.092	6	-32.12	85.9	17.9	0

[1] Represented as the ratio which body size of conspecifics was divided by that of heterospecifics

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

Discussion

Although the species recognition hypothesis (Wallace AR. 1889) can plausibly explain the mechanism driving evolution or maintenance of ornamentation in females as well as males, it has not been demonstrated previously. The results of our experiment, conducted with two Far Eastern dace species in which the females display nuptial ornamentation, provide the first 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 AR. 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). 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. Spawning aggregations consist of one species widely observed in nature, especially in fishes (Domeier and Colin 1997). 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 2017). If the nuptial coloration of *Th* contributes to sexual isolation as suggested by the present study, selection against hybridization should favour 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 a mystery in evolutionary biology (Darwin 1871; Collection et al. 2007; Tobias et al. 2012), and several explanations focusing on sexual and social selection have been proposed (reviewed in (Collection et al. 2007; Tobias et al. 2012)). If ornamentation reflects individual quality and social status within populations, it could be a target of sexual and social selection arising from intraspecific interactions. In Far Eastern daces, however, neither sexual nor social selection is likely to drive evolution of nuptial coloration in females because they spawn promiscuously in spawning schools (i.e. sexual selection should be weak) and they are non-territorial (i.e. social selection should be weak). Therefore, selection against hybridization among congeneric species may be the driver of the female nuptial coloration in these two Far Eastern dace species. 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; Letters et al. 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 social selection might also function as an effective barrier to interspecific hybridization. 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

Competing interests: The authors declare no competing interests.

Funding: This study was supported by a Sasakawa Scientific Research Grant from The Japan Science Society, by the Fujiwara Natural History Foundation, by a Grant-in-Aid for a Japan Society for the Promotion of Science (JSPS) Research Fellow (18J10096) to KA, and by a JSPS KAKENHI grant (17H03725) to OK.

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

Acknowledgments: We are grateful to T. Ishii for tremendous help in setting up the experimental system, to M. Kudoh, K. Takahashi, T. Tsuruya, and K. Tomita for sampling assistance, and to all staff members of Tomakomai Experimental Forest of Hokkaido University for their support of this research. We also thank M. Kikuchi and Salmons' Hometown Chitose Aquarium for allowing us to conduct preliminary trials by computer animation, C. Ayer for checking the English of the manuscript, and Y. Ohkubo for advice on the statistical analyses.

References

Andersson MB (1994) Sexual Selection. Princeton University Press, Princeton

Atsumi K, Koizumi I (2017) Web image search revealed large-scale variations in breeding season and nuptial coloration in a mutually ornamented fish, *Tribolodon hakonensis*. Ecol Res 32:567–578. doi: 10.1007/s11284-017-1466-z

Atsumi K, Nomoto K, Machida Y, et al (2018) No reduction of hatching rates among F1 hybrids of naturally hybridizing three Far Eastern daces, genus *Tribolodon* (Cypriniformes, Cyprinidae). Ichthyol Res 65:165–167. doi: 10.1007/s10228-017-0588-1

Clutton-Brock T. (2007) Sexual selection in males and females. Science 318:1882–1885. doi: 10.1126/science.1133311

Collection D, Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J (2007) The evolution of mutual ornamentation. Anim Behav 74:657–677. doi: 10.1016/j.anbehav.2006.12.027

Couzin ID, Krause J (2003) Self-organization and collective behavior in vertebrates. Adv Study Behav 32:1–75. doi: 10.1016/S0065-3454(03)01001-5

Dale J, Dey CJ, Delhey K, et al (2015) The effects of life history and sexual selection on male and female plumage colouration. Nature 527:367–370. doi: 10.1038/nature15509

Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London

Domeier ML, Colin PL (1997) Tropical reef fish spawning aggregations: Defined and reviewed. Bull Mar Sci 60:698–726. doi: 10.1111/j.1462-5822.2007.00989.x

Espinedo CM, Gabor CR, Aspbury AS (2010) Males, but not females, contribute to sexual isolation between two sympatric species of *Gambusia*. Evol Ecol 24:865–878. doi: 10.1007/s10682-009-9343-z

Gritsenko, O F (1982) Reproduction ecology of the Eastern redfins of genus *Tribolodon* (Cyprinidae). J Ichthyol 22:121–134

Hanzawa N, Taniguchi N, Shinzawa H (1984) Genetic markers of the artificial hybrids between *Tribolodon hakonensis* and *T.* sp. (*Ukekuchi–ugui*). Otsuchi Mar Res Cent Rep 10:11–17

Hill GE (2015) Sexiness, individual condition, and species identity: the information signaled by ornaments and assessed by choosing females. Evol. Biol. 42:251–259

Ito Y (1975) Notes on the spawning habits of three species of genus *Tribolodon* in Hokkaido. Sci Rep Hokkaido Fish Hatch 30:39–42

Klingenberg CP (2011) MorphoJ: An integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357. doi: 10.1111/j.1755-0998.2010.02924.x

Klingenberg CP, McIntyre GS (1998) Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52:1363–1375. doi: 10.2307/2411306

Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305. doi: 10.2307/2407393

Letters E, Okamoto KW, Grether GF (2013) The evolution of species recognition in competitive and mating contexts: The relative efficacy of alternative mechanisms of character displacement. Ecol Lett 16:670–678. doi: 10.1111/ele.12100

Mendelson TC, Shaw KL (2012) The (mis)concept of species recognition. Trends Ecol Evol 27:421–427. doi: 10.1016/j.tree.2012.04.001

Nakamura M (1969) Cyprinid fishes of Japan: Studies on the life history of cyprinid fishes of Japan. Research Institute of Natural Resources, Tokyo

R Development Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Roberts NS, Mendelson TC (2017) Male mate choice contributes to behavioural isolation in sexually dimorphic fish with traditional sex roles. Anim Behav 130:1–7. doi: 10.1016/j.anbehav.2017.06.005

Roelofs WL, Comeau A (1969) Sex pheromone specificity: Taxonomic and evolutionary aspects in lepidoptera. Science 165:398–400. doi: 10.1126/science.165.3891.398

Rohlf F (2013) tpsDIG2: Digitize landmarks & outlines from image files, scanner, or video. http://life.bio.sunysb.edu/morph/soft–dataacq.html/. Accessed 12 Jun 2016

Sakai H (1995) Life-histories and genetic divergence in three species of *Tribolodon* (Cyprinidae). Mem Fac Fish Hokkaido Univ 42:1–98

Sakai H, Hamada K (1985) Electrophoretic discrimination of *Tribolodon* species (Cyprinidae) and the occurrence of their hybrids. Japan J Ichthyol 32:216–224

Sakai H, Saitoh T, Takeuchi M, et al (2007) Cyprinid inter-generic hybridisation between *Tribolodon sachalinensis* and *Rhynchocypris lagowskii* in Tohoku District. J Nat Fish Univ 55:45–52

Satterthwaite FE (1946) An approximate distribution of estimates of variance components. Biometrics Bull 2:110–114. doi: 10.2307/3002019

Takakura KI, Nishida T, Iwao K (2015) Conflicting intersexual mate choices maintain interspecific sexual interactions. Popul Ecol 57:261–271. doi: 10.1007/s10144-015-0492-3

Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: Social selection, sexual selection and ecological competition. Philos Trans R Soc B Biol Sci 367:2274–2293. doi: 10.1098/rstb.2011.0280

Tobler M, Schlupp I, Plath M (2008) Does divergence in female mate choice affect male size distributions in two cave fish populations? Biol Lett 4:452–454. doi: 10.1098/rsbl.2008.0259 von Schilcher F, Dow M (1977) Courtship behaviour in *Drosophila*: Sexual isolation or sexual selection? Z Tierpsychol 43:304–310. doi: 10.1111/j.1439-0310.1977.tb00077.x

Wallace AR. (1889) Darwinism. Macmillan, London

West RJD, Kodric-Brown A (2015) Mate choice by both sexes maintains reproductive isolation in a species flock of pupfish (Cyprinodon spp) in the Bahamas. Ethology 121:793–800. doi: 10.1111/eth.12394

Zelditch ML, Swiderski DL, Sheets HD (2012) Geometric morphometrics for biologists: A primer, Second edi. Academic Press, New York