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Authors: Sekiya, Yuko, and Karino, Kenji

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# Female Mate Preference in Goby *Eviota prasina*: Do Secondary Sexual Traits Influence Female Choice?

Yuko Sekiya<sup>†</sup> and Kenji Karino<sup>\*</sup>

Department of Biology, Tokyo Gakugei University, Koganei,  
Tokyo 184-8501, Japan

**ABSTRACT**—Males of the small gobiid fish *Eviota prasina* have longer dorsal fins than females and use their dorsal fins for courtship displays and male-male competition. We examined the influence of male dorsal fin length as well as the frequency of courtship displays on female mate preference using sequential choice experiments. Females responded more frequently to courting males with longer dorsal fins than males with shorter dorsal fins. This indicates female mate preference on the basis of male dorsal fin length in this goby. In addition, the logistic regression analysis showed that male courtship frequency positively influenced female mate preference. Since males incur costs to produce and maintain longer dorsal fins and to perform frequent courtship displays, these secondary sexual traits may indicate the quality of physical characteristics. In *E. prasina*, only males conducted parental care for eggs in their nests. Therefore, females may be able to ensure greater survival rates as well as higher genetic quality of their offspring by choosing males with better qualities based on these male traits.

**Key words:** female mate choice, Gobiidae, dorsal fin, courtship display, sexual selection

## INTRODUCTION

Sexual dimorphic traits within a species have often been considered a result of sexual selection, i.e., mate choice and intrasexual competition (Andersson, 1994). In particular, the evolution of the most exaggerated traits of males has been caused by sexual selection, since males having elaborate tails or brighter coloration are frequently chosen by females as their mates (Petrie *et al.*, 1991; Møller, 1994; Houde, 1997). In addition, males that have greater weapons, such as horns or antlers, can win in male-male competition for acquiring females or resources that attract females (Andersson, 1994; Emlen, 1997). Not only these morphological traits, but also the behavioral traits of males may play an important role in sexual selection. For instance, the courtship display of males is the most important trait in female mate choice for many animals (Ryan, 1985; Hedrick, 1986; Gibson, 1989; Dugatkin and FitzGerald, 1997; Hauser and Konishi, 1999).

The fins of some fish species show sexual dimorphism. In these fishes, males usually have longer or more elaborate caudal, pelvic, anal, and/or dorsal fins (Turner, 1993).

Hence, these sexual dimorphisms in fish fins have been thought as the result of sexual selection, although only a few empirical studies have documented the relationship between fin dimorphism and sexual selection (Turner, 1993; Andersson, 1994). For example, female guppies *Poecilia reticulata* and swordtails *Xiphophorus helleri* prefer males with larger or longer caudal fins as their mates (Bischoff *et al.*, 1985; Basolo, 1990); however, females prefer males that appear to be of larger apparent sizes (body size plus caudal fin size) and do not choose males based on the size of the caudal fin itself (Rosenthal and Evans, 1998; Karino and Matsunaga, 2002). In a cichlid fish *Cyathopharynx furcifer* in Lake Tanganyika, females prefer males having longer and more symmetrical pelvic fins (Karino, 1997). However, the relationship of long and exaggerated male fins to sexual selection is unknown in most fishes.

*Eviota prasina* is a small gobiid fish and is distributed over the shallow reef zone of the Indo-Pacific Ocean (Nakabo, 1993). The first dorsal fin of *E. prasina* shows remarkable sexual dimorphism, i.e., males have longer dorsal fins than females (Sunobe, 1998). Male gobies build nests within crevices or small holes of substrate and maintain territories around their nests. The females visit male nests and spawn demersal eggs within the nests (Sunobe and Nakazono, 1999). They leave the nests soon after spawning, and only the males tend to eggs until hatching. The female mates with several males, and the male also obtains multiple

<sup>\*</sup> Corresponding author: Tel. +81-42-329-7520;  
FAX. +81-42-329-7737.

E-mail: kkarino@u-gakugei.ac.jp

<sup>†</sup> Present address: Graduate School of Science, Osaka City University, Osaka 558-8585, Japan

clutches from plural females (Sunobe and Nakazono, 1999). Males use their longer dorsal fins when they perform courtship displays toward females and also when they aggressively compete with other males (Sunobe, 1998; our personal observation). This suggests that the long dorsal fin of male *E. prasina* may play an important role in the process of the female mate choice and/or male-male competition. In the present study, we focus on the role of the male dorsal fin as well as courtship display in female mate preference.

## MATERIALS AND METHODS

### Study animals

In order to examine the degree of sexual dimorphism in *E. prasina*, we collected specimens at the shallow area of the fringing reef of Sesoko Island (26°38'N, 127°52'E), Okinawa, southern Japan in August 2002. The reproductive season of *E. prasina* lasted from March until November in Okinawa (our unpublished data). Most (87%) of the females from the collection had ripe eggs in their swollen abdomens. To evaluate sexual dimorphism, we measured the body size (standard length; SL) and the longest part (the first spine) of the first dorsal fin to the nearest 0.1 mm using a vernier caliper. The fish were anesthetized using a 2-phenoxyethanol solution at the measurement. We determined the sex of the fish based on the shape of urogenital papilla (Sunobe, 1990).

The fish that were used for the female choice experiment were collected at the shallow area of the fringing reef of Sesoko Island in June and August 2000. We carried the fish to the laboratory of Tokyo Gakugei University and reared them in 30-l aquariums with circulated water at 27–30°C with a 14:10 light and dark regime. The gobies were fed once daily with commercial food (Tetra Marine; Tetra Werke) and newly hatched brine shrimp nauplii. The fish were anesthetized using a 2-phenoxyethanol solution and their sexes were determined based on the shape of urogenital papilla (Sunobe, 1990). The fish were individually marked with subcutaneous injections of fluorescent elastomer (Northwest Marine Technology). We measured the body size and dorsal fin length of the fish just before the experiment.

### Experimental design

Because males frequently attack each other when they are in the same aquarium, female mate preference may be masked by the effect of the intermale competition when two males are simultaneously presented to the females. In order to examine female mate preferences, we chose a sequential procedure (Bakker and Milinski, 1991; Karino, 1996), i.e., two stimulus males were presented to a test female sequentially. Since female *E. prasina* visit several male nests sequentially for spawning in nature (Sunobe and Nakazono, 1999), the sequential procedure for female choice experiments may be suitable in this species.

We used a small aquarium (35×22×25 cm) for this experiment. We spread gravel of 2–3 cm thickness at the bottom of the aquarium and put opaque boards on three sides of the aquarium to minimize disturbance from outside. The aquarium was filled with circulated water at 28–30°C. An 18-watt daylight lamp was placed 10 cm above the aquarium. We divided the aquarium into two compartments using an opaque board and put a plastic pipe (1.0 cm in diameter and 7.0 cm long) at the bottom of one compartment as the nest. All test fish were fed 1 hr prior to the experiment.

We chose two stimulus males to present to a test female in each trial of the experiment. The two stimulus males were put into the compartments with nests in the different aquariums at least 24 hr prior to the trial. Before the trial, we confirmed that the stimulus males used the pipes as their nests, based on nest-cleaning behav-

ior, and we introduced the test female into another compartment of one aquarium. After a 10-min acclimation period, we removed the opaque board from the aquarium and observed the test female and the stimulus male during a 30-min trial. Then, the test female was shifted to the vacant compartment of the other aquarium with another stimulus male. We removed the opaque board after a 10-min acclimation period and observed the fish during a 30-min trial. To minimize the influence of the presentation order of males on female preference, we repeated the above-mentioned two trials using the same female on the next day but reversed the presentation order of the two stimulus males. The two days' data were combined to analyze female preference. We never used these fish again in other trials.

During the 30-min observation, we counted the number of male courtship displays toward the test female. Male *E. prasina* exhibited a typical motor pattern as their courtship displays (Sunobe, 1998): the male approached the female by simultaneously moving the pectoral fins and erecting the dorsal fins, and soon after reaching the female, the male led the female to the nest. We counted this sequence of the male approaching and leading behavior as one courtship display. We also recorded the frequency of responses by the test female to the displaying males. When the female followed the courting male, we recorded the female behavior as "following." If the female entered into the nest following the courting male, we recorded the female behavior as "nest-in."

We chose a pair of two stimulus males that had similar body sizes (<0.5 mm difference), but had different dorsal fin lengths (≥1.0 mm difference). Indeed, the body sizes did not significantly differ between the two males (mean±SD=22.9±1.4 mm and 22.8±1.3 mm, n=30 pairs, paired *t*-test, *t*=0.41, *P*=0.68), whereas the dorsal fin lengths showed a significant difference between the pair-males (6.3±0.7 mm and 4.3±0.5 mm, n=30 pairs; paired *t*-test, *t*=13.5, *P*<0.001). This difference of dorsal fin lengths occurred naturally, not by artificial cutting. We chose females having swollen abdomens with ripe eggs as the test females. When the test females spawned with the stimulus males or when the stimulus males did not perform courtship displays during the trials, we excluded those data from the analysis. We also excluded the data where females did not respond to courtships by both males.

If females would choose their mates based on the dorsal fin lengths of males, they would respond more frequently to courting males with longer dorsal fins than males with shorter dorsal fins.

### Statistical analysis

In order to examine the sexual difference of dorsal fin length, we adopted an ANCOVA with body size as a covariate, because it was expected that the dorsal fin length increased in accordance with the body size. All of these data were normally distributed (Kolmogorov-Smirnov one-sample test, *P*>0.2).

Because some data of the female choice experiment were not normally distributed (Kolmogorov-Smirnov one-sample test, *P*<0.05), these data were log-transformed (Sokal and Rohlf, 1973). Since some behavioral data contained 0-values, these data were log-transformed after adding 1 to the frequency. Subsequently, we adopted an ANCOVA with the frequency of female responses as the dependent variable, that of male courtship displays as a covariate, and the males (males with longer and shorter dorsal fins) as the independent variable.

Furthermore, to clarify the independent effects of the male dorsal fin length and courtship frequency on female mate preference, we conducted a logistic regression analysis (Menard, 2002). We chose the logistic regression analysis because the two males that were presented to the test female can be divided into binary data, i.e., preferred males (females showed following and nest-in behavior more frequently) and less-preferred males. We chose the female preference (preferred males were coded 1 and less-preferred males were coded 0) as the dependent variable and the male courtship

frequency and dorsal fin length as the independent variables (Menard, 2002). We performed a logistic regression analysis with stepwise removal procedure according to the method of Hosmer and Lemeshow (1989).

The logistic regression analysis was conducted by an SPSS 11.0J Regression Models package (SPSS, Inc.). We used StatView 5.0 (SAS Institute) for the other statistical analyses.

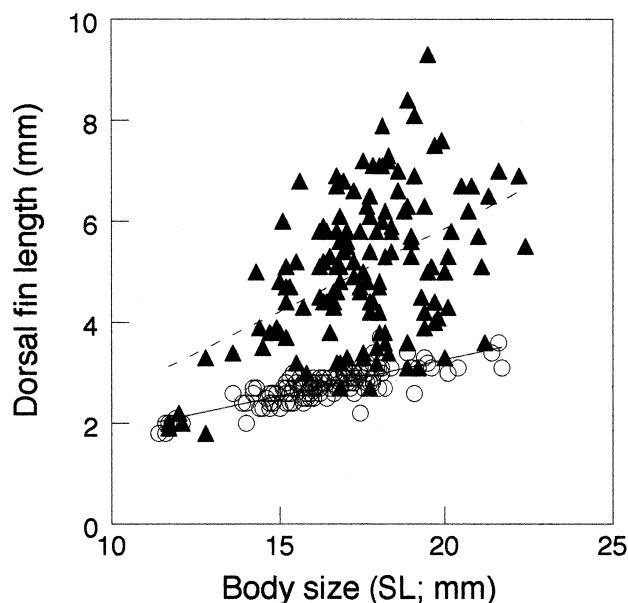
## RESULTS

### Sexual dimorphism

We collected 135 and 123 specimens of mature males and females in August 2002, respectively. The body sizes (SL) of males (mean $\pm$ SD=17.5 $\pm$ 2.1 mm) were larger than those of females (16.4 $\pm$ 1.9 mm, two sample *t*-test, *t*=5.4, *P*<0.001). The dorsal fin length increased with the body size (ANCOVA, *df*=1/255, *F*=29.4, *P*<0.001; Fig. 1). The dorsal fin length showed remarkable sexual dimorphism, i.e., the males had significantly longer dorsal fins than the females (ANCOVA, *df*=1/255, *F*=305.1, *P*<0.001; Fig. 1). The coefficient of variation (CV) of dorsal fin length was greater in the males (28.8%) than females (12.3%), indicating higher individual variation in the male dorsal fin length (Fig. 1). In contrast, the CV of the body size of males (11.7%) and of females (11.7%) were not different.

### Female choice experiment

Females exhibited the following behavior more frequently toward males with higher courtship frequency (ANCOVA, *df*=1/57, *F*=122.0, *P*<0.001; Fig. 2A). The frequency of the female-following behavior showed a significant difference between the males (*df*=1/57, *F*=5.1, *P*=0.03). The females responded more frequently to males with

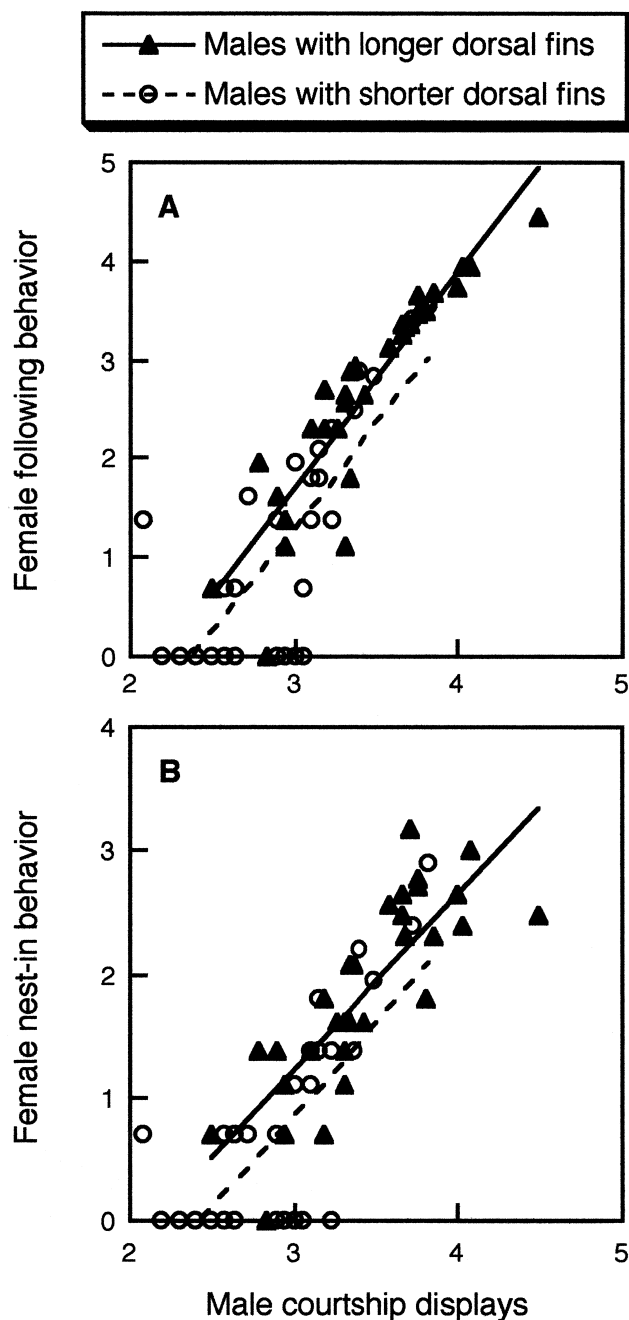


**Fig. 1.** Relationships between the body size and dorsal fin length in females (open circles) and males (solid triangles). Solid and broken lines indicate regression lines of females and males, respectively.

longer dorsal fins than males with shorter dorsal fins (Fig. 2A). The frequency of nest-in behavior also increased according to that of male courtship frequency (*df*=1/57, *F*=81.1, *P*<0.001), and differed between males, i.e., females preferred males with longer dorsal fins to males with shorter dorsal fins (*df*=1/57, *F*=5.1, *P*=0.03; Fig. 2B).

### Results of logistic regression analysis

After the stepwise removal procedure, both male dorsal fin length and courtship frequency entered the final model



**Fig. 2.** Female responses (A; following, B; nest-in behavior) to courtship displays of males with longer and shorter dorsal fins in the female choice experiment. Data were log-transformed.

and significantly influenced female-following behavior (Table 1). Similarly, both male dorsal fin length and courtship frequency showed a significant and positive effect on female nest-in behavior in the final model (Table 1). The goodness of the fit, calculated with the Hosmer–Lemeshow statistic (Hosmer and Lemeshow, 1989), of both the models showed  $P > 0.2$  ( $\chi^2 < 9.9$ ,  $df=8$ ). This indicated that both models seem to fit well, since the difference between the expected and observed results was not significant (Hosmer and Lemeshow, 1989; Menard, 2002). These results indicate a positive effect of both male dorsal fin length and courtship frequency on female mate preference.

**Table 1.** The results of logistic regression analysis with female preferences (following and nest-in) as the dependent variable and dorsal fin length and courtship frequency as independent variables. Final models of stepwise removal procedure are shown.

Variables	B	SE	Wald	P
Following (n=60)				
(-2 log likelihood=52.8, $\chi^2=30.4$ , $df=2$ , $P<0.001$ )				
Dorsal fin length	5.16	1.86	7.66	0.006
Courtship frequency	2.38	0.94	6.33	0.01
Constant	-16.03	4.07	15.51	<0.001
Nest-in (n=60)				
(-2 log likelihood=45.9, $\chi^2=37.2$ , $df=2$ , $P<0.001$ )				
Dorsal fin length	7.89	2.22	12.69	<0.001
Courtship frequency	1.95	0.98	3.97	0.046
Constant	-19.16	4.71	16.53	<0.001

## DISCUSSION

In *E. prasina*, males had longer dorsal fins than females. The male dorsal fin length also showed a greater CV than that of females. This indicates higher individual variation of male dorsal fin length within the population. Møller and Swaddle (1997) suggested that traits showing higher individual variation are often reliable indicators of the male quality. Male *E. prasina* having a better body condition and better developmental stability may be able to extend their dorsal fins to a greater degree relative to their body sizes.

The results of the female choice experiments showed a positive effect of male dorsal fin length on female mate preference. If longer dorsal fins would indicate better quality of the males, as other exaggerated traits in many animals (Andersson, 1994; Møller and Swaddle, 1997; Zahavi and Zahavi, 1997), the females could obtain several benefits by choosing males with longer dorsal fins as their mates. In *E. prasina*, only males tend to their eggs until hatching and paternal-care behavior may affect the male-body condition, because male *E. prasina* scarcely feed and move out from their nests during egg tending (Sunobe and Nakazono, 1999). It is expected that males with a poor body condition often desert or cannibalize their eggs to recover their own

condition (Marconato *et al.*, 1993; Okuda and Yanagisawa, 1996; Sargent, 1997). Therefore, female *E. prasina* should choose males with longer dorsal fins for greater survival of their offspring under the males' care, when the male dorsal fin lengths may signal the body condition of the males. In addition to the direct benefit (Andersson, 1994), females may be able to obtain an indirect benefit by mate choice on the basis of the male dorsal fin lengths. Males with longer dorsal fins may incur greater costs to produce and maintain their longer fins than males with shorter dorsal fins, similar to other exaggerated male traits in many animals (Andersson, 1994; Zahavi and Zahavi, 1997). Moreover, longer dorsal fins may increase the vulnerability of the males to predators, because male longer dorsal fins are conspicuous, especially during courtships to females (Sunobe and Nakazono, 1999) or male-male competition. Thus, longer dorsal fins may indicate a higher quality of the males (Zahavi, 1975; Andersson, 1994), and the females can ensure higher genetic quality of their offspring by choosing their mates on the basis of the male dorsal fin lengths.

Female *E. prasina* also preferred males exhibiting frequent courtship displays. In some other fish species, females also choose their mates based on male courtship frequency (cf. Andersson, 1994; Dugatkin and FitzGerald, 1997). Especially, the importance of the male courtship frequency to female mate choice is well known in fishes with paternal care (Jamieson and Colgan, 1989; Knapp and Kovach, 1991; Karino, 1995). Knapp and Kovach (1991) documented that female *Stegastes partitus* preferred males with higher courting frequency, and the survival rate of eggs in the male nest increased with the male courtship frequency. This suggests that male courtship frequency is an honest signal of male condition such as energy reserves for future parental care. Hence, female *E. prasina* can obtain a higher survival rate for their offspring by choosing males with a higher courtship frequency.

The present study demonstrated the important role that male dorsal fins play in female preference in *E. prasina*. Long dorsal fins of male *E. prasina* are used not only for courtship displays toward females, but also for aggressive displays against rival males (our personal observation). Males perform lateral displays with other males (side-by-side position) by fully erecting their dorsal fins before direct combat such as biting. In some animals, the males often assess rival males during aggressive displays based on their exaggerated traits, such as antlers or long eye span, before direct combat, to reduce the risk of injury or costs of energy and time (Blum and Blum, 1979; Andersson, 1994). In *E. prasina*, it is possible that the males assess the competitive ability of rival males based on dorsal fin lengths during aggressive displays. Further study will be needed to clarify the function of the male long dorsal fin in sexual selection.

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