

Algal-searching ability in laboratory experiments reflects orange spot coloration of the male guppy in the wild

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Summary

In the guppy *Poecilia reticulata*, females often choose their mates on the basis of the orange spot coloration of males. It is known that the colour saturation of orange spots in males is determined by the amount of intake of algae, a natural source of carotenoids. In this study, we examined the relationship between sexually selected traits in wild-caught males and their algal-searching ability in a laboratory experiment. Neither the body size nor the orange spot size of males correlated significantly with the algal-searching ability. However, males that possessed orange spots with a high colour saturation in the wild exhibited a high searching ability in the laboratory. This result suggests that the algal-searching score of males in the laboratory experiment indicated their algal-foraging ability in the natural environment. In a previous study, the algal-searching ability of guppies in a laboratory experiment was demonstrated to be a heritable trait. The result of this study further supported the hypothesis that female mate preferences for carotenoid-based coloration of males have evolved via indirect benefits such as the high foraging ability of their offspring.

Keywords: carotenoid-based coloration, foraging ability, indirect benefit, sexual selection, *Poecilia reticulata*.

Introduction

Carotenoid-based coloration is one of the major sexual signals in some animals (Andersson, 1994; Olson & Owens, 1998; Hill, 2002). Since animals

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cannot synthesize carotenoids de novo, they have to obtain carotenoids via their food (Krinsky et al., 1989; Olson & Owens, 1998). Therefore, the conspicuousness of the carotenoid-based coloration is considered as a reliable indicator of the foraging ability of individuals for carotenoid-rich foods (Endler, 1980; Kodric-Brown, 1989; McGraw et al., 2005). In addition, it is known that in animals, carotenoids play important roles in a variety of physiological processes such as the nervous, immune, and endocrine systems (Krinsky et al., 1989; Lozano, 1994; Olson & Owens, 1998; Blount et al., 2001). Hence, the conspicuousness of carotenoid-based coloration may indicate the viability of the individual. For example, carotenoid-based coloration is often an indicator of the parasite resistance ability of an individual because the conspicuousness of coloration is reduced by parasite infections (Milinski & Bakker, 1990; Houde & Torio, 1992; Hill, 2002).

In some animals, females choose their mates on the basis of the conspicuousness of the carotenoid-based coloration of males (Milinski & Bakker, 1990; Houde, 1997; Craig & Foote, 2001; Hill, 2002; Bourne et al., 2003; MacDougall & Montgomerie, 2003). It is considered that female mate preferences on the basis of carotenoid-based coloration of the males helps females to obtain some indirect benefits such as reproducing offspring with a high foraging ability or parasite resistance (Endler, 1980; Kodric-Brown, 1989; Barber et al., 2001; Hill, 2002; Saks et al., 2003). This hypothesis is based on the assumption that the foraging ability for foods that serve as a source of carotenoids or other viabilities related to carotenoid-based coloration are heritable traits.

The guppy *Poecilia reticulata* is a live-bearing poeciliid fish that is native to the streams of Trinidad, and its males exhibit bright spots such as orange, black, and iridescent coloration (Houde, 1997; Magurran, 2005). It is well known that female guppies choose their mates on the basis of the colour saturation of the orange spots of males (Kodric-Brown, 1989; Houde & Torio, 1992; Grether, 2000; Brooks & Endler, 2001; Karino & Shinjo, 2004) as well as other traits such as the size of the orange spots (Houde, 1987) and the body sizes of the males (Reynolds & Gross, 1992). Since the colour saturation of the orange spots in male guppies depends on their dietary carotenoid intake, the saturation of the orange coloration is regarded as an indicator of the foraging ability of males for algae that serve as natural sources of carotenoids (Endler, 1980; Kodric-Brown, 1989; Grether et al., 1999). In

addition, algal intake enhances the growth of both sexes and the reproductive efficiency of females in the guppy (Karino & Haijima, 2004). Algae are known to be a scarce resource in the natural environment in which guppies live (Endler, 1980; Dussault & Kramer, 1981; Grether et al., 1999). Therefore, if offspring inherit the algal-foraging ability from their male parents, females can achieve indirect benefits such as offspring with a greater algal-foraging ability and consequently a higher viability because of their mating preferences for the orange spot coloration of the males. In a laboratory maze experiment, Karino et al. (2005) have demonstrated a high significant heritability of the algal-searching ability, i.e., one component of the foraging ability, between male guppies and their offspring. However, in the natural environment, the foraging behaviour of animals may also be influenced by many other factors such as predation risk and interactions between conspecific individuals (Milinski & Heller, 1978; Abrahams & Dill, 1989; Day et al., 2001; Swaney et al., 2001; Carvalho & Del-Claro, 2004). Therefore, it is still unclear whether the searching ability in laboratory experiments reliably indicates the foraging ability in the wild. On the other hand, it is difficult to measure the heritability of the algal-foraging ability in the natural environment.

In the present study, we examined the relationship between the colour saturation of orange spots as well as other sexually selected traits of male guppies in the wild and their algal-searching ability in a laboratory experiment. Since the amount of algal intake determines the colour saturation of orange spots of males (Grether et al., 1999; Karino & Haijima, 2004), the colour saturation of orange spots of wild-caught males may indicate their algal-foraging success in their natural habitats. The detection of a positive relationship between the orange spot coloration of males in the wild and their algal-searching ability in the laboratory will further support the hypothesis, i.e., female preferences on the basis of the carotenoid-based coloration of males have evolved via obtaining indirect benefits derived from the preferences.

Material and methods

We used wild-caught male guppies from the Hiji River, Okinawa, southern Japan (26°43'N, 128°11'E). This population was identical to that used in the

study that documented the heritability of the algal-searching ability in a laboratory experiment (Karino et al., 2005). We collected male guppies at a single site in the middle of the Hiji River on March 26, 2005. At the collection site, we found several potential predators of guppies, such as gobies, carnivorous insects and newts (cf., Karino & Haijima, 2001), and algal-availability seemed to be low. Immediately after the collection, we recorded images of the right and left sides of the males by using a digital camera (Coolpix 5200, Nikon, Japan). The fish were anesthetized using a 2-phenoxyethanol solution to facilitate the recording. In order to minimize the effect of light, the recording was conducted within a closed box. The distance between the lens of the camera and the fish was fixed at 9.5 cm, and the distance and angle of the two lights (23 W, Tokin, Japan) within the box to the fish were also fixed. For calibration, scale and colour plates were displayed in the background of the fish (Karino & Haijima, 2001, 2004). After the recording, we housed the males in a laboratory of Tokyo Gakugei University. When males were infected by ectoparasites such as *Ichthyophthirius* at the collection, we excluded these males from further experiment. In order to individually discriminate, 3-5 males possessing different spot patterns were reared in an aquarium (14.0 × 14.0 × 10.0 cm) with 12 h light and 12 h dark photoperiods. They were fed newly hatched brine shrimp nauplii once daily.

The recorded images of the males were transferred to a computer (Power-Mac G4, Apple, USA) and analyzed using Photoshop 6.0 (Adobe, USA). We measured the area of all the orange spots on both the body and tail (caudal fin) as well as total body and tail areas of the male. The values of these areas were averaged between the right and left sides of the male guppies. In this study, we used the body and tail areas as the indicators of the body and tail sizes, respectively. The total area of the orange spots was divided by the total area of the body and tail for each male to obtain the relative orange spot area (%). In order to quantify the coloration of orange spots, we measured the hue (°), saturation (%), and brightness (%) at six points in each spot using Adobe Photoshop (Karino & Haijima, 2001; Skarstein et al., 2005; Tarof et al., 2005). Subsequently, we calculated the average of the six points as the score of each spot. We used the average value of all orange spots on both the right and left sides of the male guppies as the score of their orange spot coloration (see Karino & Haijima, 2001, 2004, for detailed method).

In order to measure the algal-searching ability of the male guppies, we conducted a maze experiment by using the method described by Karino et

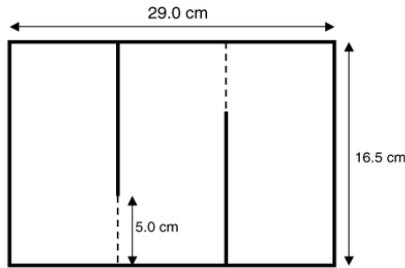


Figure 1. Experimental apparatus to examine the algal-searching ability of male guppies. Broken lines indicate the removable parts of the opaque partitions.

al. (2005). For the experimental apparatus, we used an aquarium (29.0×16.5 cm and 18.0 cm high). Two opaque boards were used to divide the aquarium into three compartments (9.7×16.5 cm). A part of each opaque board (5-cm width) could be removed by a nylon filament (Figure 1). We added 2-3 cm of gravel at the bottom of the aquarium. In order to avoid disturbance, we also placed opaque boards on three sides of the aquarium. A 15-watt daylight lamp was placed 10 cm above the aquarium, and the water temperature was maintained at 26°C .

We measured the time required by each male to swim from the compartment on one side of the experimental aquarium and reach and peck algae that were placed on the compartment on the opposite side of the aquarium. In order to evaluate the searching ability of an individual male, we conducted six trials on different days. The test males were not fed from the day prior to the trial to ensure a high motivation level for searching foods. We placed the test male in the compartment on one side of the aquarium. Further, we placed a mesh sheet covered with a species of unicellular green algae at the bottom of the centre of the compartment on the other side. These algae were derived from water from the original river (Karino & Haijima, 2004). After a 10-min acclimation period, we removed the removable parts of the opaque partitions by using the nylon filaments when the test male was located at the centre of the compartment. The test male could not see algae from its starting point. Subsequently, we recorded the fish behaviour during a 30-min experimental period by using a digital video camera (DCR-TR30, Sony, Japan). We measured the time required by the male to peck the algae on the mesh sheet. The locations of the test male and algae were reversed at the start of the next trial. A more detailed description of this experimental procedure is given in Karino et al. (2005). We calculated the mean value of the latencies

for completing the searching task in six trials as the score of the searching ability of the test male. Since some males never pecked algae in the six trials, we excluded data of these males from the analysis.

For statistical analysis, percentage data such as the relative orange spot area, saturation and brightness of the orange spot coloration were arcsine transformed. All data showed normal distributions (Kolmogorov-Smirnov one-sample test, $p > 0.7$). In order to examine the relationship between the algal-searching ability of the males in the laboratory experiment and their morphological traits in the wild, we calculated Pearson's correlation coefficients using StatView 5.0 (SAS Institute, Cary, NC, USA). A previous study (Karino et al., 2005) has demonstrated that tested males could learn the location of algae in the experimental aquarium during the six trials in the laboratory experiment. In order to exclude the effect of learning ability, we also calculated Pearson's correlation coefficients between the latency for completing the algal-searching task in the first trial in the laboratory experiment by males that could peck algae and their morphological traits in the wild.

Results

In the laboratory experiment, data on the searching ability of 30 wild-caught males were obtained. The mean latencies in six trials for completing the algal-searching tasks showed a high individual variation (mean \pm SD = 743.3 ± 500.0 s; range = 98.8-1632.8 s, $N = 30$).

Neither the body area nor the tail area of the males in the wild showed a significant correlation with regard to the mean latency for completing the algal-searching tasks in six trials in the laboratory experiment (Table 1). This latency did not correlate with either the absolute or relative areas of the orange spots of males (Table 1). The relationship between the hue and brightness of the orange spot coloration of males and the mean latency for completing the algal-searching tasks was also not significant (Table 1). The saturation of orange spot coloration in males correlated significantly with the mean latency for completing the algal-searching tasks in the laboratory experiment (Table 1). Since this relationship was negative (Figure 2a), males that exhibited orange spots with higher colour saturation in the natural environment required a shorter time to reach and peck algae in the laboratory experiment.

Table 1. Mean \pm SD of the morphological traits of the wild-caught males and Pearson's correlation coefficients (r) between the trait values and both the mean latency for completing algal-searching tasks in six trials ($N = 30$) and the latency for completing algal-searching task in the first trial ($N = 18$) in the laboratory experiment. Values of the relative area of the orange spots, saturation and brightness of the orange spot coloration were arcsine transformed before the analysis.

Traits	mean \pm SD	Mean of six trials		First trial	
		r	p	r	p
Body area (mm ²)	60.9 \pm 10.3	-0.01	0.96	0.35	0.16
Tail area (mm ²)	19.2 \pm 3.7	-0.04	0.82	0.03	0.91
Absolute orange spot area (mm ²)	5.7 \pm 2.9	-0.04	0.86	-0.37	0.13
Relative orange spot area (%)	7.1 \pm 3.2	-0.08	0.68	-0.46	0.06
Orange spot coloration					
Hue ($^{\circ}$)	35.3 \pm 9.0	-0.17	0.38	0.04	0.87
Saturation (%)	72.4 \pm 12.8	-0.45	0.01	-0.56	0.02
Brightness (%)	72.5 \pm 9.0	0.03	0.89	-0.28	0.27

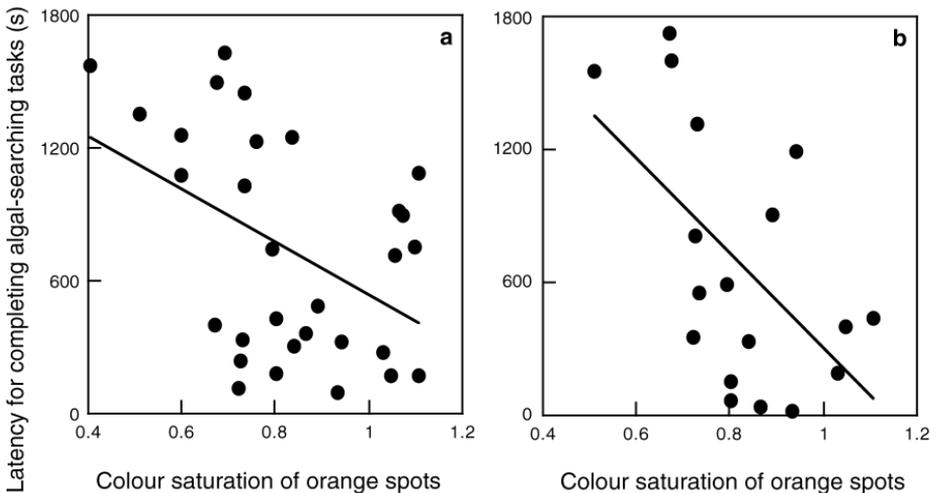


Figure 2. Relationships between the colour saturation of the orange spots in wild-caught males and the latency for completing algal-searching tasks in the laboratory experiment. (a) The mean latency in six trials, and (b) the latency in the first trial in the laboratory experiment. The value of colour saturation of orange spots was arcsine transformed.

In the first trial of the laboratory experiment, 18 males in the 30 males could reach and peck algae. The latency for completing the algal-searching task in the first trial also correlated significantly with the colour saturation of orange spots of males (Table 1, Figure 2b). This result further indicates that males with the high saturation of orange spot coloration in the wild exhibited the high algal-searching ability in the laboratory experiment. Other morphological traits of males in the wild were not significantly correlated with the latency for completing algal-searching task in the first trial in the laboratory experiment (Table 1), although males possessing larger relative orange spot area tended to peck algae within a shorter time.

Discussion

The result of the present study demonstrated that the colour saturation of the orange spots of the male guppies in the wild correlated with their algal-searching ability in the laboratory experiment. Since the saturation of the orange spot coloration in male guppies is determined by the amount of algal intake (Grether et al., 1999; Karino & Haijima, 2004), the colour saturation of the orange spots in wild-caught males may indicate their algal-foraging ability in their natural habitats. Hence, the latency for completing the algal-searching tasks in the laboratory experiment may reliably indicate their foraging ability in the wild. It is evident that in the laboratory experiment, the algal-searching ability has a heritable component (Karino et al., 2005). Therefore, females may be able to acquire indirect benefits such as offspring with a high algal-foraging ability by their mate preferences for males possessing the high colour saturation of orange spots.

Recently, Locatello et al. (2006) have revealed that male guppies possessing conspicuous orange spots produce sperm with a faster swimming velocity and longevity. In addition, Evans et al. (2003b) have documented that the fertilization success of male guppies exhibiting more conspicuous orange spots is greater than that of males having less conspicuous orange spots. It is possible that a greater intake of carotenoids, i.e., antioxidants, enhances both orange spot coloration and sperm quality of males (Blount et al., 2001; Evans et al., 2003b). Hence, the result of this study suggests that females also obtain a high fertilization success of their male offspring by mating preference based on orange spot coloration that is indicative of the algal-foraging

ability of males. In addition, Grether et al. (2004) have demonstrated that carotenoid intake enhance immune system in male guppies. Therefore, it is possible that females can produce offspring with enhanced immune system via their mate preference on the basis of the orange spot coloration of males.

In contrast to the colour saturation of the orange spots, the algal-searching ability in the laboratory was not significantly correlated with the size of the orange spots of the wild-caught males. This result was not surprising because it is well known that the orange spot size in male guppies is determined by genetic components rather than environmental factors such as the amount of algal intake (Kodric-Brown, 1989; Houde, 1992; Brooks & Endler, 2001; Karino & Haijima, 2001). On the other hand, the algal intake enhances the growth of the guppies in the laboratory (Karino & Haijima, 2004). However, the algal-searching ability in the laboratory experiment was not correlated to either the body or tail sizes of the wild-caught males. The body and tail sizes of the male guppies are also influenced by other factors such as genetic factors (Brooks & Endler, 2001; Karino & Haijima, 2001) and the amount of intake of foods other than algae (Karino & Haijima, 2004). It is also possible that the body size reflected age of the males and the age-dependent effect might mask the influence of algal intake on body size, although ages of the wild-caught males were not examined in this study. Kolluru et al. (2006) suggested that the growth of tails in male guppies was not constrained by carotenoid intake. Therefore, the body and tail sizes of male guppies in the natural environment may be determined to a greater degree by factors other than the amount of algal intake.

In the laboratory experiment in this study as well as the one performed in a previous study (Karino et al., 2005), the males could not see the location of the algae from their starting point in the experimental aquarium. Therefore, this experimental system examined the ability of the male guppies to search for algae with respect to their exploration ability in novel environments and their sensitivity to the chemical cue from algae. The result of this study suggests that the exploration ability and chemical sensitivity of the males play important roles in their foraging success in the natural environment. On the other hand, Karino et al. (2005) have documented high heritability estimates ($h^2 = 0.57-0.66$) in the algal-searching ability of guppies. Considering the female mate preference for males possessing orange spots with the high colour saturation (Kodric-Brown, 1989; Grether, 2000;

Karino & Shinjo, 2004), it is plausible that a subtle directional sexual selection toward males exhibiting a greater colour saturation of orange spots and thus having a higher algal-foraging ability might occur. However, orange spot coloration as well as the algal-searching ability showed high individual variations among the male guppies. It is possible that some behavioural patterns not only contribute to the foraging success but also incur some costs to the individuals. For instance, the exploration ability in novel environments may be a component of the foraging ability but appears to increase predation risks (cf., Lima, 1985; Gilliam & Fraser, 1987; Abrahams & Dill, 1989; Dugatkin, 1992). As a result, opposing natural and sexual selection pressures may reduce overall selection, and the large amounts of heritable variation of the algal-searching ability in the male guppies can be maintained.

In addition to the sexual attractiveness to females, the reproductive success of male guppies is influenced by sneak copulations (Matthews & Magurran, 2000; Evans et al., 2003a; Karino & Kobayashi, 2005). Therefore, it is also possible that the high variation in orange spot coloration and foraging ability among males are maintained via the sneak mating tactic by males, i.e., males possessing lower algal-foraging ability and exhibiting lower colour saturation of orange spots can reproduce by sneak copulations. It will be useful to examine the relationships between the algal-foraging ability and the sneaking behaviour or costs of predation in male guppies. This information will provide some implications on the mechanisms required to maintain a high individual variation in sexually selected traits among males.

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