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Colour patterns influence symbiosis and competition in the anemonefish-host anemone symbiosis system

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Colour patterns in fish are often used as an important medium for communication. Anemonefish, characterized by specific patterns of white bars, inhabit host anemones and defend the area around an anemone as their territory. The host anemone is not only used by the anemonefish, also by other fish species that use anemones as temporary shelters. Anemonefish may be able to identify potential competitors by their colour patterns. We first examined the colour patterns of fish using host anemones inhabited by *Amphiprion ocellaris* as shelter and compared them with the patterns of fish using surrounding scleractinian corals. There were no fish with bars sheltering in host anemones, although many fish with bars were found in surrounding corals. Next, two fish models, one with white bars and the other with white stripes on a black background, were presented to an *A. ocellaris* colony. The duration of aggressive behaviour towards the bar model was significantly longer than that towards the stripe model. We conclude that differences in aggressive behaviour by the anemonefish possibly select the colour patterns of cohabiting fish. This study indicates that colour patterns may influence not only intraspecific interactions but also interspecific interactions in coral reef ecosystems.

1. Introduction

Colour patterns in fish are known to be shaped by a variety of selective pressures, including predators, prey, competitors and mate choice (e.g. [1–6]). In particular, fish inhabiting coral reefs have the most diverse pigment cell types of any vertebrate, resulting in a wide variety of colour patterns, such as bars, stripes and spots [7]. In coral reefs with high water transparency, visual signals are effective communication tools, and the colour patterns of fish play an important role in determining the behaviour between individuals and species, such as, camouflage/mimicry [8–14], species/individual identification [15–17], courtship [10,18–20] and other social interactions [21,22]. Competition and symbiosis can be established using such visual information, and as a result, there must be rules regarding the composition of colour patterns within the local community. However, there have been no studies focusing on the role of visual information in determining the species composition of fish communities.

Anemonefish (Amphiprioninae, Pomacentridae) have conspicuous white bars against a background colour of orange, red or black, and the number of white bars varies depending on the species [7,23–25]. The evolutionary function of colour patterns in anemonefish is poorly understood, but at least three adaptive hypotheses have been proposed [7,26–29]. The first hypothesis is that the number of white bars has a recognition function since anemonefish have

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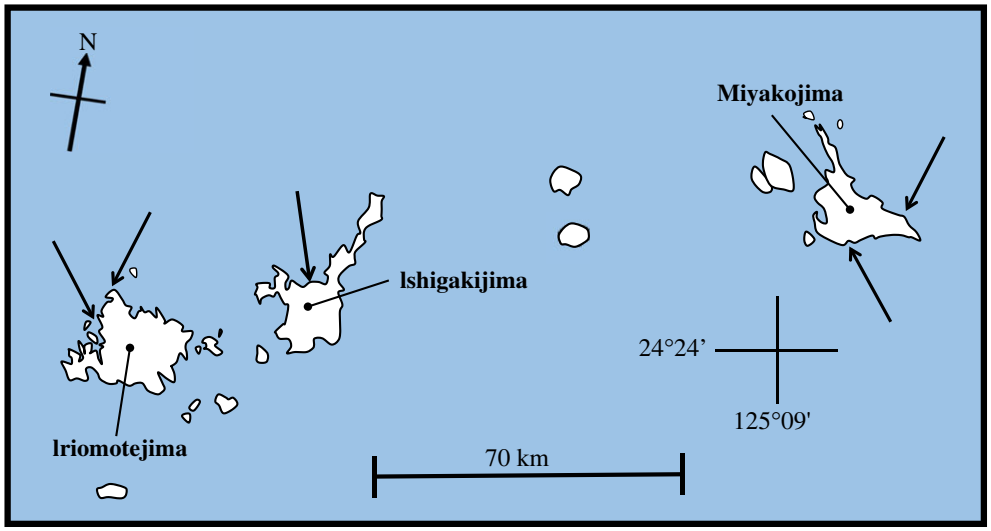

Figure 1. Map of the study site in the Sakishima Islands. Arrows indicate the study sites. (Online version in colour.)

species-specific numbers of white bars. This hypothesis is supported by the fact that the differences in the number of white bars between species inhabiting the same area are significantly greater than would be expected at random [27]. In addition, anemonefish patterns change during ontogeny in some species [7,23–25,27], and the different patterns of juveniles from adults may be a dishonest signal to conceal their presence and reduce agonistic interactions [26,27,30]. The second hypothesis is that the contrast of the bright base colour and white stripes is disruptive and functions to hide the fish silhouette. In *Amphiprion ocellaris* and *A. percula*, in particular, an indentation on the dorsal fin and the white bars extending across the dorsal fin appear to have a high disruptive colour effect [27]. The third is an aposematism hypothesis, that is, the conspicuous colour patterns serve to advertise the toxicity of the host anemone. Phylogenetic analyses have revealed that host venom strength and tentacle length are correlated with colour patterns of anemonefish, supporting their function as warning colours [29].

Anemonefish inhabit host anemones for their whole life except for their pelagic larval stage (e.g. [31–33]). Host anemones have numerous nematocytes that sting most fish, but anemonefish can use host anemones as shelter thanks to their mucus which prevents stinging [31,34,35]. Anemonefish defend their host anemone as territories and express aggressive behaviour toward other anemonefish and towards other fish species [36–39]. Despite this, the host anemone inhabited by anemonefish pair may be used as a temporary refuge by other species of fish such as damselfish (Pomacentridae), cardinalfish (Apogonidae) and wrasses (Labridae) [40–42]. In the Ryukyu Archipelago, Japan, 16 species from three families (Apogonidae, Labridae and Pomacentridae) have been observed inhabiting host anemones, and Labridae come to host anemones to clean anemonefish [42].

Dascyllus trimaculatus (Pomacentridae), which most frequently uses anemonefish colonies, has a black ground colour with three small white spots. When a model of this pattern is presented to six species of anemonefish, aggressive behaviour was observed in response to the models, but there were species differences in the frequency of this behaviour [41]. *D. trimaculatus* tended to use colonies of *A. samdaracinos*, which showed relatively less aggressive behaviour, but not colonies inhabited by *A. frenatus* or *A. polymnus*, which showed more aggressive behaviour [41]. Further, during our studies on anemonefish-host anemone symbioses in the Ryukyu Archipelago, we noticed that fish species other than anemonefish that use host anemones have stripes and spots but not patterns with vertical bars [42]. This may be because the aggressive behaviour in anemonefish is less frequent to fish without bars or stripes, such as *D. trimaculatus*, and to the fish with stripes, such as *Labroides dimidiatus* (Labridae), but more frequent towards fish with bars. Therefore, we hypothesized that anemonefish may decide whether to tolerate or exclude potential intruders based on their colour patterns, and that their behaviour may influence fish community structure.

The purpose of this study was to examine the relationships between the frequency of anemonefish aggressive behaviour and the colour patterns of fish by clarifying the following two questions: (i) Within the same habitat, is there a difference in the colour patterns of fish that live in host anemones (with anemonefish) and in scleractinian corals (without anemonefish)? and (ii) Do anemonefish differ in the frequency of their aggressive behaviour toward fish with vertical bars and horizontal stripes? The answers to these two questions will help clarify the effects of anemonefish behaviour on fish communities in the anemonefish-host anemone symbiosis system.

2. Materials and methods

(a) Colour patterns of fish using scleractinian corals and host anemones

We conducted field surveys at five study sites (two study sites on reefs around Miyakojima Island, one around Ishigakijima Island and two around Iriomotejima Island) in the Ryukyu Archipelago from September 2020 to October 2021 (figure 1). *A. ocellaris* and their hosts *Stichodactyla gigantea* were targeted in each study area, which ranged from 0 to 2 m in water depth. The fish fauna of a total of 49 individuals of *S. gigantea* and that of 49 scleractinian corals that were closest (2–10 m away) to each of the *S. gigantea* were recorded (Miyakojima: $n = 18$, Ishigakijima: $n = 6$, Iriomotejima: $n = 25$). The scleractinian corals studied ranged from *ca* 20 to 60 with a mean of 35 cm (s.d. = 6.38) in diameter, and the host anemones ranged from 20 to 47 with a mean of 31 cm (s.d. = 10.14). The areas within the outermost range of host anemone's tentacles and scleractinian coral colony branches were defined

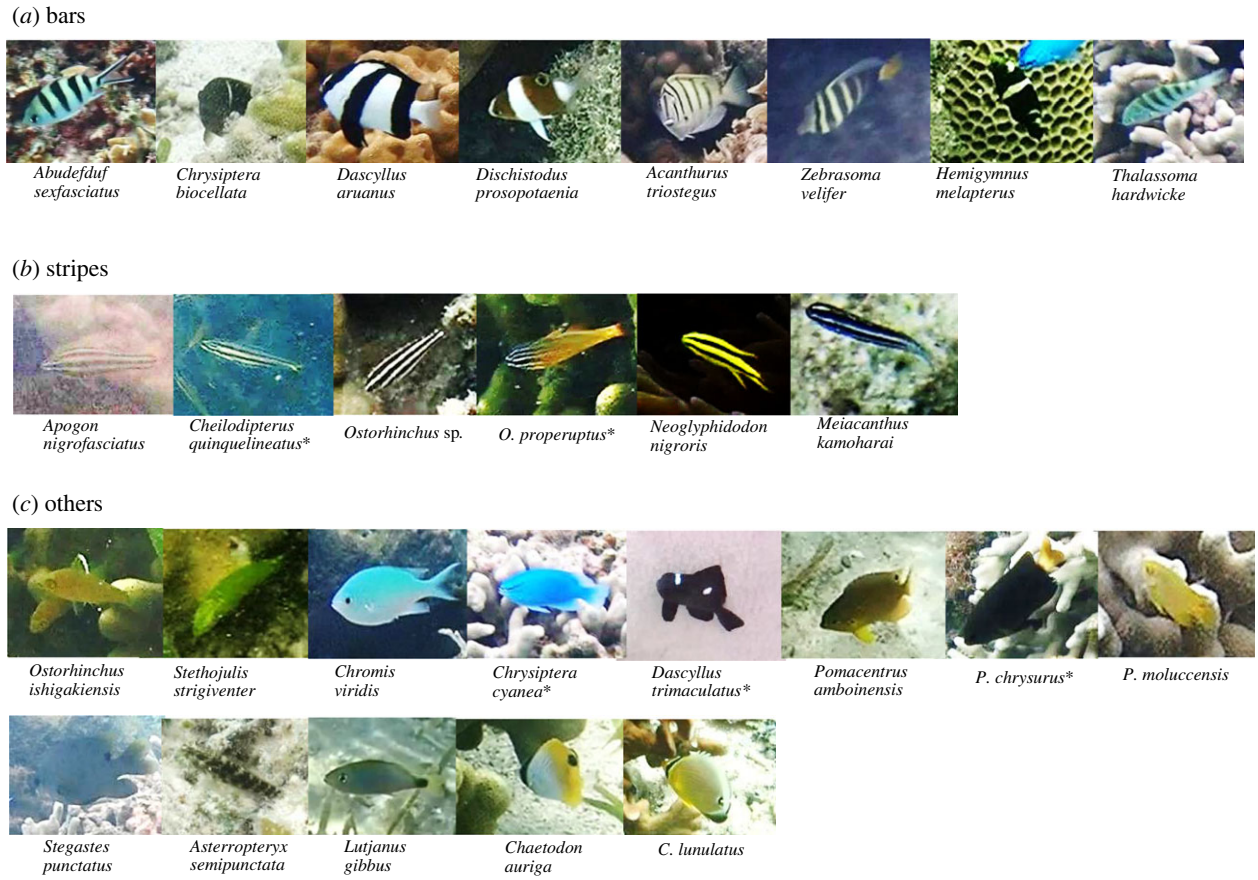

Figure 2. Photographs and colour pattern classifications of the various fish species observed in scleractinian corals and host sea anemones in the present study. Species with asterisks were found in both corals and sea anemones. (Online version in colour.)

as the target area, including spaces covered under the tentacles and branches, between the tentacles/branches, and above the tentacles/branches [42]. To minimize the influence of the observer on the behaviour of anemonefish based on the results of [43], we slowly approached the host anemone/ scleractinian corals from *ca* 2 m and started recording at a distance of 0.5 m. In order to compare the fish species using the host anemone and scleractinian corals as shelter, we recorded around the target area for 3 min using video camera (Olympus TG-6). Fish species swimming within the target area for a minimum of 2 min were identified as fish using the host anemones/scleractinian corals, thereby excluding from the analysis fish species that briefly enter the host anemones/scleractinian corals [44]. The colour patterns of fish using host anemones/scleractinian corals were divided into three categories: bars (vertical bars) (figure 2*a*), stripes (horizontal stripes) (figure 2*b*) and others (figure 2*d*). Fishes which had both bars and stripes such as butterflyfish (figure 2*c*) were counted as 'others'. Differences in the frequency of colour patterns of resident fish between host anemones and corals were compared using a χ-square test of independence conducted in IBM SPSS Statistics Ver. 28.

(b) Aggressive behaviour of anemonefish toward bar and stripe patterns

We conducted behavioural experiments on 45 *A. ocellaris* colonies (Miyakojima: 15 colonies, Ishigakijima: 6 colonies, Iriomotejima: 24 colonies). After 3 min recording fish species using host anemones as mentioned above, we started behavioural experiments by setting the model fish. Two types of fish models were prepared: one with two white vertical bars on a black background (bar model) and the other with two white horizontal stripes on a black background (stripe model). Fish-shaped plastic toys of 5 cm in total length were painted with a permanent marker (Mitsubishi Paint Marker PX-20, Mitsubishi Pencil Corporation, Tokyo, Japan) in black for the base colour and white for the bars and stripes (electronic supplementary material, figure S1). The reflectance spectra of colours were determined every 10 nm from 400 to 700 nm using 30 mm-diameter illumination by Konica Minolta Inc., CM-700D (electronic supplementary material, figure S1). The three attributes of colour (L: lightness, C: chroma, H: hue) of the model were measured by a colorimeter (NR-11A; Nippon Denshoku Industries, Tokyo, Japan). Measurements were conducted three times and the mean value of the base colour was 2.1, 1.9, 312.0 (L, C, H), and that of bar/stripe was 71.6, 6.7, 111.7 (L, C, H).

These models were dangled by a transparent fishing line and placed in close proximity to each *A. ocellaris* colony. As aggressive behaviour of anemonefish toward intruders is known to decrease gradually over 2 min [41,43], we continued recording for 3 min. To prevent habituation to the model, each colony was tested only once, and one of the two different models was randomly presented to each *A. ocellaris* colony. Following previous studies, both 'chasing' and 'biting' were collectively defined as aggressive behaviour towards the model fish [41,43,45]. To calculate the difference in frequency of aggressive behaviour between bar and stripe models, and sexual differences in the frequency of aggressive behaviour, a non-parametric Mann–Whitney *U-test* conducted in IBM SPSS Statistics Ver.28. was used.

3. Results

(a) Differences in colour patterns of fish using host anemone and scleractinian corals

Throughout all study sites, 19 out of 49 individuals (39%) of *S. gigantea* anemones were used by other fish species

Table 1. List of fish species inhabiting (a) host anemones and (b) scleractinian corals (Pocilloporidae, Acroporidae and Poritidae) in each island.

family of coexisting fish species	coexisting fish species	colour patterns	number of host anemones inhabited by each fish species			total	percentage in 49 host anemones (%)
			Miyakojima	Ishigakijima	Iriomotejima		
(a) host anemones							
Apogonidae	*Apogon nigrofasciatus*	stripes	1	0	0	1	2.0
	Cheilodipterus quinquelineatus	stripes	4	0	0	4	8.2
	Ostorhinchus properuptus	stripes	4	0	2	6	12.2
Pomacentridae	*Chrysiptera cyanea*	others	2	4	0	6	12.2
	Dascyllus trimaculatus	others	4	2	3	9	18.4
	Pomacentrus chrysurus	others	1	1	0	2	4.1
(b) scleractinian corals							
Apogonidae	*Cheilodipterus quinquelineatus*	stripes	2	0	0	2	4.1
	Ostorhinchus sp.	stripes	2	0	2	4	8.2
	O. ishigakiensis	others	0	0	1	1	2.0
	O. properuptus	stripes	2	0	1	3	6.1
Labridae	*Hemigymnus melapterus*	bars	0	0	1	1	2.0
	Stethojulis strigiventer	others	1	0	1	2	4.1
	Thalassoma hardwicke	bars	1	1	0	2	4.1
Pomacentridae	*Abudefduf sexfasciatus*	bars	0	2	0	2	4.1
	Chromis viridis	others	2	0	1	3	6.1
	Chrysiptera biocellata	bars	0	0	2	2	4.1
	C. cyanea	others	13	5	23	41	83.7
	Dascyllus aruanus	bars	15	1	8	24	49.0
	D. trimaculatus	others	0	1	1	2	4.1
	Dischistodus prosopotaenia	bars	1	0	0	1	2.0
	Neoglyphidodon nigroris	stripes	1	0	1	2	4.1
	Pomacentrus amboinensis	others	0	0	1	1	2.0
	P. chrysurus	others	1	2	8	11	22.4
	P. moluccensis	others	4	3	3	10	20.4
	Stegastes punctatus	others	2	0	4	6	12.2
Chaetodontidae	*Chaetodon auriga*	others	0	0	7	7	14.3
	C. lunulatus	others	0	0	1	1	2.0
Acanthuridae	*Acanthurus triostegus*	bars	0	0	1	1	2.0
	Zebrasoma velifer	bars	1	0	2	3	6.1
Gobiidae	*Asterropteryx semipunctata*	others	0	0	1	1	2.0
Blenniidae	*Meiacanthus kamoharai*	stripes	0	0	1	1	2.0
Lutjanidae	*Lutjanus gibbus*	others	0	0	2	2	4.1

(table 1*a*). Three species of cardinalfish and three species of damselfish were observed during the course of our study (table 1*a*). Throughout all study sites, all 49 individuals (100%) of scleractinian corals were used by other fish species (table 1*b*). A total of 26 species were observed in scleractinian corals, consisting of 4 species of cardinalfish, 3 species of wrasse, 12 species of damselfish, 2 species of butterflyfish, 2 species of surgeonfish, 1 species of goby and 1 species of snapper (table 1*b*). Five of 26 species (*Ostorhinchus properuptus*, *Cheilodipterus quinquelineatus*, *D. trimaculatus*, *C. cyanea* and *P. chrysurus*) used both host anemones and scleractinian corals (table 1, figure 2). *D. trimaculatus* and *O. properuptus* tended to use host anemones rather than scleractinian corals (table 1). Most fish observed in host anemone and scleractinian corals were immature fish.

Of the six fish species observed in host anemones, three had stripes, the other three had other types of patterns, and no fish species had bar patterns (table 1*a*, figures 2 and 3). On the other hand, 8 of the 26 species which used corals had bar patterns, 5 species had stripes and 13 species had other patterns (table 1*b*, figure 2). There was a significant difference in the frequency of colour patterns of fish using between host anemone and corals (figure 3, chi-square test, $\chi^2 = 14.27$, d.f. = 2, $p < 0.01$). In host anemones, there

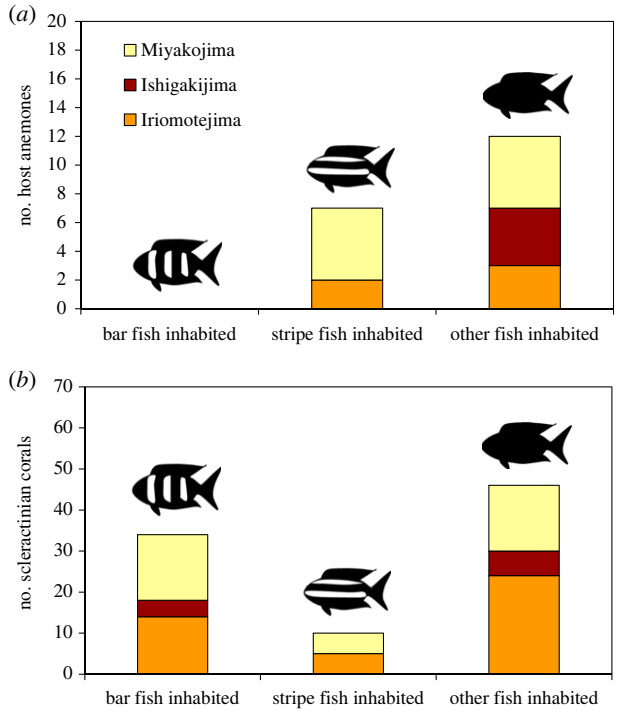

Figure 3. The number of (*a*) host anemones and (*b*) scleractinian corals that were inhabited by each colour pattern of fish. (Online version in colour.)

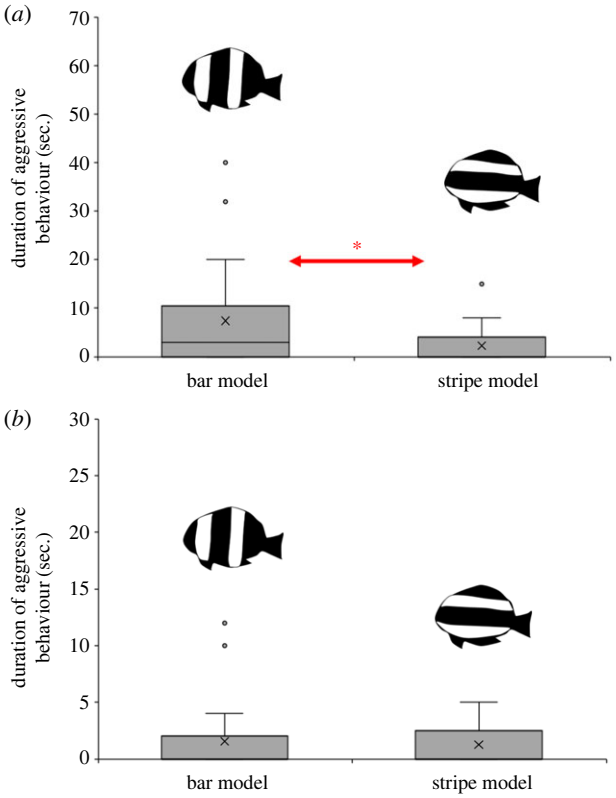

Figure 4. Box plot showing the duration of aggressive behaviour of (*a*) female and (*b*) male *Amphiprion ocellaris* towards bar model and stripe model during 180 s videos. Crosses represent means, boxes represent inter-quartile ranges (25%–75%), centre lines represent medians, whiskers represent ranges excluding outliers and circles represent outliers. Significant differences by Mann–Whitney *U-test* indicated by asterisks; $*p < 0.05$. (Online version in colour.)

were no fish species with bar patterns but fish species with stripe patterns were more abundant (figure 3*a*). On the other hands, in corals, the fish species with bar patterns were more frequently found than the fish species with stripe patterns (figure 3*b*). This tendency was similarly observed in sites at Miyakojima, Ishigakijima and Iriomotejima islands (figure 3).

(b) Differences in the frequency of aggressive behaviour toward bar and stripe models

In female anemonefish, the duration of aggressive behaviour towards the bar model was 3 s/3 min (median), 0–10.5 (25–75% quartiles), 0–40 (min-max range), and that towards the stripe model was 0 s/3 min (median), 0–4 (25–75% quartiles), 0–15 (min-max range), and the former was significantly longer than the latter (figure 4*a*; Mann–Whitney *U-test*; $U = 150$, $p < 0.05$). In males, there were no significant differences in duration of aggressive behaviour between the bar (0 s/3 min in median, 0–2 in 25–75% quartiles, 0–12 in min-max range) and stripe (0 s/3 min, 0–2.5 in 25–75% quartiles, 0–5 in min-max range) models (figure 4*b*; Mann–Whitney *U-test*; $U = 200.5$, $p = 0.84$). No aggressive behaviour was observed in immature fish. The duration of aggressive behaviour toward bar models was significantly longer in females than males (Mann–Whitney *U-test*; $U = 142.5$, $p < 0.01$). On the other hand, there were no significant differences in the duration of aggressive behaviour toward stripe models between male and female (Mann–Whitney *U-test*; $U = 174.5$, $p = 0.63$).

4. Discussion

The present study showed that fish species asides from anemonefish that use host anemones did not have vertical bar patterns, but that nearby scleractinian corals were used by a variety of fish species with various colour patterns including bars. The fact that only 39% of anemones were used as shelter by other fish, while 100% of scleractinian corals were used by fish, may be due to the lack of tolerance to the venom of the host anemone by most fishes. However, this observation may also be linked to the aggressive behaviour of anemonefish, which actively chase away many intruding fish. Therefore, we hypothesize that the reason for this difference was due to the aggressive behaviour of anemonefish, which defend host anemones as their territory. We further hypothesized that the behaviour of anemonefish differs depending on the colour pattern of the intruder fish.

When either bar or stripe models were presented to colonies of anemonefish, we observed that female anemonefish attacked the bar model more persistently and for a longer time than the stripe model. When a similar experiment was conducted on a model with white spots on a black background (=imitating *D. trimaculatus*), the duration of aggressive behaviour by *A. ocellaris* was 0 s/3 min (median) with 0–2 (25%–75% quartiles) and 0–3 (min-max range) in females and 0 s/3 min (median) with 0–0 (25%–75% quartiles) and 0–1 (min-max range) in males [41]. Although comparisons between the current study and this past research should be made with caution due to the use of different study sites, the duration of aggressive behaviour did not differ between the *D. trimaculatus* model and the striped model, but was much higher in the bar model. As expected, anemonefish responded more aggressively to intruders with bar patterns and protected the host anemone as their own

territory, indicating that only fish species without bar patterns may have access to host anemones. Our results strongly suggest that the fish community around host sea anemones is likely to be biased by the preferences of anemonefish. In order to further develop the present results, future comparisons of aggressive behaviour of anemonefish to a variety of colour patterns at different sites are needed.

Territorial and social fish, like the Ambon damsel (*Pomacentrus amboinensis*) or cichlid species (Cichlidae), have been shown to use colour patterns to distinguish between competitors and mates [1,15–17,46]. According to [1], dorsoventral bars, especially in the case of melanin pigmentation, are associated with intraspecific aggression in various species of fish. For example, the Lake Tanganyika cichlid *Tropheus* sp. has a yellow bar. *Tropheus* sp. tends to less frequently attack individuals with wider yellow bars [46]. As well, the cichlid *Neolamprologus pulcher* has been shown to attack individuals with vertical lines lighter and shorter than their own [47]. Most previous studies have focused on the significance of bar patterns in intraspecific competition. In this study, we suggest that aggressive behaviour of anemonefish towards intruders with bar patterns (presumably toward anemonefish) may also have a secondary effect on interspecific interactions.

By contrast, cranio-caudal stripes are often associated with intraspecific cooperation such as shoaling in cichlids, zebrafish and rainbow fish [1]. Moreover, stripes can be a signal of interspecific cooperation, such as cleaning behaviour [44,48,49]. In fact, five species of wrasse that clean anemonefish in the Ryukyu Archipelago have a striped pattern [42]. In the cleaner wrasse, *Labroides dimidiatus*, a longer striped pattern is known to comparatively attract more fish [49]. [50] reported that 11 parasite species (two species of copepods, six digeneans, two nematodes and one acanthocephalan) have been found in anemonefish in the South China Sea. Thus, the presence of cleaner fish, which feed on body surface parasites, should be beneficial to anemonefish. As all the cleanerfish in the study site have horizontal stripes, it was not possible to confirm whether the anemonefish recognized the cleanerfish species or judged them solely on the basis of their stripe patterns, which remains an issue to be examined in the future. It is also necessary to focus on how cleanerfish behave in response to aggressive behaviour by anemonefish.

In this experiment, females were more aggressive than males toward bar models. In most species of anemonefish, females are larger than males and are often more aggressive [41,43]. However, in laboratory conditions, female *A. ocellaris* displayed fierce aggressive behaviour toward female intruders, whereas males were more often aggressive to male intruders [39]. Thus, we theorize that if the size of the models was reduced to 3 cm, equivalent to the total length of male *A. ocellaris*, males may have become more aggressive. [39] reported that immature intruders were rarely attacked at all under laboratory condition, suggesting that size as well as colour pattern is involved in aggressive behaviour in intraspecific competition.

At least seven species of anemonefish are known to use *S. gigantea* as a host [35]. In the present study site, the Ryukyu Archipelago, *A. clarkii* and *A. perideraion* are known to use *S. gigantea*, but only *A. ocellaris* inhabited the *S. gigantea* examined in the present study. Moreover, *A. ocellaris* does not co-inhabit with other species of anemonefish [51,52]. Thus, *A. ocellaris* may be aggressive not only against competitors of the same species, but also against other anemonefish species that may use the same host species. In this experiment, we used a model with two white bars on a black background, which is similar to *A. clarkii*. However, *A. ocellaris* has a pattern of three white bars, and *A. perideraion* has one white bar. Since anemonefish are known to recognize the same species by visual cues [28], it is possible that they can identify the numbers of white lines and alter their behaviour based on such information.

The present results, together with previous reports [26,27,30], suggest that the white bars of anemonefish have two different effects: (i) they allow the regulation of the relationship within anemonefish in the case of bispecific colonies [53] or when immature fish recruits enter the host, and (ii) they act as a selection filter to tolerate other species that use the sea anemone as a shelter. Future work needs to more precisely examine the aggressive behaviour of anemonefish to various bar patterns and sizes, including patterns of the same and different species of anemonefish, as well as fish species other than anemonefish. Results from these future works may allow us to better understand how the various patterns are recognized and to pinpoint the basic rules controlling this inter- and intraspecific signalling.

On tropical and subtropical coral reefs, the presence of 'shelters' such as zooxanthellate scleractinian corals are known to be important for the coexistence of many species of fish in these ecosystems (e.g. [54–58]). Similarly, host sea anemones inhabited by anemonefish play an important role in maintaining the diversity of coral reef ecosystems [42]. Investigating the rules of colour patterns that determine the behaviour of anemonefish will help to understand the community structure in the anemonefish-host anemone symbiosis.

Ethics. All of the experimental procedures described were approved by University of the Ryukyus and Okinawa Institute of Science and Technology (OIST: FWA-2021-003).

Data accessibility. All data are available as part of supplement materials and table 1.

The data are provided in the electronic supplementary material [59].

Authors' contributions. K.H.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, visualization and writing—original draft; K.T.: project administration, supervision and writing—review and editing; J.D.R.: project administration, supervision and writing—review and editing; V.L.: funding acquisition, project administration, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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