Spacing pattern and body size composition of the protandrous anemonefish *Amphiprion frenatus* inhabiting colonial host anemones

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Abstract The protandrous anemonefish *Amphiprion frenatus* often forms a group consisting of a large female, a small male, and a smaller nonbreeder at an isolated single host anemone, where home ranges of subordinates were covered with the female's home range. Within the group, the dominant individuals suppress the growth of subordinates, resulting in large size differences. The spacing pattern and body size composition of *A. frenatus* on colonial hosts were investigated in Ishigaki Island, Okinawa, Japan. Six breeding pairs and 14 nonbreeders inhabited a colony of 157 anemones. Each pair maintained a territory in which pair members used different hosts. Nonbreeders had unstable home ranges on the outskirts of or in the pairs' territories. Body size differences between males and females in pairs and between males and nonbreeders were small. The small size differences in the colony of hosts are caused by reduced suppression of growth of subordinates by the dominant individuals. The total area of host anemones largely affects spacing pattern and social suppression of the anemonefish.

Key words Coral reef fish · Microhabitat structure · Host density · Social structure

Anemonefishes are known for symbiotic associations with sea anemones (Allen, 1972; Fautin and Allen, 1992; Moyer, 2001; Porat and Chadwick-Furman, 2004) and socially controlled protandry with a monogamous mating system (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Ross, 1978; Fricke, 1979). As they use hosts as shelter and spawning site, the density of host (the number of host in an area) is often considered to be a crucial determinant of the spacing pattern and body size composition of individuals in a certain area (Allen, 1972; Fricke and Fricke, 1977; Ross, 1978; Fricke, 1979; Moyer, 1980). For example, *Amphiprion bicinctus* inhabits isolated single hosts and forms a small group consisting of a large female, a small male, and a varying number of smaller nonbreeders at a host (Fricke and Fricke, 1977). In a group, home ranges of small fish are covered by the female's home range, and large individuals suppress the growth of small ones. When a female disappears in a group, a male acquires the vacant female breeding post and starts growing to be female, and the largest nonbreeder becomes male. Thus, the body size composition and sex of group members are socially controlled by dominant fish (Fricke and Fricke, 1977).

A few species of anemonefishes inhabit both sparsely distributed and densely distributed single hosts and colonial hosts (Allen, 1972; Moyer and Nakazono, 1978; Fautin and Allen, 1992; Moyer, 2001). *Amphiprion clarkii* is one of such fishes, but there seems to be no great difference in the spacing pattern and body size composition of individuals between habitats (Moyer, 1980; Ochi, 1989a,b; Hattori and Yanagisawa, 1991a,b; Hattori, 1994, 2002; Hattori and Yamamura, 1995). Breeding pairs have territories, occupying large hosts that are necessary for their reproduction, and nonbreeders often have home ranges including small hosts on the outsides of the pairs' territories (Moyer, 1980; Ochi, 1989a,b; Hattori and Yanagisawa, 1991a; Hattori, 1994). Even in a habitat of low host density, individuals sometimes move between hosts to acquire larger mates and/or larger hosts, and when a female disappears, a solitary large nonbreeder often acquires the vacant female breeding post (Hattori and Yamamura, 1995). Regardless of host density, dominant individuals cannot suppress the growth of subordinates, and consequently body size difference between the sexes and that between males and nonbreeders are very small (Hattori and Yanagisawa, 1991a,b; Hattori and Yamamura, 1995; Hattori, 1994; Hirose, 1995). As large hosts are necessary for anemonefish reproduction, the size composition of the host, not the density of the host, may affect the spacing pattern and body size composition of individuals in anemonefishes. Except for *A. clarkii*, however, little information is available on the ecology of anemonefishes that inhabit sparsely and densely distributed single hosts and colonial hosts (see Allen, 1972; Moyer and Nakazono, 1978; Fautin and Allen, 1992; Moyer, 2001).

Amphiprion frenatus inhabits sparsely distributed single hosts and colonial hosts (Allen, 1972; Moyer and Nakazono, 1978; Dunn, 1981; Fautin and Allen, 1992; Moyer, 2001). At a fringing reef of Sesoko Island in Okinawa, Japan, *A. frenatus* forms an isolated small group consisting of a breeding pair and a nonbreeder at an isolated single host (Hattori, 1991, 2005; Hirose, 1995). The dominant female in a group strongly suppresses the growth of her mate and is about 1.6 times larger in standard length (Hattori, 1991). The strong growth suppression is attributed to the limited living space of an isolated single host, which can harbor only three individuals (Hattori, 2005). In fringing reefs of Ishigaki Island in Okinawa, *A. frenatus* inhabits "carpets" of colonial hosts (Moyer and Nakazono, 1978; Moyer, 2001). The present study was performed to determine the spacing pattern and body size composition of *A. frenatus* inhabiting colonial hosts. The data obtained in the present study are compared with those of *A. frenatus* in the Sesoko population and those of other anemonefishes. The aim of the present study was to know the relationship between the distribution pattern of host anemone and the social structure of a protandrous anemonefish.

Materials and Methods

The field study was conducted from June to September 1994 at a coral moat of Shiraho Reef in Ishigaki Island (24°22′N; 124°15′E), Okinawa, Japan. In Ishigaki Island, *Amphiprion frenatus* inhabits colonies of the giant sea anemone *Entacmaea quadricolor* (see Moyer and Nakazono, 1978). The anemonefish was monitored on a small patch reef (1.6–1.9 m in width, 1.9 m in height), where it was easy for a snorkel diver to observe and catch fish.

A field map of the patch reef was made based on underwater photographs and measurements, and locations of individual host anemones were plotted on the map. To evaluate the host size, the area covered by the tentacles of the hosts was measured as the sum of an oval area estimated by (long axial length) × (short axial length) × π/4 (see Hirose, 1985; Hattori, 1991). The oval area often included two or more host individuals. Hosts in an oval area were counted by determining the number of mouths. The sizes of several small hosts could not be measured because of their locations in the patch reef.

All individuals of *A. frenatus* were captured with hand nets, and then their standard lengths (SL) were measured with a ruler. All fish were recognized individually by scars on fin, body color pattern, and body sizes. A snorkel diver observed fish behavior while floating on the water surface at the highest tide in the daytime (1 h). Fish in the patch reef were monitored 76 days during the study period. Fish performing egg care were regarded as males, and individuals spawning the clutch of eggs or larger than the males in their home ranges were regarded as females. Other individuals were nonbreeders. A 15-min observation was conducted twice on each fish to determine the home range. In a 15-min observation, the locations of each fish were plotted on the map at intervals of 15 s. A line encircling all points plotted in the two observations was regarded as the boundary of its home range. The host containing the most points was regarded as the activity center of a fish. If they were regarded as a pair, a line encircling the home ranges of a pair was regarded as the border of the pair's territory. In the 15-min observation, the breeding (spawning and egg care), agonistic (rushing, dorsal leaning, and ventral leaning), and appeasement (head standing, head shaking, and substrate biting) behaviors were also recorded (see Yanagisawa and Ochi, 1986). When determining the home range of a breeder, its interactions with nonbreeders could not be recorded because it was difficult to distinguish nonbreeders while observing a breeder. After the home ranges of all individuals were determined, a 15-min observation of all breeders was conducted twice to count interactions between breeders and between breeders and nonbreeders, and a 15-min observation of seven nonbreeders was also performed twice to count interactions between nonbreeders.

Results

Twenty-six individuals of *Amphiprion frenatus* (12 breeders and 14 nonbreeders) inhabited the patch reef (Fig. 1) at the beginning of the study, and none disappeared in the study period. Six breeding pairs (A–F, in order of the sum of body sizes of pair members) held territories, which did not overlap each other (Fig. 1a). Home ranges of pair members were not widely overlapped (an area overlapped between home ranges of pair/an area of pair territory = 0.462, 0.234, 0.535, 0.317, 0.477, and 0.644, corresponding to A through F, respectively). Nonbreeders had home ranges on the outskirts of or in the pairs' territories (Fig. 1b). During the observations, the locations often changed within the patch reef. A solitary nonbreeder (male-like color pattern, Fig. 1b: 3) immigrated into the home range of a solitary nonbreeder (female-like color pattern, Fig. 1b: 1), and they formed a pair. All fish involving breeders and nonbreeders used a different host as activity center.

Body sizes of breeding females were highly correlated with those of their mates (Fig. 2a; $r = 0.954$, $n = 6$, $P = 0.001$). The average size difference in pair members was 18.0 mm ± 8.9 SD ($n = 6$). Body size differences in pair members were correlated with the female body sizes (Fig. 2b; $r = 0.972$, $n = 6$, $P = 0.0011$) and male body sizes ($r = 0.858$, $n = 6$, $P = 0.028$). The ratios of the size difference to female body size were also correlated with the female body sizes (Fig. 2b; $r = 0.974$, $n = 6$, $P = 0.001$). Body sizes of both nonbreeders [47.5 mm SL ± 12.5 SD (range, 29–67 mm SL), $n = 14$] and males [66.3 ± 6.2 (54–74), $n = 6$] were widely overlapped with those of females [87.6 ± 13.9 (60–100), $n = 6$].

The average numbers of interactions between breeding pairs in a 15-min observation were significantly larger in neighbors than in no neighbors (Fig. 3a; Mann–Whitney *U* test, $U = 0$, $P < 0.01$). Breeding pairs interacted more frequently with nonbreeders whose home ranges overlapped with the territories of the former (Fig. 1b); i.e., the average numbers of interactions between breeders and nonbreeders in a 15-min observation were significantly larger when their home ranges overlapped (Fig. 3b; Mann–Whitney *U* test,

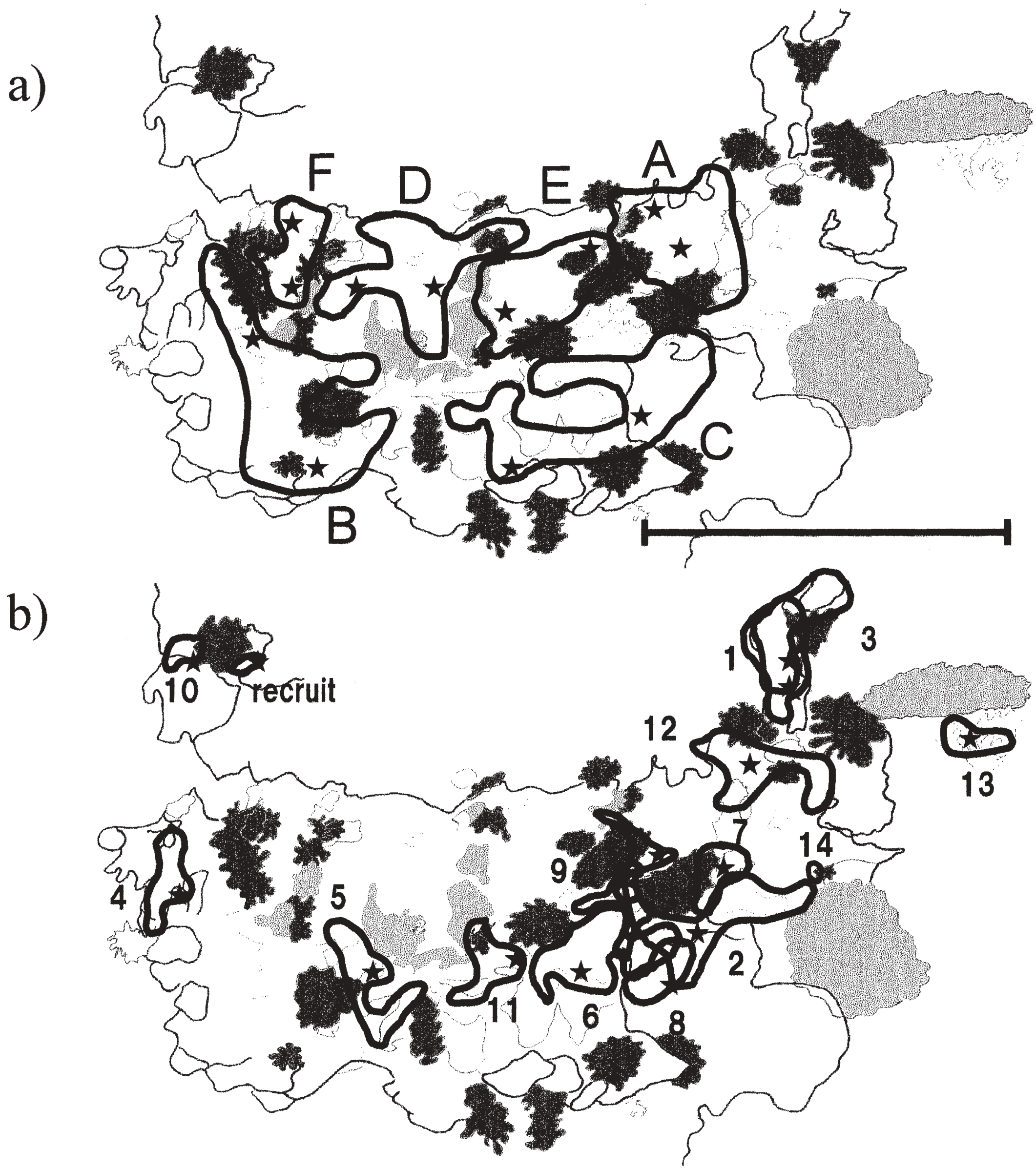

Fig. 1. Spacing patterns of breeders (**a**) and nonbreeders (**b**) of *Amphiprion frenatus* in a patch reef. *Bold lines* indicate territories of 6 breeding pairs (*A–F*, in order of the sum of body sizes of pair members) and home ranges of 14 nonbreeders (*1–14*, in order of body size) and a recruit. *Thin lines* indicate configurations of the patch reef. *Stars* indicate the "activity center" of each fish (see the definition in text). *Light shaded areas* and *darker shaded areas* are massive accumulations of algae (including *Padina minor*, *Dermonema frappieri, Liagora* sp., and *Jania* sp.) and branching algae (including *Sargassum* spp. and *Cystoseira* sp.), respectively. *Bar* 1 m

$z = 2.25$, $P = 0.001$). Interactions between nonbreeders were not restricted to neighboring nonbreeders (Figs. 1b, 3c), and the average numbers of interactions between nonbreeders in a 15-min observation were not larger when their home ranges overlapped (Fig. 3c; Mann–Whitney *U* test, $U = 28$, $P > 0.05$).

In the host colony, 157 hosts were found; their average size was 77.3 cm^2 ± 86.6 SD (range, 7.1–490.9 cm^2, $n = 150$), and the total area of the hosts was 11 598.8 cm^2. Small hosts less than 100 cm^2 comprised 79.3% of all hosts (Fig. 4a). The host density was 52.0/m^2, and one fish used an average of 6.08 hosts. Pair territories included 12–31 hosts (mean, 17.6 ± 5.3 SD, $n = 6$). The average total area of hosts in the home range of a breeder was significantly larger than that of hosts in the home range of a large nonbreeder (breeders: mean, 1152.9 cm^2 ± 620 SD, $n = 12$; largest 5 nonbreeders: mean, 453.1 cm^2 ± 433 SD, $n = 5$; Mann–Whitney *U* test, $U = 6$, $P = 0.011$). The sum of body sizes of members of a pair was more significantly correlated with the total area of hosts in the pair's territory (Fig. 4b; $r = 0.941$, $n = 6$, $P = 0.005$) than with the maximum size of host in the territory ($r = 0.824$, $n = 6$, $P = 0.043$).

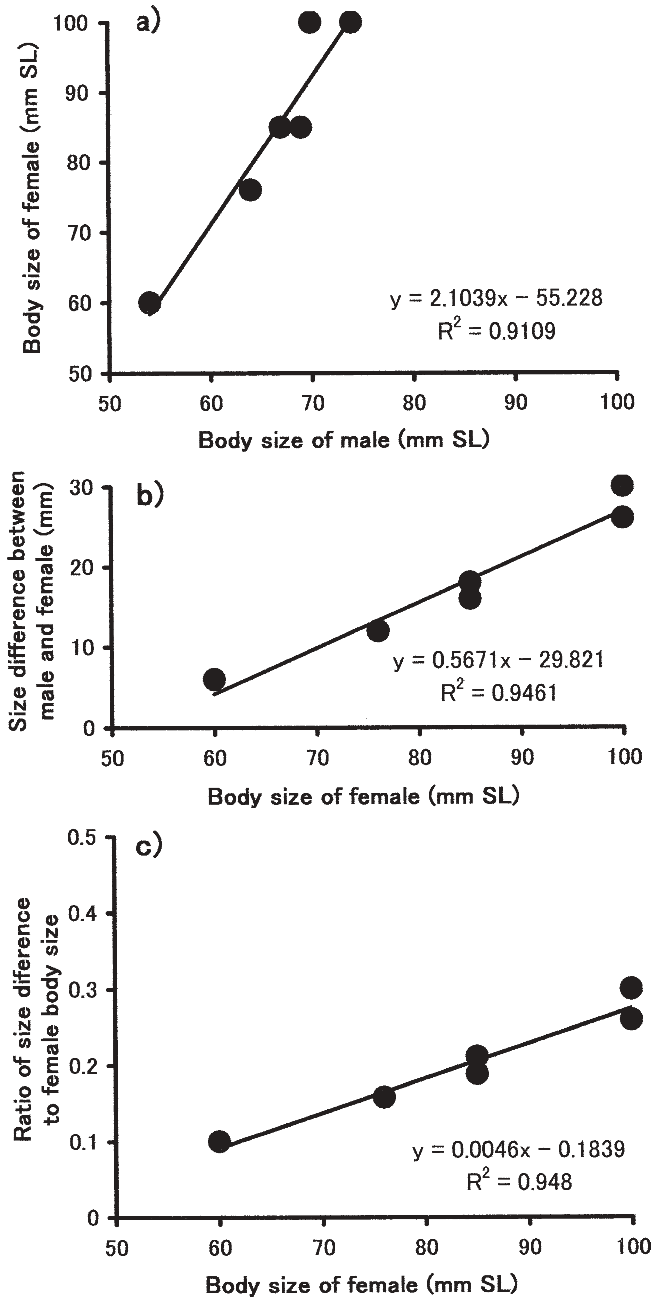

Fig. 2. Relationships between body size of males and females in pairs (**a**), between body size differences in pair members and body sizes of females (**b**), and between the ratio of the body-size differences to female body sizes and body sizes of females (**c**)

Discussion

Host utilization patterns of *Amphiprion frenatus* inhabiting colonial hosts differed markedly from those of individuals inhabiting sparsely distributed single hosts in Sesoko Island (host density, 0.0017/m^2; see Hattori, 1991). Host number per fish in the colony was much larger than that in sparsely distributed single hosts in Sesoko Island (6.04 vs. 0.33; see Hattori, 1991). In the colony of hosts, each fish used different host as activity center, and breeding pairs and nonbreeders occasionally occupied different hosts. Breeding pairs less frequently interacted with nonbreeders whose home ranges did not overlap with their territories. In Sesoko Island, in contrast, members of a group use only one host, and the home range of a nonbreeder is always included within the pair's home range (Hattori, 1991). These facts suggest that nonbreeders can escape social suppression by breeding pairs in the colony of hosts.

Breeding females were significantly smaller in this study site than in Sesoko Island [mean, 98.6 mm SL ± 8.6 SD, $n = 24$ (see Hattori, 1991); *t* test, $t_s = 2.5$, $P = 0.019$]. In contrast, nonbreeders were significantly larger in this study site than in Sesoko Island [mean, 25.1 mm SL ± 11.6 SD, $n = 21$ (see Hattori, 1991); *t* test, $t_s = 5.4$, $P < 0.000001$]. However, there was no significant difference in breeding males between the two habitats [Sesoko Island, mean, 60.5 mm SL ± 9.9 SD, $n = 23$ (Hattori, 1991); *t* test, $t_s = 1.4$, $P > 0.05$]. Body size differences between males and females in pairs in this study site were significantly smaller than those between males and females in pairs in Sesoko Island [mean, 36.8 mm SL ± 8.7 SD, $n = 34$ (Hattori, 1991); *t* test, $t_s = 5.4$, $P < 0.0001$], and those between males and nonbreeders (66.3–47.5 mm SL ± 18.8 mm) were also much smaller than those between males and nonbreeders in Sesoko Island (35.6 mm SL). These results suggest that the growth suppression of subordinates by the dominant fish is weaker in the colony of hosts.

In a habitat of low host density, anemonefishes are considered to form a small group consisting of a breeding pair and a nonbreeder(s) at a single host (Allen, 1972; Fricke and Fricke, 1977; Moyer and Nakazono, 1978). The formation of such a small group is often attributed to their low motility to migrate between isolated single hosts (Allen, 1972; Fricke and Fricke, 1977; Fricke 1979; Moyer and Nakazono, 1978; Moyer, 1980; Hattori, 1995, 2000). However, *A. frenatus* has enough mobility (Hattori, 2005), and the individuals form a small group consisting of a breeding pair and a nonbreeder (Hattori, 1991). These facts suggest that the formation of a small group in anemonefishes does not always depend on their mobility. For example, *A. clarkii* has also enough mobility, and individuals do not always form a small group at an isolated single host. In their habitat, there exist many small hosts that are not occupied by breeding pairs, and accordingly nonbreeders can move to those hosts to escape social suppression by breeding pairs (Hattori, 1994, 2002). In *A. frenatus* in Sesoko Island, in contrast, there are few small hosts and almost all large hosts are occupied by breeding pairs (Hattori, 1991). The formation of a small group of *A. frenatus* in a habitat of low host density is attributed to few recruitments and high longevity of the host anemones (Hattori, 2005). It is likely that the size composition of host anemones largely affects the spacing pattern and body size composition of individuals in the anemonefishes that inhabit single hosts. Porat and Chadwick-Furman (2004) state that the stability of anemonefish population depends in part on host population dynamics. The average size of hosts of *A. frenatus* was much smaller in this study site (77.3 cm^2 ± 86.6 SD, $n = 150$) than in Sesoko Island

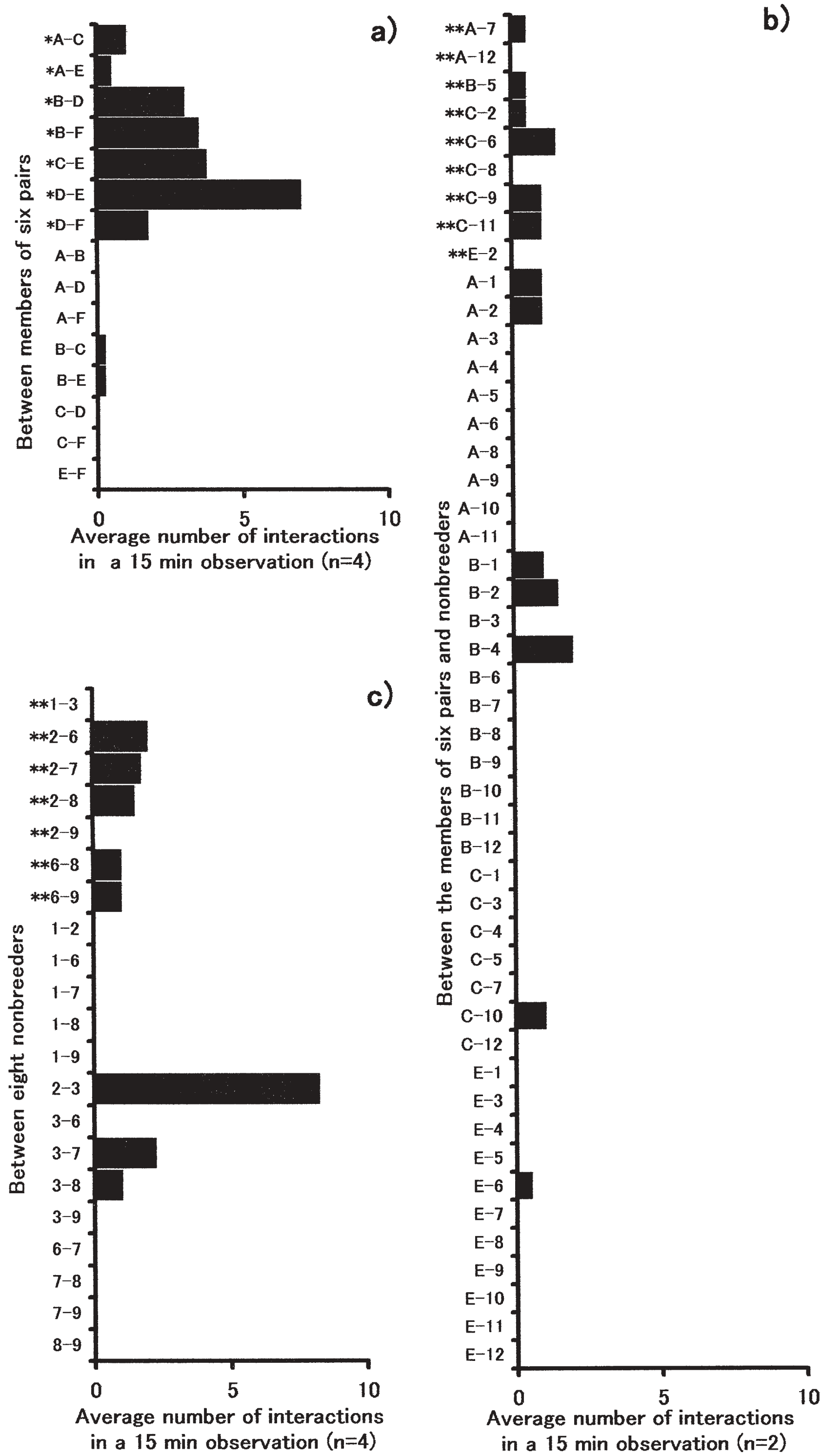

Fig. 3. Interactions between individuals in the patch reef. Average numbers of agonistic and appeasement behaviors in a 15-min observation between breeding pairs (**a**), between breeders and nonbreeders (**b**), and between nonbreeders (**c**). *Asterisks* indicate pairs whose territories were contiguous to each other. *Double asterisks* indicate individuals whose home ranges overlapped. In **b**, the numbers of interactions between nonbreeders and breeding pairs D and F were omitted, because they did not interact with any nonbreeders

[663 cm^2 ± 393 SD, $n = 36$ (Hattori, 1991); t test, $t_s = 16.09$, $P < 0.0001$]. It is likely that the "typical" group of anemonefishes, that is, a small group consisting of a breeding pair and a varying number of nonbreeders, is formed in a habitat where there are few small hosts so that nonbreeders can not escape the social suppression by breeders.

A. melanopus, which is closely related to *A. frenatus* (Allen, 1972; Fautin and Allen, 1992; Moyer, 2001), also inhabits colonial hosts (Allen, 1972; Ross, 1978). However, the spacing pattern and body size composition of individuals in *A. melanopus* are quite different from those of *A. frenatus* in this study site. Even in the largest colony of 103 hosts, *A. melanopus* forms a typical group consisting of a breeding pair and nonbreeders (Ross, 1978). The total area of a colony of hosts for *A. melanopus* even in the largest colonies was much smaller (2001–2838 cm^2; see Ross, 1978)

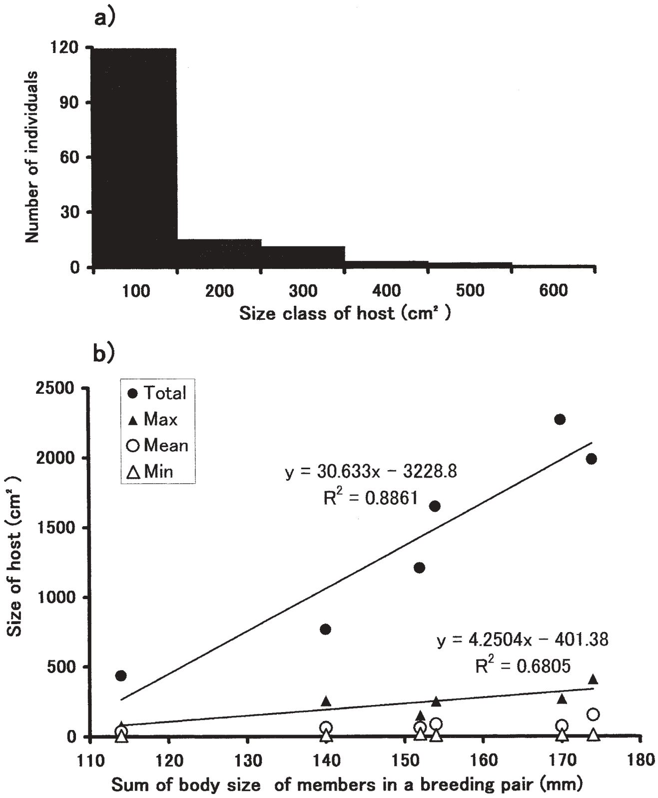

Fig. 4. Size distribution of hosts (**a**) and relationship between the sum of body sizes of pair members and the size (total, maximum, mean, and minimum) of their hosts (**b**). *The regression line* indicates where the relationship was statistically significant

than that of the colony of hosts for *A. frenatus* (11 598.8 cm^2; this study). This observation implies that two groups of *A. melanopus* could not share the colony of hosts and that nonbreeders of *A. melanopus* could not escape the social suppression by breeders because of the small area of a colony of hosts. The total area of hosts in a colony may be the crucial determinant of the spacing pattern and body size composition of the anemonefishes that inhabit colonial hosts.

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