Acclimation or Innate Protection of Anemonefishes from Sea Anemones?

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Studies have shown that anemonefishes can acquire protection from the stinging tentacles of their host anemones through a behavioral process called “acclimation.” However, some investigators have suggested that anemonefishes are innately protected from all species of host anemones. To definitively test whether anemonefishes are innately protected from anemones, we forced “naive” anemonefishes, bred and raised in complete isolation from anemones, to contact the tentacles of Macrodactyla doreensis, Heteractis crispa, and Stichodactyla haddoni. Individuals of Amphiprion clarkii were protected when forced to contact the tentacles of all three anemone species. However, individuals of A. ocellaris and A. perideraion were stung by some anemones. All fishes went through acclimation behavior after the initial forced contact with anemones. Thus, anemonefishes are innately protected from some anemone species but must acclimate to live with others.

The ability of anemonefishes to live unharmful among the stinging tentacles of large anemones in the Indo-Pacific and Red Sea has interested marine scientists since its discovery by Collingwood (1868). Sea anemones discharge nematocysts and other cnidae (intracellular capsules containing eversible tubules) in response to chemical or mechanical stimuli from their prey or predators (Hessinger and Lenhoff, 1988). However, anemonefishes are able to avoid eliciting nematocyst discharge during contact with their hosts. Early studies of this phenomenon concluded that the fishes acquire their protection from anemones through a behavioral process called “acclimation” (e.g., reviewed by Mariscal, 1971; Schlichter, 1976). These studies were usually conducted with anemonefishes that were collected from the field, then isolated from their host anemones for a period of days, and subsequently introduced into an aquarium with an anemone. The anemonefishes were generally stung upon initial contact with the tentacles of an anemone, but after repeated contacts the fish were able to swim among the tentacles of their host without harm. This acclimation process was considered necessary for the fishes to contact anemones without being stung.

In contrast to the acclimation studies, several studies with anemonefishes reared in the laboratory have concluded that anemonefishes do not need to go through acclimation behavior before they are protected from being stung by anemones (Fukui, 1973; Miyagawa, 1989; Elliott et al. 1994). Miyagawa (1989) states that the results of her work “clearly demonstrated that naive juveniles of all the Amphiprion species examined... have an innate protection against their respective symbiotic anemone species.” Elliott et al. (1995) showed that anemonefishes reared in the laboratory and then released in the field were attracted toward host anemone species, and most fishes were protected from being stung upon initial contact. Thus, the fishes did not require acclimation to be protected. However, Elliott et al. (1995) did not report this as an example of “innate protection” because the anemonefishes had been reared in the egg stage next to anemones in the field and therefore were in contact with chemical and mechanical stimuli from anemones before the experiments were conducted.

The conflicting results of the acclimation and innate protection studies may be partially a result of different authors having different interpretations of the terms used to describe the same phenomenon. Because of this, it is not always easy to interpret exactly what is meant or how each set of experiments was controlled or conducted. To help resolve this problem, as well as to explicitly state what is being discussed in this study, the following definitions are presented to provide a reference point for both future and past studies.

Acclimation is defined as a behavioral and physiological adjustment to an anemone involving a stereotyped sequence of behaviors displayed by anemonefishes during the initial stages of an encounter with an anemone, by which the fishes establish their association with an anemone. The fish may or may not be stung during initial contact with the anemone’s tentacles, but when acclimation behavior is completed, the fish is able to swim forcefully through the tentacles without being stung or causing a noticeable behavioral response by the...
anemone. Protection refers to the absence of cnida discharge during repeated contact of an anemonefish with the tentacles of an anemone, and innate protection is defined as protection resulting from normal development, and not from contact with chemical, visual, or mechanical stimuli from sea anemones. That is, the protection mechanism has a genetic basis and is not acquired through experience. Acquired protection refers to protection resulting from contact with visual, chemical, or mechanical stimuli from sea anemones (e.g., protection through acclimation).

The term protection has been used interchangeably with acclimation in many studies. Fish that are protected from being stung by anemones are called acclimated, and fish that are unprotected have been called unacclimated. However, fish that have gone through extensive periods of acclimation behavior have not always been able to become protected from being stung by a particular species of anemone. For example, Mariscal (1970) reported that only one of three Amphiprion clarkii (= A. xanthurus) was able to become protected from an unnatural host, the California sea anemone Anthopleura elegantissima, but only after 45 h of acclimation behavior. Two other A. clarkii remained unprotected during this same time period, with one individual still being stung after going through 119 h of acclimation behavior. Therefore, we suggest that the term unacclimated not be used to refer to unprotected fish but only to fish that have not gone through acclimation behavior to an anemone.

The distinction between protected and acclimated fishes was made even more relevant by the observations of Fukui (1973), Miyagawa and Hidaka (1980), and Miyagawa (1989) who found that unacclimated individuals of some anemonefish species were not stung during the initial and subsequent contacts with most species of symbiotic anemones. These authors claimed that, because the fishes were not stung initially, they did not need to go through acclimation behavior and therefore were "innately" protected. However, the results of Miyagawa (1989) clearly showed that, although some anemonefish species appeared to be protected from being stung by certain species of symbiotic sea anemones, other anemonefish species remained unprotected and were stung by particular anemone species. Miyagawa (1989) states that the anemonefishes used in her experiments were "naive." However, she never defines this term, which has resulted in confusion about the validity of her claim that anemonefishes are innately protected from sea anemones (Fautin, 1991). The fishes used in the experiments of Miyagawa (1989) were spawned and brooded next to an anemone, and the fishes were not moved to a separate rearing tank until after they hatched. Thus, the fishes remained in close proximity to anemones for at least seven days in the egg stage and for some time after hatching and were not completely naive with regard to possible influences from the sea anemones. Rather than being innately protected, it is possible that the fishes acquired some form of protection from stinging during this time, either directly from the anemone or from anemone products transferred to the egg by the parent fishes. Completely naive larval anemonefishes would be those whose eggs are laid by parents isolated from contact (visual, physical, chemical, etc.) with sea anemones and then hatched and subsequently raised in continued isolation from sea anemones.

The purpose of the present study was to test whether truly naive anemonefishes, bred and raised in complete isolation from anemones at a hatchery in Florida, were innately protected from being stung by sea anemones. We conducted tests with both natural and unnatural host anemone species to test whether the anemonefishes had comprehensive protection from symbiotic anemones, as suggested by Miyagawa (1989). Forced-contact tests were conducted to determine whether the fishes were protected upon initial contact with the anemones.

Materials and Methods

We conducted experiments at Florida State University with individuals of A. clarkii, A. ocellaris, and A. perideraion purchased from a hatchery (Dynasty Marine Associates, Marathon, FL), which breeds and raises anemonefishes in complete isolation from anemones (i.e., naive). The fishes ranged in size from 18-28 mm standard length. We purchased two individuals of each of three anemone species (Heteractis crispa, 180 mm oral disk diameter; Stichodactyla haddoni, approximately 220 mm; and Macroactyla doreensis, 120 mm) from local tropical fish stores that obtain their animals from suppliers in the Philippines. Both natural and unnatural species combinations of anemonefishes and anemones were represented with this group of test animals (Table 1).

We kept each species of fish and anemone in a separate 75-liter glass aquarium filled with a mixture of artificial and natural sea water. Water temperature was 26 C, and salinity ranged between 31‰ and 33‰. The water was filtered with Penguin BioWheel filters and charcoal. All
Table 1. Host Specificity Patterns Reported by Fautin and Allen (1992) for the Three Anemone-fish Species and Three Anemone Species Examined in This Study.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Anemone species</th>
<th>Heteractis crispa</th>
<th>Stichodactyla haddoni</th>
<th>Macrodactyla doreensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. clarkii</td>
<td>natural</td>
<td>natural</td>
<td>natural</td>
<td></td>
</tr>
<tr>
<td>A. ocellaris</td>
<td>unnatural</td>
<td>natural</td>
<td>unnatural</td>
<td></td>
</tr>
<tr>
<td>A. perideraion</td>
<td>natural</td>
<td>unnatural</td>
<td>natural</td>
<td></td>
</tr>
</tbody>
</table>

of the tanks received ambient light from a nearby window, but the tanks with the anemones were also illuminated with two high-intensity, longwave ultraviolet fluorescent lights on a 12:12 light:dark cycle. This helped to maintain the symbiotic zooxanthellae in the tissues of the anemones. The anemones were allowed to attach their pedal disks to glass Petri dishes on the bottom of the aquaria so that they could be moved without disturbing their behavior. The fish were fed once daily with Tetramin Fish Flakes and Artemia nauplii. The anemones were fed one small shrimp every seven days.

To determine whether the anemonefishes were protected from the different species of anemones, we conducted forced-contact tests in a manner similar to that of Miyagawa (1989). Individual sea anemones were brought near the surface of the water in an aquarium (by lifting the Petri dish to which they were attached) so that there was only 1 cm of water over their tentacles. Individual fishes were carefully collected from their holding tanks in a 500 ml beaker so as not to disturb their mucous coat. The contents of the beaker were then gently emptied onto the tentacles of the anemone. To determine whether the tentacles were adhering to the fishes, the anemone was moved slowly back and forth in the water so that the tentacles were moved by the water currents in relation to the fish. If the tentacles did not adhere to the fishes after they were in contact for 5 min, we considered the fishes to be innately protected. If the tentacles adhered to the fishes, we considered them to be unprotected. After a forced-contact trial, we collected the fish with a beaker and kept it in a separate tank for 24 h to determine whether it would survive.

We considered anemonefishes to be stung if they were adhered to by the tentacles of the anemones. Previous studies of anemonefish protection have also used visual criteria as indicators of whether or not a fish is stung during contacts with an anemone (e.g., Mariscal, 1970; Schlichter, 1976; Lubbock, 1981). These criteria include behavioral responses of both the fish and anemone: adhesion of the tentacles to the fish, damage to the epidermis of the fish, and the presence of patches of cnidae on the surface of the fish.

To be certain that the cnidae in the tentacles of the test anemones were capable of discharging and adhering to a fish, we forced an individual fish of a nonsymbiotic species that was known to elicit cnida discharge (Dascyllus melanurus) to contact the anemones before each series of forced contact tests with the Amphiprion species. After this control test, we conducted forced-contact tests with anemonefishes using the anemone. Individual anemonefish were used only once in the experiment.

Results

The tentacles of all three species of anemones adhered strongly to all individuals of the nonsymbiotic species of pomacentrid, D. melanurus, in control forced-contact trials. In contrast, 17/30 anemonefishes (57%) were not adhered to by the anemones in forced-contact trials (Table 2). The anemonefishes generally remained inactive for the first 10 sec to 2 min after we initially forced them to contact the anemone’s tentacles. If the tentacles did not adhere to the anemonefishes, the fishes actively swam among the tentacles and frequently rubbed their bodies against the oral disk and tentacles. These fishes did not display any behaviors indicating that they were being stung by the anemones. If tentacle adhesion occurred, the anemonefishes either remained still until adhesion ceased, or the fish attempted to swim...
The tentacles of M. doreensis adhered weakly to captured or killed when tentacle adhesion occurred. No anemonefishes were free from the tentacles. Of 11 A. clarkii tested, 10 were protected from H. crispa, S. haddoni, and M. doreensis (Table 2). The tentacles of M. doreensis adhered weakly to the mouth region of one A. clarkii, but the fish remained in the tentacles of the anemone and was not adhered to again after the first few contacts. The mucus coat near the mouth of the fish may have been damaged from the fish hitting its head against the side of the beaker during collection.

Individuals of A. ocellaris were adhered to by both H. crispa and M. doreensis, but the fish were able to break free of the tentacles, and all were alive 24 h after their encounter with an anemone (Table 2). These two anemone species are unnatural hosts for A. ocellaris (Table 1). However, this fish species was not adhered to by S. haddoni, another unnatural host species. Of the four A. perideraion tested (Table 2), two were adhered to by M. doreensis (natural host species), and two were protected from S. haddoni (unnatural host species).

**DISCUSSION**

This study indicates that naive anemonefishes are innately protected from certain anemone species. Contrary to the conclusions of Miyagawa (1989), who claimed to show innate protection, the naive anemonefishes in our experiments did not have comprehensive protection from all species of symbiotic anemones. Individuals of A. clarkii were innately protected from three species of anemones, but the protection of A. ocellaris and A. perideraion varied among anemone species.

Sabol (1992), working with anemonefishes from the same hatchery in Florida, also found that A. clarkii was innately protected from S. haddoni. In contrast to our results, he found that A. ocellaris was not innately protected from S. haddoni. These conflicting results were not caused by a lack of stinging ability by the anemones, because the tentacles of the S. haddoni used in the two studies were both shown to be strongly adhesive to other nonsymbiotic species of fishes. Such conflicting results with the same species combination, suggests that individual variation within a species of anemonefish or anemone may also be a factor in causing conflicting results among the various studies of anemonefish protection.

Even though most of the naive anemonefishes examined in the present study were protected from anemones during the initial encounter, the fish still displayed the acclimation behaviors described by previous workers (e.g., Davenport and Norris, 1958; Mariscal, 1971; Schlichter, 1976). These appear to be stereotyped behaviors that the fishes invariably exhibit upon initial contact with an anemone, whether or not the fishes are already protected. The anemonefishes that Miyagawa (1989) referred to as being innately protected displayed behaviors that were very similar to those described by other workers as acclimation behavior. Miyagawa (1989) states that, although acclimation may be required by some fish species to acquire protection, the sequence of behaviors from the initial encounter until association is achieved should not be interpreted as acclimation per se but should be considered an innate characteristic behavior pattern of anemonefishes. Her claim that acclimation does not occur because some anemonefishes are not stung upon initial contact ignores previous usage of this term to describe the same behaviors that she reports in her study. Previous workers have all called this sequence of behaviors acclimation, even though some fish were not observed to be stung upon initial contact with anemones but continued to display the same acclimation behaviors (Davenport and Norris, 1958; Lubbock, 1980). Even if anemonefishes are protected from stinging during the initial encounters with an anemone, the fish are often hesitant and only gently contact an anemone’s tentacles. Therefore, the acclimation process may provide the fishes with further protection, eventually allowing them to swim unrestricted through the tentacles and display their normal association behaviors. Thus, acclimation may result in both a behavioral and physiological change in the fish.

In the present study, those fishes that were stung by anemones during initial contact required a period of acclimation behavior to become fully protected from an anemone. Innate protection may thus involve a different mechanism(s) than acclimation. Fish that are innately protected presumably must produce their own protective, or inert, mucous coat at some time during their larval development. On the other hand, it has been suggested that during acclimation the fishes may acquire protection by complexing compounds from the water in close proximity to the anemone (Mariscal, 1971; Schlichter, 1975). Alternatively, they may coat themselves with anemone mucus during repeated contacts with the body wall of anemones during the acclimation process (Schlichter, 1967, 1968, 1972). Elliott et al. (1994) found that individuals of A. clarkii do not produce a mucous coat containing antigens similar to those of
A. clarkii has anemone antigens in its mucous coat. It is not known whether these anemone antigens are acquired from the anemone directly or whether they provide any additional protection to the fish. The level of innate protection and the amount of acclimation required to become fully protected from an anemone probably varies, depending on the species combination of fish and anemone.

Fautin (1991) suggests that there is a "spectrum of mechanisms by which fish locate and adapt to hosts." Our results support this claim, because some anemonefishes were innately protected and others were not. Fautin also predicts that host generalist species of anemonefishes (such as A. clarkii) would be more likely to rely on behavioral mechanisms (such as acclimation) to become protected from anemones, whereas host specialist species (such as Premnas biaculeatus) are more likely to be innately protected by a chemical mechanism of some sort. A phylogeny for anemonefishes proposed by Allen (1972), places host generalists, such as A. clarkii, in an ancestral position and host specialists in a more derived position. This suggests that innate protection evolved in more specialized anemonefishes from an ancestral species that was protected through acclimation. However, the results of this study show that the host generalist fish, A. clarkii, was innately protected from all three anemone species. In contrast, the host specialist anemonefish species were stung by some of the same anemone species, which had to acclimate to their hosts to become protected. Thus, the evolution of a more specialized mechanism of protection in host specialists than host generalists is not supported by our study. Mebs (1994) also reports interspecific variation in the sensitivity of anemonefishes to toxins from different symbiotic anemone species. Further studies of interspecific variation in protection of anemonefishes are needed to provide insight into the evolution of the protective mechanism(s) and the symbiosis in general.

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LITERATURE CITED


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