

CLOWNFISH'S MUTUALISM WITH SEA ANEMONES TRIGGERED ITS ADAPTIVE RADIATION

Kajal Manjhi

Research Scholar

Deptt. of zoology

CMJ University Meghalaya

Shakshi Pushker

Research Supervisor

Deptt.of zoology

CMJ University Meghalaya

ABSTRACT

In evolutionary ecology, one of the most basic questions is: how does one's physical size become determined? The size of vertebrates is believed to be mostly fixed and influenced by factors like as genetics, nutrition, habitat, and stress. But in reactions to predator-prey and competitive dynamics, vertebrate development may be malleable. The flexibility of vertebrate development may be induced by these types of partnerships; however, the nature of mutualistic relationships is yet unknown. A classic vertebrate When anemones and anemonefish work together, it's called a mutualism. Size of anemones and anemonefish tend to go hand in hand in this relationship. A correlation between anemone area and changes in standard length and body depth were confirmed by mixed model analysis. To a greater extent than in smaller anemones, fish in bigger ones flourished. Despite getting individuals in large anemones consume the same amount of food as small anemones managed to succeed in this remarkable feat. The capacity of clownfish to tailor their pace of development to the dimensions of anemones might be an adaptation mechanism that allows where the area of anemones is a good indicator of the availability of resources, allowing the fish to maximum reproduction without compromising survival.

KEYWORDS: Ecological , Comparative method, Evolutionary rate, Motion, clownfish, Sea-level

INTRODUCTION

The clownfish, the little fish species known as anemonefish is really a member of the Pomacanthidae family superclass Pisces. Worldwide, around 29 different species of clownfish have been identified, with one species being members of the *Premnas* genus and the other members of the *Amphitryon* genus. In the aquatic environment, they establish symbiotic, mutualistic relationships with sea anemones, as their name suggests. Some species may be uniformly yellow, orange, reddish, or black, while others may have more subtle patterns like patches or bars. While most species don't grow to be more than 10 centimeters in length, the biggest ones may reach 18 cm. It is the clownfish that takes center stage in the hit Pixar/Disney film *Finding Nemo*.

Clownfish may be found across the Indian and Pacific oceans, as well as the Great Barrier Reef and the Red Sea. Although some species have a broad distribution, the vast majority are known to inhabit more localized regions. While most are host-specific, a few species exhibit cooperative behavior. It is believed that they reside in the sheltered environments of coral reefs or lagoons, far below the surface of the ocean. Their natural habitat is a couple. Additionally, you may find them in the Indo-Malaysian area, Japan, and the northwest of Australia, but you won't find any of them in the Caribbean. If they don't eat little invertebrates, they could hurt sea anemones. Sea anemones get their nutrition from the waste products of these fishes. They consume 20-25% algae in their diet, and their gut content has shown that they are exclusively omnivores. In addition to algae and zooplanktons, the diet also includes copepods. In addition, they consume tiny mollusks and crustaceans

for food. When housed in captivity, they are fed a diet of fish flakes and pellets. In addition, they eat the sea anemones' undigested food.

The only fish species known to be immune to the sea anemone's poison are clownfish and several types of damselfish. This idea has been backed by several hypotheses. One theory is that the sea anemone doesn't see the fish as food sources because their mucus covering is made of carbohydrates instead of proteins. According to an alternative theory, clownfish may have evolved a resistance to the sea anemone's poisons as a result of their shared evolutionary history. It is well-known that anemones often live in couples, with the male changing its sex to that of a female once the female dies. Such a transformation is called sequential hermaphroditism. Clownfish are protandrous hermaphrodites since they are born with a male sex. The reproductive female is at the head of the food chain, followed by the male, but this whole system topples over when the female dies. A group's biggest member is often a female, with the male coming in as a close second. The lack of completely formed sex organs in clownfish indicates that they are neuter.

Clownfish provide nutrients (ammonia, sulphur, and phosphorus) to the anemone's endosymbiotic zooxanthellae algae while the anemone protects them from predators that they excrete. This dynamic is a captivating example of a mutualistic relationship. A defence attack mechanism for clownfish in response to the release of nematocysts from the anemone is necessary for this mutualism to occur. Two major, non-exclusive theories have emerged from the many investigations into clownfish defence mechanisms: first, that clownfish benefit their skin's natural protective mucus; and secondly, that clownfish must cover themselves in anemone mucus for chemical camouflage. It is interesting to note that the clownfish's immune profile changes to resemble the anemone's during acclimation (i.e., before initial physical contact) with the anemone. Therefore, it is believed that the epithelium mucus of clownfish serves as a "chemical camouflage" to avoid "not-self" detection that is linked to the release of nematocysts. Most interestingly, it was shown that the clownfish skin mucus began to undergo chemical change prior to physical contact with the anemone, as the transfer of A few hours of physical and remote involvement was all it took to evaluate the amino acid transfer from the clown fish's skin mucous to the anemones.

LITERATURE REVIEW

Mélanie Audet-Gilbert, François-Étienne Sylvain, Sidki Bouslama, and Nicolas Derome. (2021) The relationship between clownfish and sea anemones is intriguing and has not been fully explained. The current theory of tolerance suggests that the anemone's nematocysts cannot be released because clownfish epithelium mucus molecularly mimics its own. Recent research has tested a new theory by examining how distant contact affects the reorganization of the epithelium microbiome in both individuals. The study used metataxonomics to investigate the epithelial microbiota dynamics in 18 pairs of percula clownfish and the symbiotic anemone *Heteractis magnifica* over four weeks. The findings showed that partners' epithelium microbiota converged with time, and the interaction signal persisted in both the physical and remote interaction categories. This suggests that the symbiotic partners' potent chemical communication via water caused long-lasting changes to the skin microbiota, which persisted even after the fish and anemone couples were separated. The research suggests a possible correlation between the reorganization of the microbiota in fish and anemones, namely the simultaneous recruitment of three strains of Flavobacteriaceae that are close relatives of Cellulophaga tyrosinoydans, a tyrosinase-producing cellulophage. This research disproves the idea that just physical interaction is necessary for bacterial community restructuring during adaptation and casts doubt on the long-held theory of unidirectional chemical camouflage.

Michael Berumen, Benjamin Titus, John Majoris, and Morgan Bennett-Smith. (2021). The study aimed to determine the variety of host anemone species in the fringe reef systems of the Red Sea, which provide habitat for sea anemones and clownfish. The researchers examined over 1600 km of coastline, from the northern Saudi Arabian Red Sea to the Gulf of Aden, and identified seven distinct species of host anemones, six of which were *A. bicinctus* hosts. *Stichodactyla haddoni* and *Stichodactyla mertensii* were included in the *A. bicinctus* symbiotic list. Two species that might hybridize in the region were also examined. However, *Stichodactyla gigantea* was not found in the Red Sea, and it is improbable that the Red Sea is home to *S. gigantea*. The findings provide light on Red Sea host anemones, call into doubt the presence of *S. gigantea* in the Indian Ocean, and update previous work on the host specificity of *A. bicinctus*.

Mélanie Audet-Gilbert, François-Étienne Sylvain, Sidki Bouslama, and Nicolas Derome. (2020) The mutualistic connection between clownfish and sea anemones is a fascinating phenomenon. The current theory of tolerance suggests that the anemone's nematocysts cannot be released due to clownfish epithelium mucus mimicking its own. However, recent research suggests that a generalist clown fish's skin microbiota may undergo a reorganization upon first contact with its symbiotic anemone. The study used metataxonomics to study the dynamics of the epithelium microbiota in symbiotic anemone *Heteractis magnifica* and eighteen pairs of percula clownfish (*Amphiprion percula*). The results showed that the epithelium microbiota of both fish and anemones converges after spending only fifteen minutes in the identical water system. This reorganization was more closely linked to the simultaneous entry of three strains of Flavobacteriaceae that are closely connected to *Cellulophaga tyrosinoydans*, a tyrosinase-producing cell. The research disproves the idea that just physical interaction is necessary for bacterial community restructuring during adaptation and casts doubt on the long-held theory of unidirectional chemical camouflage. Instead, the convergence of the epithelial microbiota of the two couples is crucial to the development of mutual acceptance.

G. Litsios & C. Sims & Rafael Wüest & Peter Pearman & Niklaus Zimmermann & Nicolas Salamin. (2012). Adaptive radiation occurs when a single ancestral species produces offspring that can adapt to different environments. Mutualism, which is a key factor in species diversity, can help species access resources that rivals cannot. Clown fishes, for example, were able to spread adaptively across Indian and western Pacific seas due to their obligatory mutualism with sea anemones. The study reveals that clown fishes' physical traits are related to ecological niches occupied by sea anemones, supporting the ecological speciation theory. They also show a higher rate of morphological development and species diversity compared to their closest relatives without mutualistic connections. The study highlights the importance of mutualistic touch in the evolution of biodiversity. Marine ecosystems are relatively free from ecological speciation due to dispersion obstacles. Clown fishes likely facilitated fast species diversification due to unique life-history traits that fostered reproductive isolation among populations.

Benjamin Titus and Laroche Sergio and his associates, Christopher Meyer, Herman Wirshing, and Estefania. (2020). In order to carry out their most fundamental metabolic processes, all forms of eukaryotic life depend on symbioses with a wide variety of bacteria. Many eukaryotic species develop secondary symbioses with other large eukaryotic organisms. Microbiome convergence across taxa with very distant relationships is possible due to the chances for microbial transmission created by the physically connected interactions that characterised many macroscopic symbioses. This, in turn, affects the variety and role of human microbiomes. The microbiomes of five species of sea anemones that inhabit coral reefs in the Maldives are sequenced here. These anemones house clownfish. We review the effects of environment, evolutionary history, and clownfish symbiont association on the taxonomic and expected functional diversity of the microbiome, and we look for indicators of microbiome convergence in anemone species that have evolved symbioses with clownfish independently. Host anemone microbiomes converge in predicted functional microbial diversity

studies, indicating more functional variety than in persons who do not host clownfish. Clownfish affiliation and host identification affect the majority of the taxonomic diversity in the sea anemone microbiome, according to our findings that hosts clownfish. In addition, we find expected microbial activities that are elevated, suggesting that clownfish presence may impact these processes. Our findings, when interpreted as a whole, may indicate a more profound metabolic relationship between clownfishes and the anemones that host them, as well as a yet unseen mutualistic advantage for the anemones.

CLOWNFISH AND SEA ANEMONE RELATIONSHIP

National Geographic published an article by James Prosek, never venturing more than a short distance from their host anemone, clownfish spend their whole lives in close proximity to it. Eggs are laid on the closest hard surface, hidden by the anemone's thick base, about twice a month, and the growing embryos are fiercely protected. After hatching, clownfish spend about a week or two floating near the water's surface as a small, translucent larva. Afterwards, it undergoes a metamorphosis into a little clownfish that is less than half an inch in length and makes its way to the reef. Within a day or two, the juvenile fish will perish unless it locates an anemone and adjusts to its new environment.

When clownfish feel threatened, they swiftly retreat to the protection of the anemone's tentacles, even if they do venture out to graze on zooplankton. Clownfish are known to inhabit about ten different types of anemones. Many clownfish species are acceptable to some. Some are exclusive to certain species. Clownfish are no different. Although some are exclusive to certain anemone species, others have found new homes with other anemones.



The average clownfish colony consists of one host anemone, two offspring, or perhaps six. Larger fish naturally take precedence over their smaller counterparts in a pecking order when many fish inhabit the same host. Here, mating females deposit their eggs at the anemone's base, while their lover keeps a close eye on them until they hatch. After emerging, the bony fish larvae swim with the currents in search of new hosts.

When selecting a host anemone, juvenile anemonefish rely on both chemical and visual signals. They are quite cautious when initially approaching a sea anemone, but after they get used to their surroundings, they skilfully avoid the toxic tentacles. Upon the anemone's nighttime closure, the clownfish may sometimes scuttle within its tentacles. Nighttime is clownfish's prime time for fin flapping and wriggling. Evidence suggests they do this to enhance aeration and water movement during periods of low oxygen levels, such as nighttime when photosynthesis is inhibited.

The sea anemone shields clownfish from potential dangers. In exchange for the anemone's protection and sometimes crumbs of food, clownfish clean up after it and sometimes eat the little parasites that afflict it. While clownfish do entice fish and other prey for sea anemones, they may also deter fishlike butterflyfish—which are immune to the poison—from eating the tentacles of sea anemones.

THE ADAPTIVE RADIATION OF CLOWNFISH WAS TRIGGERED BY THEIR MUTUALISM WITH SEA ANEMONES

Biologists have relied on Darwin's research on Galapagos finches to establish the notion of adaptive radiation. An overarching theory behind this process is that ecological opportunities, which provide resources that competing species do not have access to, will accelerate ecomorphological change and species diversity. Colonization of physically isolated places with depleted fauna (like Great Lakes cichlid fish in East Africa) is the most often cited of the four primary reasons why ecological opportunities might emerge. The same thing happens when a large-scale extinction occurs; the remaining species are able to quickly fill the vacant niches and spread outward.

Lupinus organisms that arose during the Andean uplift and are located at high elevations is an example of how resource modification may also promote radiation of native species. Lastly, a chance for species radiation may arise when a feature that permits novel environmental interactions or a critical innovation appears. Take Antarctic notothenioid fishes as an example. It is believed that their adaptive radiation was driven by the development of antifreeze glycoproteins, which allowed them to survive in very harsh settings. Similar to how significant discoveries may unlock previously unavailable resources, mutualistic relationships can evolve to do the same. One example is the role of mutualistic microorganisms in the ingestion and degradation of plant components by phytophagous insects.

Case studies using mutualism are rare, in contrast to the abundance. Several case examples that show how alternative options' ecological benefits inspire adaptive radiation. In mutualistic systems, ecological speciation may occur, since hosts alterations have enabled it in many animals, including coral-dwelling fish. Concerning the impact of mutualism on species diversity, however, findings from both theoretical and practical investigations provide conflicting data. Therefore, further case studies are necessary and the matter is still up for dispute.

The clown fishes, also known as anemonefishes, are representative of coral reefs and include thirty different species of damselfish (Teleostei: Perciformes; Pomacentridae) (Figure 1A & 1C). The Indo-Malay Archipelago has the greatest species richness, with up to nine species seen in sympatry, and their range stretches starting in the Indian Ocean and ending in the western Pacific (Figure 1B). When they intricate relationship with sea anemones serves as a prime illustration of a mutualistic connection. Even though sea anemone tentacles contain nematocysts that are toxic to most fish, clown fish are immune to their effects. Clown fishes are able to settle within sea anemones because of a protective mucous covering that stops the emission of nematocysts. Clown fishes benefit directly from the sea anemones' ability to shield them from predators. Just as clown fishes pursue

sea anemone predators, so do other fish species. The relationship is reciprocal; the clown fishes release ammonia, sea anemone tissues use it for the benefit of their endosymbiotic dinoflagellates. Clown fishes, which are protected by sea anemones, have an exceptionally high life expectancy—about 30 years for *Amphiprion percula*—that is double the average for damselfish and six times longer than what one would anticipate from a fish of that size.

There is a lot of diversity in the way clown fish use sea anemones as a host, even though certain clown fish species may have mutualistic relationships with as many as 10 different species (Table 4.1). Although two of the host sea anemones, the distributions of *Heteractis malu* and *Macrodactyla doreensis* are less extensive; both species inhabit areas close to the clown fishes' diversity core, therefore it is feasible for the majority of clown fishes to interact with these anemones regardless of their location. Sea anemones are found all around the world, however they have different environmental preferences (such as different types of reefs, substrates, and depths). Because different species make different use of their habitats, it was shown that numerous clownfish species may coexist. Because of this, the emergence of mutualism may have been the revolutionary change that enabled clown fishes to diversify into ecological niches occupied by various kinds of sea anemones. But this theory has never been put to the test in a controlled environment

DESPITE THE SEA ANEMONE'S STINGING TENTACLES, CLOWNFISH MANAGE TO STAY ALIVE.

The poisonous tentacles of sea anemones provide a suitable habitat for to hunt, mate, and raise their young, including clownfish and anemonefish. Their unique skin mucus induces the secretion of poisons by sea anemones, allowing them to withstand the stinging tentacles of these aquatic creatures. Species of fish that do not possess the same physiological adaptations as clownfish are preyed upon by sea anemones. If a clownfish goes too far away from an anemone, it will have to build immunity by returning to the anemone and getting stings from its tentacles. Clownfish have a mucus covering that shields them from sea anemone poisons. Scientists are looking at this coating as a possible medicinal element.

Before clownfish may develop immunity to anemone stings, they must undergo a period of acclimatization. In this technique, as it swoops over the anemone, the fish caresses its tentacle tips with its belly and ventral fins. "It's a slime that inhibits the anemone from firing off its stinging cells," according to Allen. When a young anemonefish first approaches an anemone, it makes these hesitant touches. To initiate this chemical reaction, they must come into touch. With its protective shell in place, the clownfish serves as an extra line of defense against fish that prey on anemones, such as butterflyfish. In general, anemones benefit from clownfish, and vice versa.



Clownfish and anemonefish develop a mucus covering that contains compounds that protect them against anemone stings as they become older. In addition to their unique swimming style, these fishes are known to have adapted to life in anemones. Not even clownfish and anemonefish can withstand the stinging of anemones. They are protected by a mucus covering and trigger the firing of the nematocysts, which are stinging cells. They often settle onto coral branches if they don't want to dwell in an anemone. "Animal Diversity Web" (ADW) is the source.

CONCLUSION

In experimental aquaria, we were able to compare the microbiological signatures of the two symbionts both before and after the symbiotic interaction began. Our findings showed that microbiota were unique to each symbiont and that sym-biosis led to substantial changes in these microbial fingerprints. The organisms and the seawater both have very dynamic microbial compositions, as we also saw. On a broader scale, our findings provide light on an underexplored topic: the possible involvement of the microbiota in symbiotic connections among eukaryotic creatures. Therefore, it would be fascinating to learn whether microbiota are involved in the beginning and/or continuation of symbiotic partnerships in various contexts (such as carapids/holothurians, shrimp, and fish). The function of the microbiota in symbiotic interactions is an intriguing area to study in many marine environments. Below, we give a rundown of the present taxonomy and nomenclature of sea anemones that host clownfish. This includes recently accepted changes as well as conclusions drawn from molecular data from Titus et al. (2019). We take into account and reject Rowlett's (2020) changes because his conclusions were based solely on images, which, as mentioned, can be misleading for actinarians when not supported by anatomical and molecular examinations. The most recent updates to the approved taxonomy and nomenclature may be found in the publication by Rodríguez et al. in 2024 of the World List of Actiniaria belongs to the World Register of Marine Species (WoRMS). We are crossing our fingers that more publications

will publish their findings after including the sea anemone host data their findings after using this guidance to improve field identification certainty.

The "camouflage hypothesis" states that when two hosts interact physically or remotely, their microbiomes reorganize. However, it is unclear whether this community remodeling is a necessary condition for mutual acceptance or a byproduct of a partner's use of an unnamed remote biochemical monitoring system. Here, we offer key findings that bolster Shapira's multi-layered model of microbiome structuring. This model suggests that holobionts are able to adapt to changing environments on a generational scale through the use of a flexible microbial pool called transient microbiota, which is influenced by environmental variables. We postulate that the transitory epithelial microbiome of both symbiotic partners gradually converges toward one another as a result of a distant encounter, which may help to build mutual acceptance. So, our findings point to the protection strategy of the *A. percula* clownfish, which has a limited range of host sea anemone species, involves a chemical conversation between symbiotic partners prior to first physical contact. Coating the fish's skin with components of the host anemone's mucus is also part of this procedure.

REFERENCES

1. Audet-Gilbert, Émie & Sylvain, François-Étienne & Bouslama, Sidki & Derome, Nicolas. (2021). Microbiomes of clownfish and their symbiotic host anemone converge before their first physical contact. *Microbiome*. 9. 10.1186/s40168-021-01058-1.
2. Bennett-Smith, Morgan & Majoris, John & Titus, Benjamin & Berumen, Michael. (2021). Clownfish hosting anemones (Anthozoa, Actiniaria) of the Red Sea: new associations and distributions, historical misidentifications, and morphological variability. *Marine Biodiversity Records*. 14. 10.1186/s41200-021-00216-6.
3. Audet-Gilbert, Émie & Sylvain, François-Étienne & Bouslama, Sidki & Derome, Nicolas. (2020). Microbiomes of clownfish and their symbiotic host anemone converge before their first physical contact.. 10.21203/rs.3.rs-25472/v1.
4. Litsios, Glenn & Sims, Carrie & Wüest, Rafael & Pearman, Peter & Zimmermann, Niklaus & Salamin, Nicolas. (2012). Mutualism with sea anemones triggered the adaptive radiation of clownfishes. *BMC evolutionary biology*. 12. 212. 10.1186/1471-2148-12-212.
5. Titus, Benjamin & Laroche, Robert & Rodriguez, Estefania & Wirshing, Herman & Meyer, Christopher. (2020). Host identity and symbiotic association affects the taxonomic and functional diversity of the clownfish-hosting sea anemone microbiome. *Biology Letters*. 16. 20190738. 10.1098/rsbl.2019.0738.
6. Dixon, Austin & Needham, David & Al-Horani, Fuad & Chadwick, Nanette. (2013). Microhabitat use and photoacclimation in the clownfish sea anemone *Entacmaea quadricolor*. *Journal of the Marine Biological Association of the United Kingdom*. 94. 473-480. 10.1017/S0025315413001719.
7. Garcia, Alberto & Gaboriau, Théo & Fitzgerald, Lucy & Heim, Sara & Marcionetti, Anna & Schmid, Sarah & Bertrand, Joris & Rangseethampanya, Ploypallin & Ruttanachuchote, Phurinat & Aunkhongthong, Wiphawan & Pongsakun, Sittiporn & Sutthacheep, Makamas & Frederich, Bruno & Cortesi, Fabio & Yemin, Thamasak & Salamin, Nicolas. (2024). Specialization into Host Sea Anemones Impacted Clownfish Demographic Responses to Pleistocene Sea Level Changes. 10.1101/2024.07.12.603135.

8. Gaboriau, Théo & Marcionetti, Anna & Garcia, Alberto & Schmid, Sarah & Fitzgerald, Lucy & Micheli, Baptiste & Titus, B. & Salamin, N.. (2024). Host-use Drives Convergent Evolution in Clownfish and Disentangles the Mystery of an Iconic Adaptive Radiation. 10.1101/2024.07.08.602550.
9. Titus, Benjamin & Bennett-Smith, Morgan & Chiodo, Tommaso & Rodriguez, Estefania. (2024). The clownfish-hosting sea anemones (Anthozoa: Actiniaria): updated nomenclature, biogeography, and practical field guide.. Zootaxa. 5506. 1-34. 10.11646/zootaxa.5506.1.1http://zoobank.org/urn:lsid:zoobank.org:pub:AFDFAEE4-9B4A-4792-80E7-27DC9ECC23D.
10. Zhou, Yang & Liu, Helu & Feng, Chenguang & Lu, Zaiqing & Liu, Jun & Huang, Yanan & Tang, Huanhuan & Xu, Zehui & Pu, Yujin & Zhang, Haibin. (2023). Genetic adaptations of sea anemone to hydrothermal environment. Science Advances. 9. 10.1126/sciadv.adh0474.