

that between the anterior NSC and the place of emergence of the NCC I, as the descending limb of the MNSP (Figure 2). In the adult, however, the condition is greatly changed. Firstly, the axons of even the MNSP are not as readily and fully stainable in the adult as they are in the pupa and secondly, the adult MNSP is not distinguishable into its ascending and descending limbs. Instead, its fibres run inwards and backwards (not forwards) to constitute only one (descending) limb which after forming the chiasma emerges directly as the NCC I (Figure 3). Consistent with the demands of a metamorphic stage, the pupal brain synthesizes and transports through its intracerebral (neurosecretory) axons larger amounts of the neurosecretory material (NSM). This is reflected in

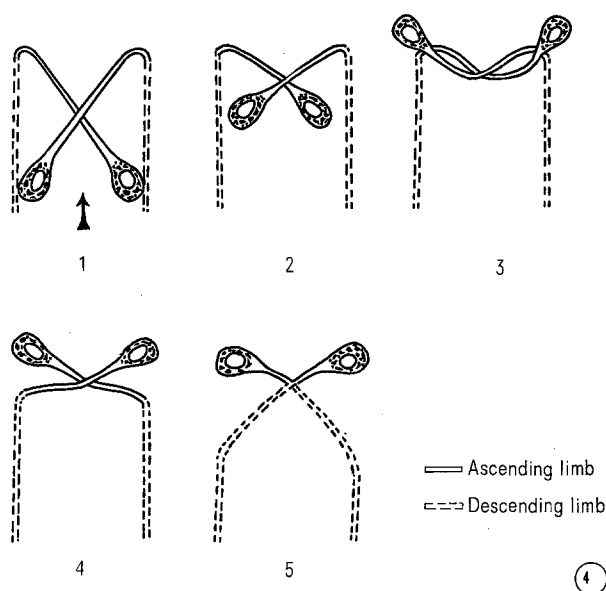


Fig. 4. Hypothetical diagrams depicting the possible steps in the change from the pupal (diagrams 1-3) to adult (diagrams 4-5) condition of the axonal pattern under a supposed anteriorly directed squeeze (arrow).

the maximum intensity that the pupal NSC show in the aldehyde-fuchsin (AF) stain. Since, NSM is the actual stainable component of the axons, there is greater possibility of the latter's staining in this stage than in the adult where the synthesis and transport of the material are relatively less. This, therefore, may account for the first difference. The cause of the second difference may lie in the changes that take place during metamorphosis in the anatomy of the brain itself. It is seen that the pupal brain is dorsally much flatter compared to the adult brain. This increase in the surface-convexity of the adult brain – obviously the result of an increase in the size and complexity of the brain¹⁴ – seemingly brings into play an anteriorly directed squeeze on the dorsum of the brain under which the superficially located NSC move forwards and in so doing dispense with the ascending limb of the MNSP. This hypothesis is supported by the fact that both median and lateral groups of NSC in the adult brain are indeed very much anteriorly displaced (compare Figures 1 and 3). Such a change from the pupal to adult condition could also be reconstructed on an axonal model prepared out of thread pieces. Diagrams of Figure 4, depicting the steps possibly involved in this transformation, are based on such a model¹⁵.

Zusammenfassung. Untersuchungen am Gehirn von Puppen und Imagines des Schmetterlings *Papilio demoleus* zeigen, dass die Axone der neurosekretorischen Zellen der Pars intercerebralis in den Puppen vorerst nach innen vorwärts, dann nach rückwärts laufen, in Imagines aber direkt nach innen rückwärts ziehen. Die notwendigen ontogenetischen Lageänderungen der NSZ und ihrer Axone werden an einem Modell demonstriert.

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¹⁴ R. H. NORDLANDER and J. S. EDWARDS, *J. Morph.* 126, 67 (1968).

¹⁵ Thanks are due to Prof. J. P. THAPLIYAL for providing facilities and to Atomic Energy Commission and C.S.I.R. for financial support.

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Protection of a Hermit Crab by its Symbiotic Sea Anemone *Calliactis tricolor*

For over a century, the symbiosis between hermit crabs and sea anemones has been studied by various workers, the results of which have recently been reviewed¹. In these partnerships, one or more anemones, usually of the same species, attach by their pedal disc to a gastropod shell inhabited by a hermit crab. The sea anemone has been suggested to protect the hermit crab from predators²⁻⁴, but virtually no experimental work has been conducted to test this hypothesis, with the exception of Ross' recent study⁴.

The purpose of the present study is to present experimental evidence that the presence of a sea anemone on its shell does appear to protect a hermit crab from one of its natural predators.

Materials and methods. *Pagurus pollicaris* SAY, which bears the sea anemone *Calliactis tricolor* (LESEUR) on its shell, is found in the shallow inshore waters of the Western Atlantic and the Gulf of Mexico. Occurring with this hermit crab is its remarkable predator, the oxystomatid crab, *Calappa flammea* (HERBST). One cheliped of *Calappa* (usually the right one) is modified for snipping open gastropod shells and in this fashion the crab is able to very

effectively remove and eat hermit crabs, as well as gastropods⁵.

Numerous preliminary observations in our laboratory suggested that *Calappa* was unable to prey successfully on those hermit crabs carrying anemones, while those without anemones appeared to be readily eaten, thus the reason for the present study.

It was necessary to standardize as closely as possible the 'hunger levels' of the various *Calappa* used. Each *Calappa* was maintained in a 20 gallon recirculating water aquarium and was fed to satiation with frozen squid once per day for 5 days (average of 4.94 g of squid per crab per day) and then starved for the 24 h preceeding an experimental

¹ D. M. ROSS, *Oceanogr. Marine Biology Annual Review* (Ed. H. BARNES; George Allen and Unwin Ltd., London 1967), vol. 5, p. 291.

² L. BERNER, *Bull. Soc. Zool. fr.* 78, 221 (1953).

³ B. B. BOYCOTT, *Pubbl. Staz. zool. Napoli* 25, 67 (1954).

⁴ D. M. ROSS, *Nature, Lond.* 230, 401 (1971).

⁵ J. B. SHOUR, *Science* 160, 887 (1968).

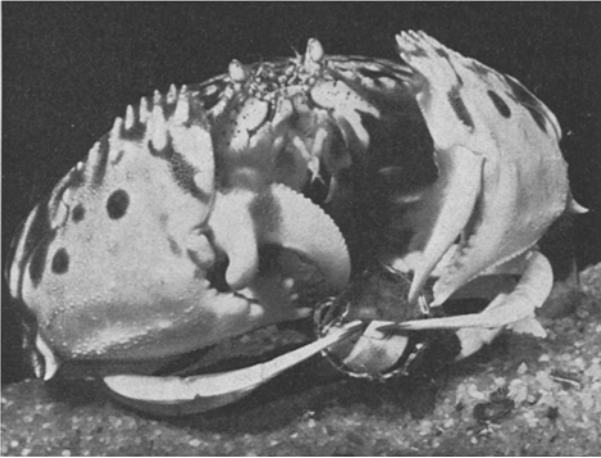


Fig. 1. *Calappa flammea* manipulating gastropod shell containing a *Pagurus pollicaris* hermit crab.



Fig. 2. Dactyl tooth of cheliped of *Calappa flammea* inserted into aperture of shell during shell opening.

series. Previous experiments had shown that this period of starvation was sufficient to produce a 'hungry' crab without undue stress.

Two hermit crabs of approximately equal size, one with an anemone on its shell and one without, were presented singly to each *Calappa* during consecutive 45 min trials. *Pagurus pollicaris* hermit crabs inhabiting only *Polinices duplicatus* (SAY) shells were used in order to control for shell thickness, size and shape and because of their common occurrence together in the field.

Results. A striking difference was observed between the behavior of a *Calappa* attempting to feed on a hermit crab with and without an anemone. In the absence of an anemone, the *Calappa* used its chelipeds and the tips of its walking legs to pick up and manipulate the shell containing the hermit crab (Figure 1). The dactyl tooth of the cheliped was then inserted in the aperture of the shell and opening was initiated (Figure 2). The opening occurred due to a combination of shearing and crushing pressure brought to bear on the shell by the closure of the dactyl tooth against two shorter and more massive protuberances on the propodus of the same cheliped.

If the anemone was present on the shell, the preliminary manipulation by *Calappa*, or the actual opening itself, caused the anemone to contract and extrude its nematocyst-laden acontia. Upon contact of the thread-like acontia with its sensitive walking dactyls or mouthparts, the *Calappa* immediately dropped the hermit crab and in some instances, wiped its chelipeds and walking legs against its mouthparts. Although a *Calappa* frequently made repeated attempts to remove such a hermit crab from its shell, contact with the sea anemone always resulted in the hermit crab being quickly released.

Fragments of acontia which happened to break off and adhere to the mouthparts of the *Calappa* during shell-opening frequently elicited a sudden dropping of the hermit crab and a rapid extension of both chelipeds away from the body in what might be described as a pushing or rejection motion (Figure 3). The *Calappa* then quickly backed away and generally made no further attempts to contact the hermit crab for several minutes. In the presence of an anemone on the shell, this rejection behavior occurred in about 67% of the experiments. When no anemone was present, this behavior was never observed. In addition

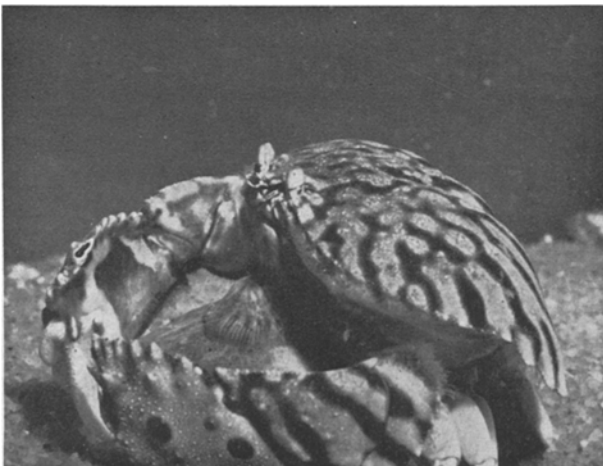


Fig. 3. Rejection motion of *Calappa flammea* after contact with acontia of anemone *Calliactis tricolor* attached to gastropod shell.

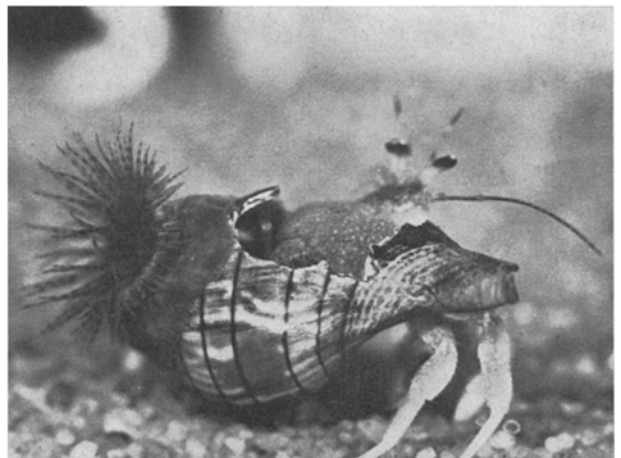


Fig. 4. A *Pagurus pollicaris* hermit crab which barely escaped predation by *Calappa flammea* during the preliminary series of experiments.

the presence of an anemone on the shell caused the *Calappa* to drop the hermit crab a total of 130 times or approximately 11 times per trial. On the other hand, hermit crabs without anemones were dropped only a total of 5 times or 0.42 times per trial (Table I).

The main series of experiments, however, were conducted in an effort to determine if the presence of the anemone did protect the hermit crabs from predation by *Calappa*. In over 91% of the experiments, the presence of an anemone on the shell of the hermit crab did in fact prevent its being killed by the *Calappa* (Figure 4; Table II). On the other hand, approximately 83% of the hermit crabs without anemones were either killed or partially eaten by *Calappa*.

Discussion and conclusions. Crabs of the genus *Calappa* prey on hermit crabs both in the field⁵ and in the laboratory. Presumably *Calappa flammea* is a natural predator of the *Pagurus pollicaris* hermit crab since both have been collected together in the same area and *Calappa* readily preys on the hermit crab in the lab.

Results of the experiments showed that the *Calappa* can be an effective predator when given a hermit crab,

Table I. 'Rejection' reactions and release of *Pagurus pollicaris* by 5 *Calappa flammea* during 24 trials

	Anemone present	Anemone absent
No. of trials	12	12
Total No. of 'rejection' reactions	8	0
Total No. of times hermit crab was dropped	130	5
Average No. of times hermit crab was dropped/trial	10.8	0.42

Table II. Results of predation attempts by 5 *Calappa flammea* on the hermit crab *Pagurus pollicaris* with and without the symbiotic sea anemone *Calliactis tricolor*

	No. of hermit crabs not killed	No. of hermit crabs killed or partially eaten
Anemone present	11	1 ^{a, b}
Anemone absent	2 ^c	10

^a $P < 0.005$ that the results are independent of the presence or absence of the anemone. ^b Anemone broken off on shell fragment. ^c Two hermit crabs escaped predation by blocking the aperture of their shell with their chelae.

in that about 83% of the hermit crabs without anemones were eaten. The only hermit crabs which emerged unscathed from these experiments were those whose cheliped happened to block the aperture in such a way that *Calappa* could not insert its dactyl tooth far enough into the aperture to initiate opening. This is in striking contrast to only one hermit crab being eaten when an anemone symbiont was present. This single occurrence was one in which the preliminary shell opening activities of the *Calappa* happened to break off that portion of the shell bearing the anemone. Thus, this case might be considered to be one of those rare exceptions which tends to 'prove' the rule.

It is obvious that *C. flammea* is not an effective predator when preying on the *P. pollicaris* hermit crab in the presence of the symbiotic sea anemone, but the proof of how the anemone protects the hermit crab remains to be shown. However, the fact that the anemone's acontia contain numerous stinging nematocysts⁶ and that the rejection reactions of the *Calappa* were often preceded by contact of the *Calappa*'s mouthparts with these acontia, suggest that the acontial nematocysts play a role in this protection.

Dropping of the hermit crab by the *Calappa* and the subsequent movement away from the prey, could possibly remove the predator far enough from the vicinity of the prey to allow escape of the hermit crab in the field. The hermit crab also was able to retreat from the *Calappa* after being dropped.

These observations, although necessarily preliminary, due to the difficulty in obtaining a suitable supply of *Calappa*, strongly suggest that the association of the sea anemone *Calliactis tricolor* with the hermit crab *Pagurus pollicaris* does in fact confer selective protection from at least one of its predators, *Calappa flammea*⁷.

Zusammenfassung. Experimenteller Nachweis, dass Einsiedlerkrebse, *Pagurus pollicaris*, mit symbiontischer Seeanemone, *Calliactis tricolor*, auf ihrer Schale vom natürlichen Feind der Krabbe, *Calappa flammea*, weitgehend gemieden werden.

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⁶ R. N. MARISCAL, I. erp. mar. Biol. Ecol. 8, 217 (1972).

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⁸ The authors wish to thank GEORGE KIRVIN of Quality Sea Foods, Port St. Joe, Florida, for providing boat time, and ROSS McWILLIAMS for help in collecting the experimental animals.

Shoot Initiation on Cauliflower Roots Caused by Morphactin

Adventitious bud formation on stem, leaf, and roots of various plants has been observed. A review of adventitious bud formation on excised leaves shows that leaves of 30 species were able to form roots which formed buds¹. In vivo roots of *Phlox* sp. are known to be stimulated to form adventitious buds. It has been reported that cabbage could be propagated by means of root cuttings obtained from the base or crown of the plant². This preliminary communication reports shoot formation on

intact roots of cauliflower plants treated with morphactin.

Seven-week-old cauliflower (*Brassica oleracea* Linn. var. *botrytis* c.v. Snowball) plants (having 11–13 leaves) raised 45 cm apart in the field were dabbed on the lamina with absorbent cotton soaked with aqueous morphactin (Chlorfluoreneol IT 3456) solution, the shoot apex was thoroughly wetted with it. The concentrations used were 100, 250, 500, and 1000 ppm (0.02% Tween 80 served as