The response of the sea anemone *Calliactis parasitica* to shells of the hermit crab *Pagurus bernhardus*

BY D. M. ROSS AND L. SUTTON

Department of Zoology, University College London and
*Department of Zoology, University of Alberta, Edmonton, Canada**

(*Communicated by P. B. Medawar, F.R.S.—Received* 30 *March* 1961—*Revised* 30 *May* 1961)

The five stages in the behaviour pattern by which *Calliactis parasitica* transfers to shells of *Buccinum*, normally occupied by *Pagurus bernhardus*, are described. The first of these, the clinging of tentacles to shell, is a trigger for the pattern as a whole; it occurs in *Calliactis* already settled on glass or plastic, and more consistently, in *Calliactis* whose pedal disks are unattached.

Tests on the frequency and speed of the clinging response under various conditions gave this information: (*a*) individual shells used by *Pagurus* vary greatly in potency; (*b*) *Calliactis* cling to shells of *Pecten* and *Mytilus*, but only erratically and after long delays; (*c*) *Buccinum* shells that have never been occupied by crabs, evoke the clinging response most consistently and rapidly; (*d*) removal of periostracum from *Buccinum* shells reduces, but does not abolish, clinging, though *Calliactis* responds to isolated strips of periostracum as to a shell; (*e*) *Calliactis* does not cling to dummy shells, to shells boiled in alkali, or to shells coated with a thin plastic layer; (*f*) *Calliactis* does not cling to inactivated shells when these are re-occupied by crabs. It is concluded that the clinging response depends entirely on a general molluscan shell-factor. In the settlement of the foot on the shell (the fourth stage of the behaviour pattern), a specific response to shell-factor is also involved.

Vertical incisions, even complete bisection, do not abolish clinging but merely slow down the orderly pattern of the transfer to a shell. Horizontal incisions impair the pattern more. Complete horizontal section, including removal of the pedal disk alone, abolishes the clinging response and the whole behaviour pattern.

Interposed stimuli show features consistent with a 5-stage programme in which the completion of each stage is a trigger for the next. But each stage may be greatly prolonged, and, if interrupted, starts again. The neuromuscular links required to co-ordinate this behaviour pattern are listed and discussed.

INTRODUCTION

The sea anemone, *Calliactis parasitica*, is normally found in European Atlantic waters living on shells of the whelk, *Buccinum undatum*, inhabited by the hermit crab, *Pagurus bernhardus*. Ross (1960) described how *Calliactis* which have settled on glass, plastic or stone, transfer both to empty and *Pagurus*-occupied *Buccinum* shells and showed that the anemone establishes the association alone, without the crab assisting. The present paper describes further observations on this phenomenon. The objects were to discover the factors involved in the initial response of *Calliactis* to a shell, and in the movements by which the anemone eventually settles on the shell.

FURTHER OBSERVATIONS ON THE TRANSFERENCE BEHAVIOUR

Since the earlier paper, many more transfers of *Calliactis* from plastic plates to *Buccinum* shells have been observed, and many of these have been filmed by

---

* Present address of both authors.

time-lapse cinematography at 1 frame every 2 or 3 seconds. It is now possible to give a more accurate description of the phenomenon. We now recognize five distinct stages in a typical transfer.

(1) *The attachment of the tentacles and oral disk to the shell*

On contact with a shell, some of the anemone's tentacles cling to the surface and move over it by excited serpentine movements. If these tentacles remain in contact, others soon adhere also and explore the surface more and more actively. Within a few seconds, waves of tentacular fluttering travel around the margin as the animal extends and more tentacles touch the shell. Occasionally the animal retreats momentarily by a quick marginal contraction, a sign of strong general stimulation, and then quickly extends to re-establish contact with the shell. Soon the entire ring of tentacles becomes closely applied to the shell, and the margin, by partial contraction of the sphincter, grips the shell, forming the rim of a sucker comprising the oral disk and mouth. This tentacular and marginal hold on the shell exerts a strong pull. A crab is often held fast by an anemone at this stage, unable to free itself except by its most powerful movements. An empty shell is frequently lifted or dragged towards the anemone. In this situation, the state and position of the tentacles and oral disk are no longer visible. Many tentacles continue to adhere but usually they are shorter than they were at the beginning of the response. Sometimes the mouth also grips the shell at this time, but usually it is hidden from view.

(2) *The detachment of the pedal disk*

Once the tentacles and margin have a secure hold on the shell, peristaltic waves travel down the column towards the foot. The column gradually thins and sometimes twists. As a rule, several minutes elapse before any change can be detected in the pedal region and this change, when it happens, is variable; the pedal edge may be drawn in towards the centre, the whole basal region may swell noticeably, or the disk may lift off the plate at one or more points around the outer rim. At this stage the mucous envelope which surrounds the basal region of the animal is often shed. The ensuing period, when the pedal disk is being detached, may last for many minutes, or it may pass quickly. Sometimes the anemone is firmly held by the cementing deposits of its pedal disk, often by only a small area of attachment. In such cases, the pedal disk, after becoming partly detached, may re-attach, and again partly detach and re-attach several times before becoming completely free. Sometimes the animal fails altogether to detach itself after several such attempts, and then it eventually relaxes its hold on the shell and the pedal disk again settles firmly on the plate.

This release of the pedal disk is achieved by co-ordinated movements of circular and parietal muscles, assisted perhaps by the longitudinal muscles of the mesenteries pulling the pedal disk towards the oral disk, which is by this time closely attached to the shell. However, the detachment is not a stereotyped predictable process. Its form varies according to the animal's position in relation to the shell, and to the firmness of the foot's attachment to the surface from which it is transferring.

(3) *The movement of the detached pedal disk to the shell*

The moment the pedal disk is detached, the column thins and bends to bring the foot over towards the shell. As this flexure develops, the basal region below the line of the cinclides swells immensely. This swelling may produce a grotesque effect, with the foot resembling the cap of a mushroom. These movements in the transfer are carried out quite rapidly and are just perceptible to the eye, taking only 1 to 3 min to complete.

While these events are taking place, the tentacles and margin continue to grip the shell, but often they slowly change position to reach an area on the shell away from that with which the foot makes contact. Frequently the swollen pedal edge first touches the shell at a point immediately adjacent to the area to which the tentacles are attached, and the animal is bent into a tight U-shape. It often happens, however, that the shell is not reached by the foot at the first attempt, or it may encounter another anemone already occupying the site towards which it has moved. In such cases the flexed animal unbends and, after a pause, the movement is repeated usually in another direction. But once even a tiny area of the foot touches the shell it begins to adhere and a firm attachment is formed as if aided by local adhesive elements, perhaps by nematocysts (p. 279).

This movement of the foot to the shell, like the detachment of the foot from the plate, shows a remarkable degree of adaptation to the circumstances of the transfer. It is adapted particularly to the position taken up on the shell by the tentacles and oral disk. If this is high on the shell, the anemone usually swings over by a spectacular vertical movement above the shell. If the tentacles are attached low down on the shell, the anemone may drop down on the floor of the container and climb on the shell lazily from a semi-prone position. If the tentacles have become attached at a position midway between the top and bottom of the shell, the detached anemone usually moves across in a horizontal position. Even if the foot first contacts the shell at some restricted surface such as the apex or the siphonal groove, ultimate success is usually achieved by behaviour adapted to these special circumstances.

(4) *The settling of the pedal disk on the shell*

However small it is initially, the area of contact between the pedal disk and the shell provides a base from which it slowly extends and consolidates. This consolidation may take from 5 to 15 min. It is achieved by further swelling of the whole basal region and the films show it as a highly dynamic process of advance and retreat, of settling, lifting and re-settling, again and again. During this period the foot often encroaches on the area to which the tentacles and oral disk have been attached heretofore. At the same time, the upper column usually constricts.

(5) *The release of the tentacles and oral disk and the resumption of normal posture*

Once the pedal disk has settled securely, the tentacles and oral disk, by now much shrunken and distorted, suddenly release their hold on the shell. Like the flexure which brought the foot to the shell, so the final straightening movement, which brings the animal back to its normal vertical posture, is rapid enough to be visible to the eye, usually taking only 1 to 2 min. But for some time after the anemone has straightened, its appearance is most abnormal; the tentacles remain deflated and the upper column is constricted and contorted. The foot continues the movements described in (4) above, usually greatly swollen and spreading over the shell to take up its final position.

The films show that this final period is one of great activity. From time to time, the animal bends and sweeps its tentacles and oral disk over the shell. The foot continues to inflate and deflate, often sliding across the shell to occupy precisely the area to which the tentacles and oral disk became attached at the beginning. Up to half an hour may elapse before these activities cease and before the anemone assumes its typical resting appearance, with its tentacles inflated, its oral disk expanded, its column relatively short and its basal disk firmly bedded into the irregularities of the shell surface. For some time after this, the attachment of the foot is relatively weak and the anemone can be easily prised off. Many hours or days later, however, the attachment is so firm that great force must be used to remove *Calliactis* from the shell. Presumably this is due to the deposition of cementing secretions as a slow final stage in the process of the settling of the foot on the shell.

This account brings out the purposiveness and flexibility of this behaviour pattern. Compared to a simple reflex satisfying an immediate need, as for food or protection, this is a complex manoeuvre from which the animal can gain only some long-term advantage in the future. Experiments now to be described were carried out to find the factors involved at various stages in the transfer to a shell.

EXPERIMENTS ON THE FREQUENCY OF TRANSFER

The shell variable

The earlier work showed big differences amongst *Calliactis* in the tendency to transfer to shells from other surfaces. Some individuals never transferred, others did so in every trial (Ross 1960). In the present study, especially in making films in London, we found that this anemone variable was often related to the time spent by the animals in the laboratory. About 2 weeks after arriving from Plymouth (perhaps several weeks after capture if not freshly caught), the tendency to transfer to shells fell off noticeably. Later at Plymouth, we found that many specimens, which had been living on crabs for some weeks crowded into a large tank, failed to display the phenomenon. For lively and consistent activity, *Calliactis* freshly collected from the sea were needed. When these fresh animals were then kept at Plymouth under good conditions they remained active as long as we were able to test them, 16 days in all. It seems, therefore, that conditions of capture and maintenance in the laboratory are responsible for much of the variability observed in the activity of different *Calliactis*. In our experience, this activity once lost never returns.

At the same time, other observations suggested that some shells evoked the typical pattern of transference behaviour much more effectively than others. It seemed also that this was a persistent feature of such shells. To test this, an experiment was set up in which four fresh *Calliactis* were allowed to settle within each of ten jars, then a recently crab-occupied *Buccinum* shell was introduced into each jar, and after 12 h, the number of anemones transferring to each shell was recorded. The anemones were then detached and allowed to re-settle, the shells were changed around, and this procedure was repeated until each shell had been offered to each group of four *Calliactis*. We thus obtained a record of the number of transfers by the forty anemones to each of the ten shells over a period of 7 days. The results are shown in table 1.

TABLE 1. NUMBERS OF *CALLIACTIS* TRANSFERRING FROM GLASS TO EACH OF TEN *BUCCINUM* SHELLS RECENTLY OCCUPIED BY *PAGURUS BERNHARDUS*

Rotating trials carried on over 7 days presenting forty *Calliactis* to each shell in groups of 4. See text for details

shells	no. of transfers to shells (out of 40 possible)
1	10
2	13
3	14
4	20
5	11
6	14
7	17
8	28
9	23
10	24

The above remarks about the decline of activity in *Calliactis* in the laboratory make one ask whether the anemones were as active at the end as at the beginning of the experiment. The totals for the ten presentations as a time series were: 23, 18, 16, 22, 14, 13, 17, 22, 17, 12. A slight decline is indicated by these figures but it is not a major effect. The possibility of a slight change in 'flavour' of the shells by these repeated transfers is also indicated by these figures.

The frequency of transfer in this experiment was 174 out of 400, compared with approximately 50 % in earlier tests (Ross 1960). Inspection of table 1 shows a wide scatter with no central clustering in the numbers of transfers to these shells. If the shells were all alike in their capacities to evoke activity, transfer to them by the anemones should follow a binomial distribution. In that case, the probability of getting ten or fewer transfers in forty trials as in shell 1 would be 0·014 and of getting twenty-eight or more transfers, as in shell 8, would be 0·00067. The results for shells 1 and 8 are unlikely therefore to be due to chance, so besides the anemone factor there seems to be a variable shell-factor which influences the tendency of *Calliactis* to transfer to a shell from another surface.

Shells of different species

The same forty anemones were tested against *Buccinum* shells in various conditions and against shells of two other species. Ross (1960) showed that *Calliactis* will transfer to the living whelk, *Buccinum undatum*. Table 2 shows how frequently it can effect this transfer and shows also that the anemone transfers to both *Pecten maximus* and *Mytilus edulis*. These recorded frequencies of transfers to living

Buccinum and *Pecten* are misleading, however. The powerful sudden movements which these two species can perform prevented a number of transfers from being completed. Twelve *Calliactis* were seen at various times attempting to settle on the living *Buccinum* but only three succeeded. The rest were thrown off by the vigorous rotatory movements of this animal through 180 to 270°.

TABLE 2. NUMBER OF TRANSFERS BY FORTY *CALLIACTIS* (TESTED IN GROUPS OF 4) FROM GLASS TO VARIOUS MOLLUSCAN SHELLS

The trials were carried out in the above order over seven days. See text for remarks on results with asterisks. *Uo* unoccupied; *Ut* untreated.

shell	occupied by	formerly occupied by	treatment	transfers to shell from glass (max. 40)
Buccinum	*Pagurus bernhardus*	—	*Ut*	14
Buccinum	*Uo*	*P. bernhardus*	alkali-cleaned	1
Buccinum	*Uo*	*B. undatum*	*Ut*	20
Buccinum	*B. undatum*	—	*Ut*	3*
Pecten	*Pecten maximus*	—	*Ut*	6*
Mytilus	*M. edulis*	—	*Ut*	9
Buccinum	*P. bernhardus*	—	*Ut*	17

Table 2 shows that *Calliactis* has no special preference for shells recently occupied by *Pagurus bernhardus*. In this experiment, the most effective shell was one direct from *Buccinum* in which no crab had ever lived. This experiment also lasted about 7 days. The *Calliactis* were kept out-of-doors in running sea water and the response to pagurid shells was about the same at the end as at the beginning. Difficulties were arising towards the end, however, because the anemones were taking longer and longer to re-settle on the glass between trials.

EXPERIMENTS ON UNATTACHED *CALLIACTIS* AND THEIR BEHAVIOUR IN RESPONSE TO SHELLS AND OTHER OBJECTS

The procedure just described to determine the numbers of transfers by attached *Calliactis* under various conditions is both tedious and difficult to standardize. The need to re-settle the anemones after every trial limits observations to two trials every 3 days. At best only about half the animals respond and they may do so only after a considerable delay.

We have observed many times that *Calliactis* lying unattached on their sides cling to shells much more readily than *Calliactis* which have already settled on another surface. To an active shell, such anemones respond almost without fail, and go through the whole process of climbing on the shells from the prone position very quickly (5 to 10 min). Such animals, therefore, offer a much more standardized basis for comparative trials. Their behaviour is essentially like that described above with stage (2) left out. Also once a trial is completed, the anemones can be removed from the shells and within a short time they may be used again.

The ready tendency of detached *Calliactis* to cling to shells, and to climb on them unaided, was observed by Faurot (1932) working with *Calliactis* from the Mediterranean which normally live on a different species, *Dardanus arrosor* Herbst

(= *Pagurus striatus* Latreille). The more active clinging response to shells of detached animals has lately been studied by Davenport, Ross & Sutton (1961) and shown to be due to a lower threshold to nematocyst discharge in this condition. It is therefore a phenomenon of considerable interest in its own right.

In these experiments, ten fresh *Calliactis* were placed in a plastic tank and prevented from settling on the bottom. From time to time, test objects, usually three shells, were introduced. The *Calliactis* were then brought into contact with these objects to see whether the tentacles adhered and whether the animals subsequently climbed on. The events were timed so that we have records, not only of the numbers of animals that cling to the shells and subsequently settled, but also how quickly they did so.

Table 3 shows the programme of experiments and the results. Beginning with untreated *Buccinum* shells, with and without *Pagurus bernhardus* or *Buccinum* itself, and comparing these with *Pecten* and *Mytilus* shells, we proceeded to test the effects of various treatments on these shells and the responses to various substitutes, which in turn were treated in various ways.

From the results of trials 1 to 8 it is clear that the clinging response and the subsequent settling are evoked most consistently and most rapidly by *Buccinum* shells. Trials 1 to 3 gave positive results for clinging and settling in virtually every case. The rapidity of the clinging response with *Buccinum* itself was remarkable (trial 4) but, for the reason already given, the anemones were usually thrown off before they could settle securely. The reaction-times with the various *Buccinum* shells (crab-occupied or *Buccinum*-occupied or empty) may not be significantly different from one another. It is evident, however, that the response is not absolutely specific to *Buccinum* shells since both *Pecten* and *Mytilus* have some activity, though the intensity is much lower (trials 5 to 8). In all cases, the clinging response, and the entire behaviour pattern, are responses to the shells and not to the animals inhabiting them. The complete failure of re-occupation by *P. bernhardus* to restore activity to inactivated shells (trials 22 to 24) makes this point clearly. *Calliactis* shows no tendency to adhere to the soft parts of the whelk in spite of its sensitivity to the shell. Occasionally it does cling to the legs or chelae of a crab; this might be due to simultaneous contact with the shell but it needs further investigation. We already know that *Calliactis* fails to transfer to shells that have been boiled in alkali to remove superficial organic deposits (Ross 1960). It might be argued that such a treatment acts merely by rendering the shell unpalatable from the traces of caustic that remain. The shells were neutralized, however, after boiling, and trial 9 shows that most of the active clinging of *Calliactis* to the shell is abolished. Trials 13 and 14 supplement this observation and show that thin coats of plastic also virtually abolish the clinging response and the settling behaviour, presumably by preventing any chemical factor in the shell from coming in contact with the anemone.

Trial 10 shows that *Calliactis* still respond, though less actively and after long delays, to *Buccinum* shells from which the periostracum has been removed. More extensive superficial cleaning, by a power-driven wire brush, may reduce the activity still more but by no means abolishes it altogether (trials 11 and 12). The

TABLE 3. RECORDED BEHAVIOUR OF TEN DETACHED *CALLIACTIS* ON CONTACT WITH *BUCCINUM*, *PECTEN* AND *MYTILUS* SHELLS, AND OTHER TEST OBJECTS, IN VARIOUS CONDITIONS AND SUBJECTED TO VARIOUS TREATMENTS

trial no.	test objects	occupied by	formerly occupied by	treatment	no. *Calliactis* giving clinging response (out of 10)	no. *Calliactis* settling on object (out of 10)	mean reaction time of *Calliactis* clinging (if > 5 out of 10) (min)	remarks
1	3 *Buccinum* shells	*Pagurus bernhardus*	—	*Ut*	10	10	6·8	—
2	3	*Uo*	*Pagurus bernhardus*	*Ut*	10	10	7·6	—
3	3	*Uo*	*B. undatum*	*Ut*	9	9	11·8	—
4	3	*B. undatum*	—	*Ut*	10	1	2·2	failed to settle due to whelk movements
5	3 *Pecten* shells	*Pecten maximus*	—	*Ut*	8	1	13·6	failed to settle due to *Pecten* movements
6	3 *Pecten* ½-shells	*Uo*	*Pecten maximus*	*Ut*	6	6	75·0	—
7	3 *Mytilus* shells	*M. edulis*	—	*Ut*	5	4	24·6	—
8	3 *Mytilus* ½ shells	*Uo*	*M. edulis*	*Ut*	3	2	—	—
9	3 *Buccinum* shells	*Uo*	*Pagurus bernhardus*	alkali-cleaned	1	1	—	sole *Calliactis* responding did so in 60 min
10	3	*Uo*	*B. undatum*	periostracum removed	8	8	22·5	periostracum removed by hand
11	3	*Uo*	*P. bernhardus*	surface-clean by wire brush	10	8	53·0	machine-cleaned
12	3	*Uo*	*B. undatum*	surface-clean by wire brush	5	3	38·4	machine-cleaned
13	1 *Buccinum* shell	*Uo*	*P. bernhardus*	coated with 'tensol' plastic	0	0	—	3 trials with 4, 3, 3 *Calliactis*
14	1	*Uo*	*P. bernhardus*	coated with 'vinalak' plastic	2	0	—	3 trials with 4, 3, 3 *Calliactis*
15	1 glass model shell	*Uo*	*P. bernhardus*	water washed	0	0	—	3 trials with 4, 3, 3 *Calliactis*
16	3 plaster model shells	*Uo*	—	*Ut*	0	0	—	—
17	3	*Uo*	—	rubbed on *Buccinum* and crabs	0	0	—	—
18	3	*Uo*	—	rubbed on *Buccinum* and shell and periostracum	0	0	—	—
19	3	*Uo*	—	rubbed on squid surface	0	0	—	tentacles active but not adhering as to shell
20	3 plaster blocks	—	—	*Ut*	0	0	—	—
21	3	—	—	impregnated with powdered shell	2	1	—	—
22	3 *Buccinum* shells	*P. bernhardus R*	*P. bernhardus*	alkali-cleaned	0	0	—	—
23	1 *Buccinum* shell	*P. bernhardus R*	*P. bernhardus*	coated with 'tensol' plastic	0	0	—	3 trials with 4, 3, 3 *Calliactis*
24	1 glass model shell	*P. bernhardus R*	*P. bernhardus*	*Ut*	0	0	—	3 trials with 4, 3, 3 *Calliactis*

Symbols: *Uo*, unoccupied; *Ut*, untreated; *R*, re-occupied.

use of plaster-of-paris models moulded from a *Buccinum* shell, and reproducing the shell surface very accurately, provide additional evidence that simple tactile contact with a surface having this form and topography is inadequate to evoke the clinging response (trial 15).

No method has yet been discovered which gives inactive substitutes or inactive cleaned shells, the capacity of eliciting the tentacular response and the subsequent settling behaviour. The plaster blocks impregnated with powdered shell showed some activity (trial 21) but spraying or coating plaster models or cleaned shells with scrapings and exudates from crab, whelks and shells had absolutely no effect (trials 17 and 18).

Trial 19 is instructive in showing the difference between the tentacular responses to shells and to food. Tentacles adhere to food and *Calliactis* clinging to food, say to a large piece of squid, can be detached only with difficulty. In passing it can be noted that *Calliactis* clinging and feeding on squid or crabs, unlike *Calliactis* clinging to shells, show no tendency to detach their pedal disks, or, if they are detached already, to go through the typical bending and swelling movements which follow adhesion to a shell. In trial 19 the ten *Calliactis* were offered cleaned shells and models smeared with mucus and scrapings of squid on which other *Calliactis* were feeding. There was much tentacular activity in response to these shells and models but no clinging of the tentacles or flexure of the column, as happens with the untreated *Buccinum* shell.

Some additional observations, not carried out as part of the above experiment, gave further information about the material factors involved in the clinging response. It has been stressed already (Ross 1960) that contact with the shell is necessary to elicit the response; no substance diffuses from the shell to stimulate the anemone at a distance even when it is very close to the shell. We have extracted a number of shells with acetone, ethyl alcohol and ether for several weeks and tested both the materials extracted, and the shells, after the extraction. The extracted materials showed no activity but *Calliactis* responded perfectly normally to the shells, clinging to them and climbing on in the usual way in a very few minutes. This would be consistent with an active agent on the shell that is highly stable and insoluble, perhaps a quinone-tanned protein or a muco-polysaccharide. It is worth noting that this active shell-factor is longlasting. Occasionally we have tested shells that have been kept dry in the laboratory for as long as 18 months and found them to be still quite active. This is important in the natural relationship with the crab. Shells must retain activity long after they have been cleaned of all tissue remnants from the *Buccinum* which deposited the active shell-factor on and in the shell. In life such shells often become encrusted with barnacles and hydroids. Barnacles on the surface do not seem to block activity but they seldom cover the original shell so completely that some areas of contact with the original shell would not be available to the anemone. We have never seen *Calliactis* clinging to shells already inhabited by *Hydractinia*, so this may form an effective barrier.

Although trial 10 showed that peeling off the periostracum from a *Buccinum* shell does not abolish activity, we have found that periostracum alone is highly active. If a strip of this material is brought into contact with *Calliactis*, it is held by the tentacles and soon afterwards the typical column flexure develops and the foot swells, as if to attach to the non-existent shell. This phenomenon has been filmed but no attempt has been made to identify the active component of the periostracum.

EXPERIMENTS ON OPERATED ANIMALS

A number of operations were carried out on *Calliactis* in order to study how the programme of responding and settling on shells was affected by removing parts of the body or separating parts normally connected horizontally or vertically. Operations were performed without anaesthesia on fresh animals known to respond very actively to shells. Various vertical and horizontal cuts were made; each operation was performed on two specimens except where the animal was vertically bisected. The animals recovered well in running sea water and were presumed ready for testing 24 to 36 h later. The appearance of the more drastically operated animals usually differed from normal because the cut edges tended to close up and seal off the exposed coelenteron.

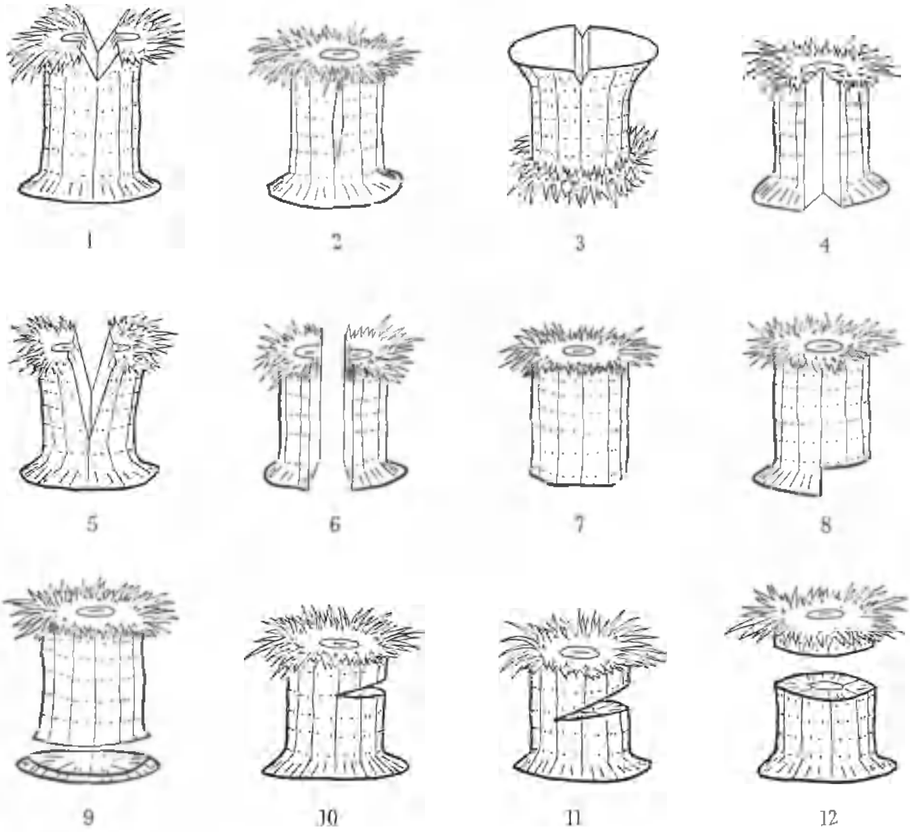


FIGURE 1. Diagrams of operations 1 to 12 performed on Calliactis as described in table 4.

The operations are shown diagrammatically in figure 1. The specimens were tested for their capacity to give the clinging response to shells typical of detached *Calliactis*, and for their ability to proceed from the clinging position to the somersaulting manoeuvre by which the foot finally settles on the shell. The results are shown in table 4.

It is evident that *Calliactis* still retains its capacity to respond to, and settle on, *Buccinum* shells after most vertical incisions. Only when the incision passed through both oral and pedal disks (4 and 6) was any serious disfunction observed in the complete behaviour programme. These deeper incisions (4 to 6) made bending and setling movements slow and laboured, but even when the animal was fully bisected (6), both halves clung to the shell and one half-animal eventually succeeded in settling.

TABLE 4. RECORDS OF ATTACHMENT OF OPERATED *CALLIACTIS* TO SHELLS OF *BUCCINUM*. TWO *CALLIACTIS* USED AND TESTED FOR EACH OPERATION

Stages as in pp. 267–269.

direction of main incision	operation no.	description of operation	results		remarks
			clinging	settled	
vertical	1	oral disk and margin divided	+ +	+ +	all stages very fast
	2	mid-column cut	+ +	+ +	stage 3 slow
	3	pedal disk divided	+ +	+ +	stages 4, 5 slow
	4	animal half-bisected	+ −	+ −	stage 3 very slow
	5	animal divided to pedal disk	+ +	+ +	stage 3 slow
	6	whole animal bisected	+ +	− +	stage 3 slow/failed
horizontal	7	pedal edge removed	+ +	+ +	all stages very fast
	8	half-pedal disk removed	+ +	+ +	stage 4 slow
	9	pedal disk removed entire	− −	− −	tentacles withdrew on contact
	10	body half-sectioned	+ +	+ +	stage 3 very slow
	11	body three-quarters sectioned	+ −	− −	stage 3 failed
	12	body sectioned entire	− −	− −	tentacles withdrew on contact

Horizontal section had more serious consequences. The oral halves of animals divided horizontally at any level (9 and 12) showed no tendency to cling to shells. The tentacles of these never became fully expanded, but in our opinion this was not the cause of failure. After some vertical incisions, e.g. bisection, the tentacles failed to expand, yet the clinging response to shells was not abolished. Perhaps the most instructive experimental result in table 4 is the abolition of the clinging response after removal of the pedal disk (9). Yet when only half the pedal disk was present (8) the operated animals attached themselves successfully, though with some difficulty. Removal of the pedal edge, i.e. the outer rim of the pedal disk, had no effect whatsoever on the settling behaviour (7).

We conclude from these observations that some organic connexions between tentacles and pedal disk are necessary for initiating and developing this pattern of behaviour. Certainly the vertical pathways, interrupted by horizontal incisions, seem to be the more important. This corresponds with the observations of Davenport, Ross *et al.* (1961) on the control exercised by the pedal disk over the discharge of the tentacular nematocysts, shown by them to be involved in the response of *Calliactis* to shells.

EXPERIMENTS ON *CALLIACTIS* DURING CLINGING, FLEXURE AND SETTLING

In clinging to shells *Calliactis* are often assisted by movements of the sphincter which grip the shell as described above (p. 267). These movements stiffen the column and help the animal to detach, or if already detached, lift it off the floor of the tank so that it becomes supported solely by its hold on the shell.

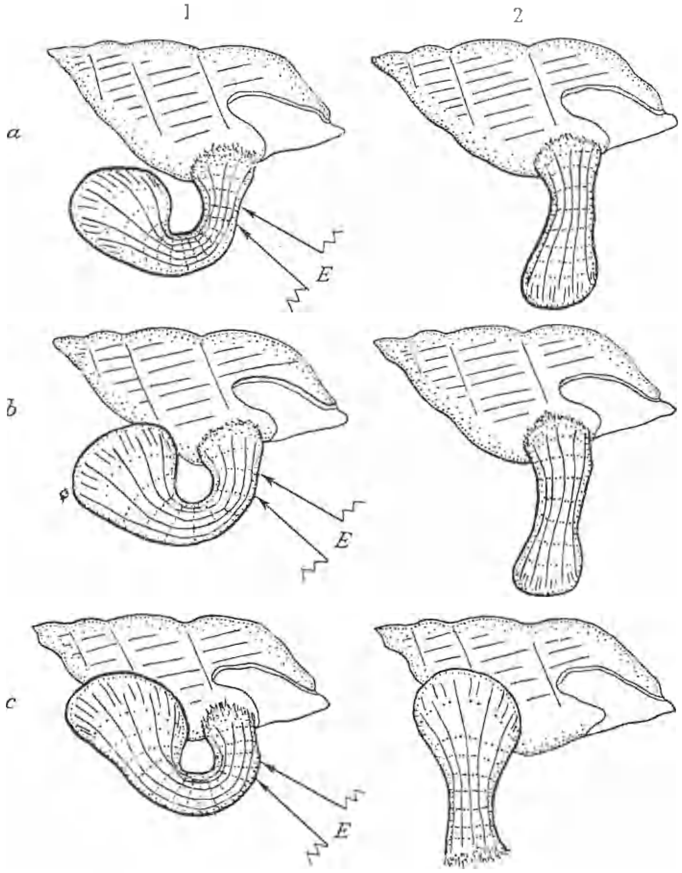


FIGURE 2. Position of *Calliactis* before (1) and after (2) electrical stimulation at three different stages during the movement of the pedal disk to the shell: (*a*) pedal disk not yet in contact with the shell; (*b*) about one-tenth of pedal disk in contact with shell; (*c*) about one-third of pedal disk in contact with shell. In (*a*) and (*b*) the animal straightens but remains attached to shell by tentacles; in (*c*) the animal straightens but remains attached to shell by pedal disk. *E*, electrodes. Time interval between (1) and (2) in each case about 30 s.

Electrical and mechanical stimulation of an animal that is beginning to cling to a shell has effects like those just described, reinforcing its hold on the shell by the partial contraction of the sphincter. Prolonged stimulation closes the margin so much, however, that the contact of the tentacles with the shell is broken and the anemone falls off.

Calliactis continues to respond to stimuli during the subsequent stages of transference and settling. Stimulation (figure 2*a* 1) interrupts flexure and causes the column to become erect again (figure 2*a* 2). After a pause, however, the animal once again undergoes flexure to bring the pedal disk over to the shell. Flexure can be interrupted several times in this way without affecting the eventual completion of the movement towards the shell.

Once the foot has established contact with the shell, stimulation may have one of two effects. If the area of contact is still very small (figure 2*b* 1), the responses to stimuli, by undoing the flexure, pull the foot away from the shell and straighten the column to bring the animal to the position shown in figure 2*b* 2. But if the foot is more securely attached (figure 2*c* 1), the straightening of the column caused by stimulation causes the tentacles and margin to let go and brings the animal into its normal settled position (figure 2*c* 2). This movement resembles the movement that normally concludes the transfer behaviour (our stage 5) but electrical stimuli at the frequency used here (1/s) give a jerky movement whereas the natural straightening movement is smooth. At lower frequencies which do not elicit quick responses (Pantin 1935; Ross 1957), the response is almost identical in timing and character with the normal movement. The natural movement could be due, therefore, to an outburst of low-frequency excitation producing a slow symmetrical response of the musculature, e.g. circular, parietal and longitudinal mesenteric muscles. This is, however, the only one of the five stages in the behaviour programme that can be evoked by the more obvious forms of external stimulation. It must be presumed that the other stages arise from more subtle forms of excitation at each transition point in the behaviour pattern.

Experiments were also carried out on the adhesion of the pedal disk to the shell. When the foot is swinging over to make contact with the shell, it is possible to present other surfaces to it as substitutes for the shell it is approaching. We have tried smooth and rough plastics, stones, plaster-of-paris models of shells, and alkali-cleaned shells, in this way. All of these were rejected. On encountering such surfaces, the pedal disk withdraws, the flexed column of the anemone becomes straight and then, after a pause, the animal bends toward the shell a second time, as it does when it encounters another anemone on the shell. If, instead of substitutes, a second *Buccinum* shell is brought up to meet the pedal disk during flexure, the disk sticks to it at once even though it is not the shell to which the tentacles are attached.

Once a small portion of the pedal disk makes contact with a shell the behaviour of the whole disk changes completely. The unattached area will then adhere to any surface presented to it so that an anemone can easily be made to settle partly on shell and partly on another surface. Moreover, if the animal is removed from the shell during settling, or soon afterwards, the pedal disk will then adhere very readily to any surface on which it is placed. *Calliactis* in this condition settle firmly on a smooth glass plate within a very few minutes, whereas an unattached *Calliactis* usually takes many hours or even days to stick securely to such a surface.

It seems to us that when the foot first touches the shell a condition which we describe as 'stickiness' is produced over the whole pedal disk. This facilitates adhesion and is so effective even when only a small area of the foot is on the shell that quite strong pressure is required to dislodge it. Although the nature of this suddenly occurring 'stickiness' is not know with any certainty, it resembles the 'stickiness' of *Calliactis* tentacles on touching a shell. This 'stickiness' was shown by Davenport *et al.* (1961) to be due to the discharge of nematocysts. Perhaps pedal 'stickiness' is due also to nematocysts, as these are left behind in considerable numbers on cover glasses which are brought into contact with a 'sticky' pedal disk.

Apparently the movement when the foot first makes contact with the shell marks a second point in the transference behaviour of *Calliactis* at which a specific response to shell is called for. Like the first contact of the tentacles, it seems to be a response to some component in the shell. Cleaned shells do not evoke it nor do shell models. Perhaps it is also a response to the 'shell-factor' postulated above, and by Davenport *et al.* (1961), but this could be settled only when more is known about the nature of the 'shell-factor'.

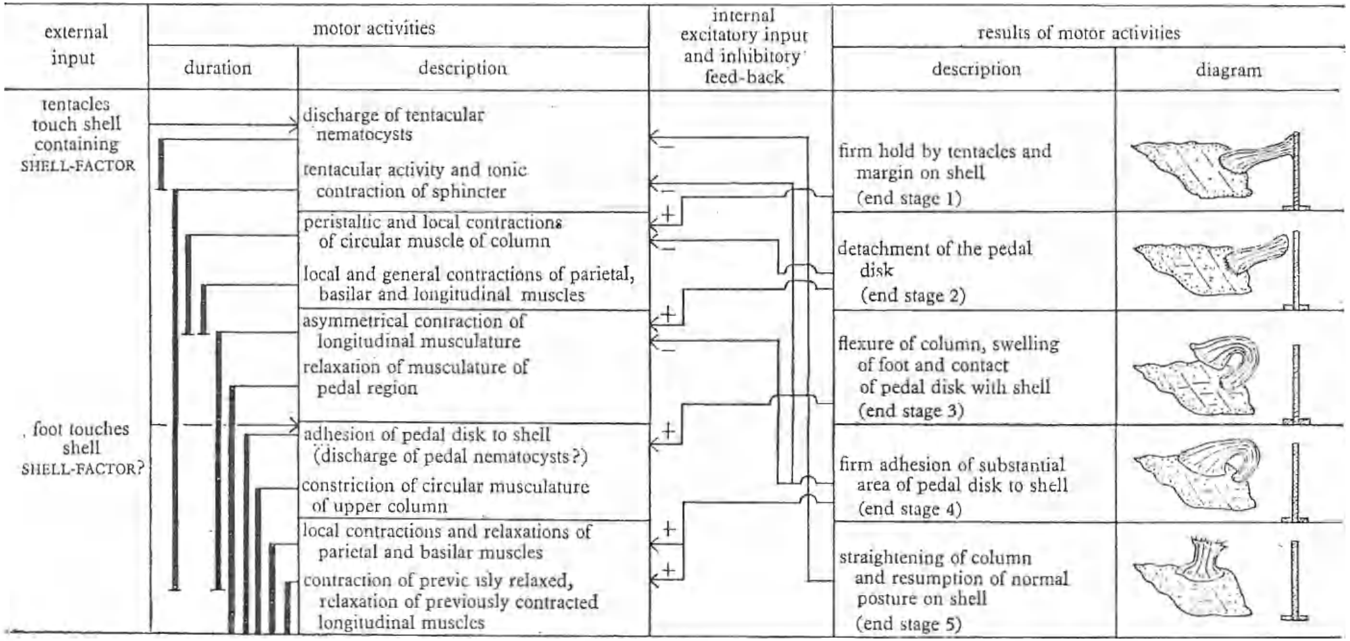
Discussions and conclusions

This analysis of the behaviour of *Calliactis parasitica*, as found at Plymouth living mostly on *Buccinum* shells inhabited by *Pagurus bernhardus*, has clarified a number of points that were previously obscure. The primary stimulus that elicits the behaviour pattern by which *Calliactis* transfers to, or climbs on, shells, is undoubtedly a shell-factor of molluscan origin. The whelk, *Buccinum undatum*, contributes this shell-factor in its most potent form but it is present also in some other molluscan shells. At present we can only say that this shell-factor is some highly stable, insoluble organic component of both shell and periostracum.

One of the unsuspected features of this association is that the anemone gains its position on the shell by responding, not to the crab, but to traces of the mollusc that laid down the shell long before. This is a good example of the indirect route by which the behavioural basis of an ecological relationship can be established.

The parallel work of Davenport *et al.* (1961) on the role of the nematocysts in the sticking of the tentacles on the shell has been mentioned. They showed that some remote control emanating from the pedal disk affected the threshold for the discharge of these nematocysts. This has been supported here by the absence of the tentacular clinging to shells when the pedal disk is removed. This nematocyst response is the first recognizable event in the transfer to a shell. It may, therefore, trigger off the whole subsequent behaviour pattern, though it cannot be proved that there is not a prior chemosensory response that triggers off the nematocyst response itself. In any event, this response of *Calliactis* suggests possible relationships between the shell-sensitive nematocysts and the nervous system, in contrast with most other nematocysts which function as independent effectors (Parker & van Alstyne 1932; Pantin 1942; Hyman 1940; Ewer 1947).

Some final comments are necessary also on the phenomenon as a co-ordinated pattern of behaviour. We have seen that it proceeds in five distinct stages, each one having to reach a certain degree of fulfilment before the next one begins. Interruption of any stage does not disrupt the programme as a whole. It sends the interrupted stage back to its starting point, whence it begins again and proceeds to complete itself.



external input	motor activities		internal excitatory input and inhibitory feed-back	results of motor activities	
	duration	description		description	diagram
tentacles touch shell containing SHELL-FACTOR		discharge of tentacular nematocysts tentacular activity and tonic contraction of sphincter	− −	firm hold by tentacles and margin on shell (end stage 1)	
		peristaltic and local contractions of circular muscle of column local and general contractions of parietal, basilar and longitudinal muscles	+ −	detachment of the pedal disk (end stage 2)	
		asymmetrical contraction of longitudinal musculature relaxation of musculature of pedal region	+ −	flexure of column, swelling of foot and contact of pedal disk with shell (end stage 3)	
foot touches shell SHELL-FACTOR?		adhesion of pedal disk to shell (discharge of pedal nematocysts?) constriction of circular musculature of upper column	+	firm adhesion of substantial area of pedal disk to shell (end stage 4)	
		local contractions and relaxations of parietal and basilar muscles contraction of previc isly relaxed, relaxation of previously contracted longitudinal muscles	+ +	straightening of column and resumption of normal posture on shell (end stage 5)	

FIGURE 3. Diagram to show: (*a*) sequence of motor events in the transference behaviour of *Calliactis parasitica*; (*b*) the timing and relationships of the excitatory (+) and inhibitory (—) influences presumably involved in this sequence. Horizontal divisions in col. 3 separate stages 1 to 5 (see text). Each stage begins with the first-named activity out of which the second phase develops.

In principle this behaviour is not difficult to interpret as a system of reflexes. Some of these form a chain in which each completed reflex initiates the next, and inhibits an earlier one. Thus, freeing the pedal disk starts flexure, and stops peristalsis, of the column. But the primary reflex in the behaviour pattern, the adhesion of the tentacles and oral disk to the shell, overlaps several stages and may govern much of the programme. The resumption of a stage thrown into reverse (as in the straightening of the flexed column by stimuli) may be due to the control exercised by excitation from the tentacular-oral region, which remains adhering to the shell almost to the end.

It is not easy to assess correctly the significance of this behaviour pattern of *Calliactis* in relation to current views about the nature of the actinian nervous system. Figure 3 presents a diagram of the minimal sensory and motor links required to account for the behaviour described above. This is a functional and not a morphological scheme, but it implies more differentiation in the nerve net than one usually expects, even allowing for the intricacy revealed in the recent paper by Batham *et al.* (1960). Yet the scheme in figure 3 shows only direct sensory-motor and motor-motor links. It does not reflect adequately the flexibility of the behaviour pattern in relation to the specific problems of transference in each case. One has to admit degrees of sensory discrimination, and capacities for acting on information, that imply association neurones working in concert.

This behaviour pattern of *Calliactis* strongly reinforces Pantin's (1952) remarks on the unexpected complexity of actinian behaviour, when seen against the background of an apparently simple nervous system. It is remarkable that this support for the general argument should come from *Calliactis*, which, compared with *Metridium*, was once described as 'an apparently inert anemone...in a state...of continual though slight movement' (Batham & Pantin 1950).

REFERENCES

Batham, E. J. & Pantin, C. F. A. 1950 *J. Exp. Biol.* **27**, 290–301.
Batham, E. J., Pantin, C. F. A. & Robson, E. A. 1960 *Quart. J. Micr. Sci.* **101**, 487–510.
Davenport, D., Ross, D. M. & Sutton, L. 1961 *Vie et Milieu* (in the Press).
Ewer, R. F. 1947 *Proc. Zool. Soc. Lond.* **117**, 365–376.
Faurot, L. 1932 *Arch. Zool. exp. gén.* **74**, 139–54.
Hyman, L. H. 1940 *The invertebrata. Protoza through Ctenophora.* New York: McGraw-Hill.
Pantin, C. F. A. 1935 *J. Exp. Biol.* **12**, 139–155.
Pantin, C. F. A. 1942 *J. Exp. Biol.* **19**, 294–310.
Pantin, C. F. A. 1952 *Proc. Roy. Soc.* B, **140**, 147–168.
Parker, G. H. & Van Alstyne, M. A. 1932 *J. Exp. Zool.* **63**, 329–344.
Ross, D. M. 1957 *J. Exp. Biol.* **34**, 11–28.
Ross, D. M. 1960 *Proc. Zool. Soc. Lond.* **134**, 43–57.