

Experimental habituation of aggression in the sea anemone *Actinia equina*

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Abstract

Behavioural plasticity in *Actinia equina* (L.) was examined in experimental contests using a range of pedal disc colour phenotypes, which characterize 3 known, ecologically distinct morphs. With repeated pairing of individuals in auto-phenotypic encounters, habituation was easily induced in the 2 mid-shore morphs, but was not obvious in the less aggressive, low-shore form. Subsequent pairing with a different partner revealed that anemones remained aggressive towards a novel opponent. Following novel contact, re-pairing of the dark red pedal phenotype with the original partner provided some evidence of retention of habituation to a previous opponent, and thus of a specific inducible memory.

Introduction

Although the intraspecific aggressive response of *Actinia equina* (L.) has been well documented (Bonnin, 1964; Brace *et al.*, 1979), it is only relatively recently that its prime functional role on the shore has been ascertained. From a long-term study of anemone spacing on a vertical rock face, it has been concluded that in this situation it acts to enhance adult survivorship through the competitive occupation of surface concavities, which must offer some degree of protection during the winter period (Brace & Quicke, 1986). Once in concavities, however, anemones may experience crowding, a feature facilitated by a subsequent reduction in aggressiveness between juxtaposed individuals (Brace, 1988), which ensures reciprocal maximization of over-winter survivorship. Data from experimental contests have indicated that this lowering of aggressiveness is largely or wholly attributable to behavioural habituation.

Additionally, it has been shown that when paired subsequently with novel partners, these anemones display aggression (Brace, 1988).

The present work demonstrates that habituation can be readily induced in laboratory-based contest sequences. To this end, autophenotypic contest series, using a range of pedal disc colour (PDC) phenotypes, which characterize collectively the 3 ecologically distinct morphs known to occur on British shores (Quicke *et al.*, 1985), were staged. Evidence for a specific inducible memory of a previous opponent was forthcoming from experiments in which following contact with a novel opponent, contestants were re-introduced to their original partners. These findings are discussed with regard to the current knowledge of self/non-self recognition processes in Anthozoa (Francis, 1973; Bigger, 1980; Lubbock, 1980; Lubbock & Shelton, 1981; Sauer *et al.*, 1986).

Material and methods

Collection and maintenance

Adult anemones (15–25 mm diam.) exhibiting red/red-brown column coloration were collected from Burniston, N. Yorkshire in October 1988 and January 1989. Samples of 3 particular PDC phenotypic groups were obtained (dark red (DRd), light red/pink (LRd/P) and green/grey (H/G)) since differences in acrorhagial morphology and aggressiveness exist between these groups (Donoghue *et al.*, 1985; Brace & Reynolds, 1989). Moreover, these 3 groups reflect the presence of 3 multi-locus genotypes which occur at Burniston and elsewhere, which are regarded as morphs of a single species (Quicke *et al.*, 1985). It was ensured that all samples were genetically diverse (see Brace & Quicke, 1985), and that only anemones out of contact with their nearest neighbours were selected. They were transported to the laboratory in individual plastic bags.

Staging of contests

Undamaged anemones which adhered to small pebbles were housed in a circulating seawater system at 12 °C, and allowed 4–5 d to acclimatize. Each encounter was staged by transferring 2 expanded anemones to an experimental arena. The initiation and monitoring of contests were as described in Brace & Reynolds (1989), except that aggression was scored as soon as acrorhagial expansion (one or both anemones) was observed. In all 3 contest series conducted, the inter-anemone contact (contest) and inter-contest periods were 1 h and 4 h respectively, except where otherwise stated. Anemones participating in those initial pairings (trial 1) in which aggression was not recorded were discarded at that stage. Individuals of all the remaining pairs were re-introduced to each other twice to bring about habituation (trials 2 and 3). Anemones drawn from those contests in which aggression was no longer evident were then introduced to novel partners (trial 4).

Series 1. Initially each of the 3 phenotypic groups was examined to assess aggressiveness and the extent to which habituation could be induced. Since statistically significant habituation and subsequent restoration of aggressiveness by pairing with a novel opponent was detected solely with the DRd-PDC phenotypic grouping, only this phenotype was used in Series 2 and 3.

Series 2. In this series, trials 1–4 were conducted as described above, but then contact with the original partner was re-established (trial 5). The time interval between trials 4 and 5 was again 4 h. Only anemones from contests in which aggression had occurred were allowed to proceed to trial 5.

Series 3. This series was essentially a repeat of series 2, but with the interval separating trials 4 and 5 increased to 48 h, and with the addition of

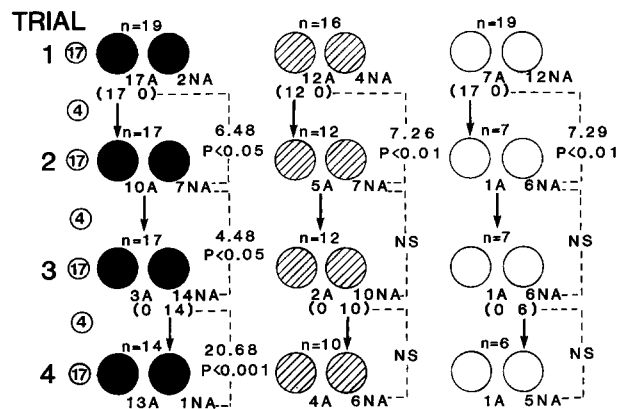


Fig. 1. Flow diagrams of contest sequences in Series 1. Experimental pairings of anemones are represented by juxtaposed circles: filled circles, DRd phenotype; cross-hatched circles, LRd/P; unfilled circles, H/G phenotype. Trial numbers are shown on the left, together with the contest and inter-contest periods (in circles). Novel partners were introduced in Trial 4. For full details of sequences see text.

The number of contests staged appear above the pairs of circles; breakdowns into the numbers in which aggression was either recorded (A) or not observed (NA), appear below. Pairs of numbers denoted in parentheses are indicative of the selection of combatants (A or NA) for subsequent trials: such selection is further highlighted by displacement (left or right) of the vertical (flow) arrows. χ^2 comparisons of the incidence of aggression between trials are shown – χ^2 value and significance, or NS (not significant): associated dashed lines depict precisely which comparisons were made.

a second re-pairing with the original partner (trial 6).

Results

In contest series 1, significant habituation was achieved with anemones from each of the phenotypic groupings (Fig. 1), but in 63% of trial 1 contests involving anemones with H/G-PDC, aggression was not scored. The relative unaggressiveness of this phenotype confirms previous findings (Brace & Reynolds, 1989). When faced with a novel partner (trial 4), significant restoration of aggressiveness was exhibited by the DRd phenotype only, with aggression similar to that of trial 1.

Contest series 2 and 3 using the DRd phenotype alone, resulted in significant habituation and restoration of aggressiveness (Fig. 2). As in

series 1, in only a few contests was aggression not evident in trial 1. In series 2, in which the interval separating trials 4 and 5 was 4 h, a significantly lower degree of aggression was detected when the 22 pairs of anemones drawn from contests with novel opponents (trial 4) in which aggression was evident, were re-introduced to their original partners (trial 5) in which aggression was evident, were re-introduced to their original partners (trial 5). This was not the case, however, in series 3 in which the inter-trial (4–5) period was extended to 48 h. Nevertheless, a second pairing (trial 6) with the original partner (after a 4 h separation) resulted in significantly less aggression, with the reduction in aggression being proportionately greater than that occurring between trials 1 and 2 in both series 2 and 3.

Discussion

The experiments demonstrated that habituation of the aggressive response of *A. equina* can be induced in a series of laboratory-based contests. That such behavioural plasticity can be so readily observed was anticipated since Brace (1988) demonstrated that anemones juxtaposed to one another on the shore display little aggression towards their respective partners. However, Brace also showed that these anemones behaved aggressively when confronted by novel opponents, with a large proportion initiating the aggressive interaction, a feature indicative of sensitization. Such behaviour was not evident in the H/G-PDC phenotype (low-shore morph), which is less aggressive than either the DRd or LRd/P phenotypes (mid-shore morphs) (Brace & Reynolds, 1989).

Successful recognition of a wide range of novel allogeneic partners implies a high degree of discrimination of the relevant histocompatibility markers. Immunocompetence in both corals and sponges has been well documented (see Klein, 1982 for review), but few studies in this context have been conducted on anemones. In common with Bigger (1980) (who used *Anthopleura krebsi* (Duchassaing & Michelotti)) and Sauer *et al.* (1986) (who used *Anemonia viridis* (Forskål)), earlier work on *A. equina* (Brace *et al.*, 1979) dem-

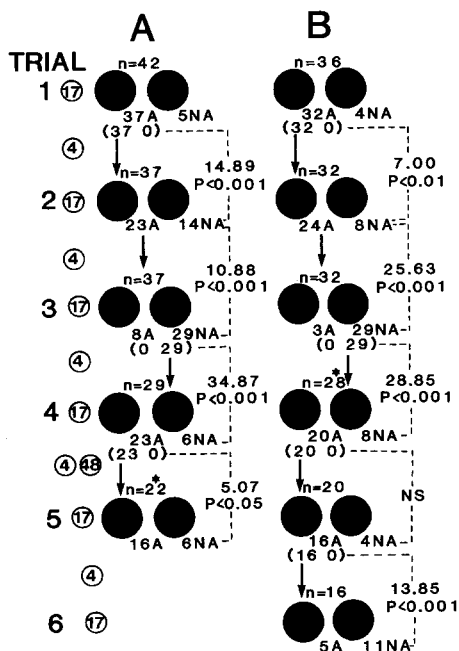


Fig. 2. Flow diagrams of contest sequences using the DRd-PDC phenotype: (A), Series 2; (B), Series 3. Novel partners were introduced in Trial 4; re-introduction of the original opponent in Trial 5 in A and B was after 4 and 48 h, respectively. For full details of sequences see text, and for details of depiction of contest results and analysis, see Fig. 1. *denotes that one potentially available pair of anemones from the preceding trial was not used.

onstrated by briefly touching anemones with isolated allogeneic tentacles that, with repetition of the same stimulus (10 min intervals), the number of contacts necessary to initiate an aggressive response declined. This sensitization is one of the necessary criteria put forward by Hildemann *et al.* (1979) for a response to be considered immunological in nature, but of course, as Bigger (1980) commented, 'while self/not-self recognition is the...cornerstone of immunology, not all self/non-self phenomena are immunologic'.

Indeed, sensitization in response to relatively brief (s/min) stimuli, which Sauer *et al.* (1986) have cautiously termed 'alloimmune memory', and the habituation observed here in response to allogeneic contacts of much longer duration (h), can be explained in terms of neural plasticity.

The 2 contest series staged using the DRd-PDC phenotype alone have provided evidence that an inducible memory is associated with allo-recognition. When previously habituated anemones were re-introduced to their original partners after being separated for 25 h (i.e., 4 + 17 + 4 h) (trial 5 in series 2), significantly less aggression was observed in comparison to the level noted in trial 3 when faced by a novel opponent (see Fig. 2A). However, the level of aggression in trial 5 was higher than that observed in trial 3 (i.e., following habituation). Re-introduction after 69 h (i.e., 4 + 17 + 48 h), however, resulted in the loss of this apparent 'carry over' of habituation, but nevertheless when the original partners were subsequently brought together again for a second time (following a standard 4 h separation period), the extent to which habituation was evident was far in excess of that seen in the equivalent trial during initial habituation (i.e., trial 2).

It is perhaps worth noting that at Burniston, DRd-PDC is characteristic of the upper mid-shore morph (see Brace & Reynolds, 1989), which, in more exposed locations, is the morph which dominates vertical faces where over-winter crowding into surface concavities has been demonstrated (see Introduction and Brace, 1988).

Further experimental work is required before the memory capability can be confirmed fully. It will be necessary first of all to run control contest

series in which following trial 4, individuals are confronted with a second novel partner, in order to check for possible non-specific habituation or fatigue. Assuming that such effects are not detected, further information concerning the persistence of habituation will be required. With regard to the rate of onset of habituation, unpublished data indicate that it is highly dependent upon the temporal schedule of the experimental paradigm used.

Brace (1988) found with some novel contest permutations using previously habituated *A. equina*, that little aggression was exhibited. A reduction in surface antigenicity following juxtapositioning (in the field) was espoused as perhaps being responsible. The present series of contests showed, to the contrary, that equivalent contest permutations almost invariably produced aggression. Thus no further evidence for this postulate has been forthcoming, though it is pertinent to point out that most juxtaposed anemones taken from the shore will almost certainly have been in contact with each other for much longer periods than those experienced during the contests described here. Presumably some mechanism other than habituation accounts for the occasional reports of non-aggression in inter-specific encounters (e.g., Francis, 1973; Williams, 1978).

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