Selective cannibalism in the rotifer *Asplanchna sieboldi:* Contact recognition of morphotype and clone

(polymorphism/predator–prey interaction)

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ABSTRACT Populations of *A. sieboldi* reproduce primarily by diploid female parthenogenesis. Females may exhibit a pronounced, nongenetic polymorphism which is controlled by the diet. The cruciform and especially the campanulate morphotypes are larger than the saccate morphotype and are cannibalistic. Direct observations of various predator–prey interactions between the different morphotypes of two taxonomically distinct clones show that the feeding responses of cannibals after actual prey contact are predictable and extremely selective. Campanulates from clone B respond regularly to saccate but only rarely to cruciform or campanulate clonemates; the same predators typically respond to cruciforms and campanulates from clone C. Cruciforms from clone C rarely respond to saccate and cruciform clonemates but readily attack all morphotypes from clone B. In contrast, campanulates from clone C show no selectivity. Morphotype- and clone-specific recognition responses, which are mediated by coronal contact chemoreceptors, permit efficient prey discrimination and protection without involving prey handling or development of energy-demanding, defensive structures, such as the body-wall outgrowths of the cruciform morphotype. Selective feeding of cannibals may increase the fitness of a clone, both by effecting a more adaptive distribution of its morphotypes and by enhancing its ability to compete directly and indirectly with co-occurring clones. The magnitude of cannibalism in this rotifer may be dependent upon a complex suite of heterogeneous predator–prey interactions and greatly affected by shifting densities and distributions of different clones and female morphotypes.

Asplanchna sieboldi is a large, planktonic, ovoviviparous, and omnivorous rotifer. Populations reproduce primarily by diploid, female parthenogenesis. The females exhibit a pronounced, nongenetic polymorphism and may be saccate, cruciform, or campanulate in shape (Fig. 1). These morphotypes and the dietary factors responsible for their induction and maintenance have been described in detail (1–4).

Cruciform and, especially, campanulate females are much larger than saccates and are cannibalistic (1, 3, 5). This report shows that the cannibalism in these rotifers may be extraordinarily selective. Some cannibals rarely respond to contact with clonemates of their own or another morphotype but readily attack females of comparable morphotypes from another clone. The character and selectivity of such adaptive responses appear to be unique among predatory zooplankton and demonstrate that small animals—even those relatively low on the phylogenetic scale—may have unsuspected capabilities for prey recognition and for the control of feeding behavior.

MATERIALS AND METHODS

The various morphotypes of *A. sieboldi* were induced from two clones isolated in 1967 by C. W. Birky, Jr. from a sewage disposal plant in Davis, California—clones 12C1 and 10C6, hereafter termed clones B and C, respectively. These clones are different in several respects (3) and probably belong to separate races and perhaps even species. Clone C rotifers have all the characteristics of forms called *A. sieboldi;* clone B females,

however, lack a tooth on the scapus of the jaw and appear to be similar to forms that have been called *A. intermedia.* This species has either been considered synonymous with *A. sieboldi* or included with it in all ill-defined species or supraspecies (6). Clones B and C will mate with each other (Gilbert, unpublished), and reciprocal cross-fertilizations result in the production of resting eggs that have a normal appearance but whose viability is unknown (C. W. Birky, Jr., unpublished).

Saccate females were cultured on *Paramecium aurelia* (1, 3, 6). Cruciforms of both clones were initially induced by culturing saccates with 10^{-7} M emulsified d-α-tocopherol. Large cruciforms from clone C and campanulates from both clones were maintained on *Asplanchna brightwelli* with 10^{-7} or 10^{-8} M α-tocopherol (3, 5). Clone B cruciforms have relatively slight body-wall outgrowths and produce campanulate offspring when transferred to an *Asplanchna* diet (3). In clone C the potential for campanulate transformation is rare (3), and only several campanulates were found in cultures of *Asplanchna*-fed cruciforms; from these individuals a line was initiated. Cultures with tocopherol contained 300 μg/ml of penicillin G to reduce bacterial growth and tocopherol degradation. All cultures were changed daily.

In all feeding experiments the *A. sieboldi* predators were gravid, adult cruciforms or campanulates isolated from mass cultures, washed, and then starved in food-free medium for 2–6 hr at 25 or 28°. The *A. sieboldi* prey were: (*a*) gravid, adult saccates removed from mass cultures and (*b*) unfed, newborn cruciforms and campanulates selected from dishes containing parents isolated in food-free medium.

Feeding experiments were conducted by placing a single predator into a vessel with prey and watching, at 18 magnifications with a Wild M-5 stereomicroscope, the behavior of the predator after its corona (head) made a direct contact with a prey organism. The vessels used were either 1-ml capacity wells of glass depression slides or 5-ml capacity, 35 mm × 10 mm, plastic petri dishes. When the two types of prey used in an experiment were easily distinguishable by the observer, both types were placed in the same vessel; otherwise, two separate vessels were used. Predators were observed while making variable numbers of contacts and were discarded as soon as a prey item was ingested. In mixed prey populations with prey likely to be attacked and ingested, the prey type to which the predator was least responsive was usually made relatively abundant; in this way, each predator would be more likely to make a number of contacts before ingesting a prey and thus to demonstrate the degree to which the more avoided prey lacked the ability to induce a feeding response. Similarly, when two types of prey were tested in succession, the prey type that induced the most feeding responses or that was readily ingested was offered last. All of the predators used were responsive to contact with at least one of the prey types. Some of the neonate prey from the cannibalistic morphotypes exhibited feeding responses when they contacted the predator; such responses were not recorded.
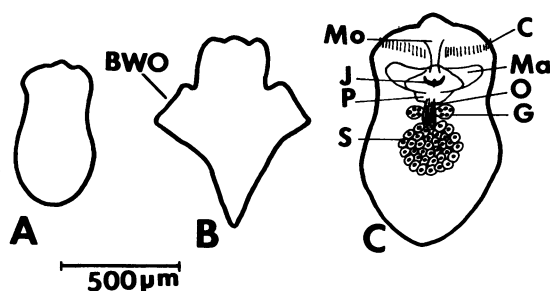
FIG. 1. Sketch of the saccate (A), cruciform (B), and campanulate (C) morphotypes of *A. sieboldi* females (dorsoventral aspect) showing the lateral body-wall outgrowths (BWO) of the cruciform morphotype and the digestive system of the campanulate morphotype: corona (C), mouth (Mo), mastax (Ma), jaws (J), pharynx (P), esophagus (O), gastric glands (G), and blind stomach (S).

Predator feeding behavior was subdivided according to a hierarchical scheme of four progressive alternatives: no response to prey, response to prey, capture of prey, and ingestion of prey. The predators, which swim randomly and show no ability to orient towards nearby prey, may or may not respond to a prey that they contact with their coronae. A response involves directed coronal movements, opening of the mouth, and frequent working of the pincer-like jaws. All of these motions serve to capture the prey in the pharynx. Many of the prey used in the experiments elicited very strong responses but were too large to be captured. Ingestion is facilitated by the jaws, which can push the prey down the esophagus towards the blind stomach. The digestive system of *Asplanchna* is sketched in Fig. 1.

The behavior of predators to two prey types was compared using row-by-column tests of independence and *G*-tests with Yates' corrections (7). Probabilities for *G*-values were calculated from the chi-square distribution using a program (8) for the HP-65 calculator.

EXPERIMENTS: RESULTS AND DISCUSSION

Morphotype Recognition. In a set of three experiments, 28 clone B campanulate predators (about 1000 μm in length) were placed in vessels containing both saccate adults and campanulate neonates from clone B. The age difference of the two prey types resulted from an attempt to have the two food items of a comparable size. However, the young campanulates (about 750 μm in length) were still larger than the adult saccates (about 600 μm in length). The results are presented in Table 1. Of the 68 contacts with saccate adults, 57 or 0.838 resulted in feeding responses; of the 136 contacts with campanulate neonates, only six or 0.044 resulted in feeding responses. This difference is highly significant (G = 138.2, $P < 1 \times 10^{-9}$). It is clear that adult campanulates regularly attack adult saccate clonemates but rarely attack newborn campanulate clonemates.

Of the 57 saccate prey that were attacked, 29 or 0.509 were captured; 28 or 0.996 of those captured were swallowed. None

of the six campanulate prey attacked was captured; this failure may have been due to large prey size or possibly weak feeding responses.

The effect of the age of clone B saccate prey on the feeding responses of clone B campanulate predators was studied by placing 14 campanulate adults singly into a vessel with both neonate and adult saccates. Of the 15 neonates contacted, 14 elicited feeding responses and were swallowed. Of the seven adults contacted, all seven elicited responses but only three were captured and swallowed. It is clear that newborn as well as adult saccates trigger feeding responses and, therefore, that the failure of the campanulate predators to respond to neonates of their own morphotype cannot be attributed to the young age of these prey.

The presence of α-tocopherol and possibly penicillin in the newborn campanulates also cannot account for their failure to elicit feeding responses. An experiment using 10 clone B campanulate predators and *A. brightwelli* prey from a population cultured with α-tocopherol and penicillin for several generations showed that feeding responses occurred after each prey contact and that almost all of these responses resulted in prey capture and ingestion.

Clone Recognition. In one set of experiments, 25 clone B campanulate predators were presented with: (*a*) clone C cruciform neonates (about 750 μm in length) and (*b*) clone B cruciform and campanulate neonates (about 750 μm in length). The results are shown in Table 2. In Exps. 1 and 2, the predators were placed in a vessel with clone B cruciform and campanulate prey and then in one with clone C cruciform prey. The clone B cruciforms in these experiments were actually cruciform-campanulate intermediates and possessed much less than maximal body-wall outgrowths. The 13 campanulate predators responded to only 11 out of 88 (0.125) contacts with prey from their own clone but 49 out of 53 (0.925) contacts with those from clone C. This difference is highly significant (G = 93.29, $P = 2 \times 10^{-9}$). The predators seemed to respond no differently to the cruciform and campanulate neonates of their own clone, but too few observations were made to resolve this question further. A separate experiment showed that clone B campanulate predators rarely responded to cruciform clonemates that had maximal body-wall outgrowths for the clone; four very responsive predators, which had been starved for 9 hr, responded to only six out of 34 (0.176) contacts with neonates of such cruciforms. Two of these responses resulted in prey capture and ingestion.

In Exps. 3 and 4, the clone B campanulate predators were placed in a mixture of clone B campanulate and clone C cruciform neonates. The 12 predators responded to only 13 out of 84 (0.155) contacts with neonates from their own clone and morphotype but 96 out of 101 (0.950) contacts with clone C cruciform neonates. This difference is highly significant (G = 133.77, $P < 1 \times 10^{-9}$). The results from all of the experiments in this set show that clone B campanulates rarely exhibited a

Table 1. Feeding behavior of starved, adult, clone B campanulate predators in a mixture of clone B saccate adults and campanulate neonates

Behavior of predator after prey contact	Prey							
	Clone B saccate adults				Clone B campanulate neonates			
	Exp. 1	Exp. 2	Exp. 3	Total	Exp. 1	Exp. 2	Exp. 3	Total
Response	34	11	12	57	3	1	2	6
No response	9	2	0	11	71	5	54	130
No. of contacts	43	13	12	68	74	6	56	136
No. of predators	11	6	11	28	11	6	11	28

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Table 2. Feeding behavior of starved, adult, clone B campanulate predators presented with clone B cruciform and campanulate neonates and clone C cruciform neonates

| Behavior of predator after prey contact | Neonate prey | | | | | | | | | |
| | Clone B cruciforms and/or campanulates | | | | | Clone C cruciforms | | | | |
	Exp. 1*	Exp. 2*	Exp. 3†	Exp. 4†	Total	Exp. 1*	Exp. 2*	Exp. 3†	Exp. 4†	Total
Response	0	11	13	0	24	15	34	44	52	145‡
No response	25	52	42	29	148	0	4	4	1	9
No. of contacts	25	63	55	29	172	15	38	48	53	154
No. of predators	5	8	7	5	25	5	8	7	5	25

* Predators placed first in vessel with clone B cruciform and campanulate neonates.
† Predators placed in vessel with both clone B campanulate and clone C cruciform neonates.
‡ One of these responses led to prey capture and ingestion.

feeding response after contact with either a cruciform or a campanulate individual from their own clone, but almost always did so after contacting a clone C cruciform.

In another set of experiments, 12 large (about 1100 μm in length) clone C cruciform predators from a population raised on *A. brightwelli* were placed in a mixture of clone B campanulate and clone C cruciform neonates. The results are shown in Table 3. The predators responded to only three out of 130 (0.023) contacts with cruciforms from their own clone but 148 out of 155 (0.955) contacts with campanulates from the other clone. This difference is highly significant ($G = 301.81, P < 1 \times 10^{-9}$). Thus, the clone C cruciform predators behaved just like the clone B campanulate predators, rarely responding to contacts with individuals of their own morphotype and clone.

In the next set of experiments, 29 clone C cruciform predators (about 1000 μm in length) were tested with saccate adults (about 600 μm in length) from clones B and C. The results are presented in Table 4. The predators responded to 148 out of 172 (0.860) contacts with clone B saccates but only 41 out of 201 (0.204) contacts with saccate clonemates. This difference is highly significant ($G = 171.48, P < 1 \times 10^{-9}$) and shows that clone C saccates, just like clone C cruciforms, are protected against attack by cruciform clonemates.

In the final sets of experiments, comparisons were made between the responses of campanulate predators from both clones to contacts with neonate, campanulate prey from both clones. The results with the clone B predators are presented in Table 5. The 20 predators responded to only 10 out of 151 (0.067) contacts with the prey from their own clone but 55 out of 68 (0.809) contacts with those from clone C. This difference is highly significant ($G = 122.35, P < 1 \times 10^{-9}$). Clearly, clone B campanulates rarely responded to neonates of their own

Table 3. Feeding behavior of starved, adult, clone C cruciform predators in a mixture of clone B campanulate and clone C cruciform neonates

| Behavior of predator after prey contact | Neonate prey | | | | | |
| | Clone B campanulates | | | Clone C cruciforms | | |
	Exp. 1	Exp. 2	Total	Exp. 1	Exp. 2	Total
Response	105	43	148	2	1	3
No response	3	4	7	89	38	127
No. of contacts	108	47	155	91	39	130
No. of predators	7	5	12	7	5	12

morphotype and clone, as described earlier (Tables 1 and 2), but generally did respond to campanulate neonates from clone C. These experiments also show that newborn campanulates are large enough to avoid being eaten by adult campanulates; only two out of a total of 65 responses led to prey capture and ingestion.

Campanulate predators from clone C (about 1500 μm in length) behaved very differently from those of clone B. They attacked both cruciform and campanulate neonates from their own clone. In a set of three experiments, 16 predators responded to 83 out of 86 (0.965) contacts with newborn campanulate clonemates. Only three of these responses led to prey capture, again demonstrating the effectiveness of the large birth size of campanulates in preventing mortality from cannibalism. In another experiment, two clone C campanulate predators responded 16 times to as many contacts with newborn cruciform clonemates. The body-wall outgrowths of these prey were seen to be extremely effective in preventing predators from trapping them; none of the 16 responses led to prey capture.

GENERAL DISCUSSION

The results of these experiments demonstrate that the cruciform and campanulate morphotypes of *A. sieboldi* may be extremely selective cannibals. They exhibit very strong feeding responses after their coronae contact some types of females but fail to respond following contact with others. They clearly have the ability to recognize very quickly what would seem to be subtle differences in the surface characteristics of different morphotypes and clones. A summary of the predator–prey interactions investigated in this study is presented in Table 6.

While clone B campanulate predators will definitely respond to saccate clonemates (Table 1), they rarely respond to similarly sized cruciform or campanulate clonemates (Tables 1, 2, and 5 and *text*). This highly selective feeding behavior shows that in this clone saccate females have different surface properties from cruciform and campanulate females and that these differences somehow are recognized by campanulate predators. Further experiments demonstrated that the morphotype-recognition response of clone B campanulate predators is clone-specific, because these predators readily respond to contact with both cruciform and campanulate neonates from clone C (Tables 2 and 5). Two similar, clone-specific feeding responses were exhibited by clone C cruciform predators, which rarely attack saccate and, especially, cruciform clonemates (Tables 3 and 4). Clone C campanulate predators, however, did not show any discrimination between prey of clone C and B.

The precise basis for the morphotype- and clone-recognition responses is not known. Failure of a predator to respond to contact with a conspecific prey may be due either to the absence of a required releasing factor or to the presence of an inhibitor

Table 4.   Feeding behavior of starved, adult, clone C cruciform predators presented
with adult saccates from both clones C and B

| Behavior of predator after prey contact | Adult saccate prey | | | | | | | |
| | Clone C | | | | Clone B | | | |
	Exp. 1	Exp. 2	Exp. 3	Total	Exp. 1	Exp. 2	Exp. 3	Total
Response	11	18	12	41	48	62	38	148
No response	52	61	47	160	9	8	7	24
No. of contacts	63	79	59	201	57	70	45	172
No. of predators	9	11	9	29	9	11	9	29

on the prey surface. The general stimulus for a feeding response, however, has been shown to be chemical; filtrates of homogenized clone B saccates regularly induce definite feeding responses in starved clone B campanulates (Gilbert, unpublished). There are many sensory organs on the corona of *Asplanchna* (9, 10), but it is not known which ones are involved in prey recognition. The ability of *Asplanchna* to recognize prey characteristics upon initial contact must be adaptive, because it permits prey discrimination without prey handling.

There are several other reports of the ability of coronal contact receptors in rotifers to recognize very specific stimuli. Gilbert (11) found that *Brachionus* males exhibited mating behavior only when their coronae contacted the surfaces of conspecific females. The stimulus was shown to be chemical. Wallace (Ph.D. dissertation, Dartmouth College, 1975, 274 pp.) observed that larvae of the sessile rotifer *Ptygura beauchampi* would settle and metamorphose only on one of four co-occurring species of the carnivorous macrophyte *Utricularia*. Furthermore, only the trap-door regions of one of the three trap-types on this species (*U. vulgaris*) were colonized. The active factor inducing settling was associated with glandular trichomes and is probably chemical. Recognition of this factor by the larvae required coronal contact (12).

The ability of *Asplanchna* to feed selectively has been reported by other investigators, but much of the evidence is indirect and does not permit a distinction between an active selection, in which certain potentially ingestible food types are avoided or rejected, or a passive selection, in which the food types most readily eaten are simply those most easily captured and ingested. For example, examination of the stomach contents of *Asplanchna* by Erman (13) and Ejsmont-Karabin (14) showed that animal food is ingested to a greater extent than algal food. However, some of this difference in electivity may only reflect the greater ability of *Asplanchna* to capture relatively large food items, such as animal prey.

Some direct evidence for selective feeding in *Asplanchna* was provided by Pourriot (15), who found that *A. brightwelli*

would reject small benthic rotifers, such as *Lepadella* and *Lecane*, and the empty loricae (shells) of *Brachionus*. It was not clear from his brief comments whether such rejection followed an initial, tactile feeding response. Active selection has also been observed by Gilbert (unpublished), who found that *A. sieboldi* campanulates (clone B) responded less frequently to contacts with the colonial alga *Volvox aureus* than they did to those with similarly sized, saccate clonemates.

The selective cannibalism of *A. sieboldi* described in this report is one of the most highly specialized cases of selective feeding known in any rotifer or member of the zooplankton. It remains to be seen whether this rotifer exhibits great selectivity with regard to other types of prey. Its very diverse diet (13, 14, 16) suggests that it is not an especially selective species and that the discrimination in cannibalism may be a unique adaptation to protect the rotifers from members of their own population.

The selective feeding of *A. sieboldi* cannibals confers a kind of self-immunity on certain individuals of the clone and represents the third type of defense against cannibalism known in this rotifer. Two other mechanisms have been considered previously—the body-wall outgrowths of the cruciform morphotype (16) and the large birth size of the campanulate morphotype (5); both of these were shown to be effective in this study. Selective feeding responses probably are an energetically more efficient way to prevent cannibalism than developing protective structures. However, unlike morphological devices, they may only protect individuals from cannibals of the same or similar clones. Many clones from a variety of races must be tested before this aspect of the subject can be elucidated.

The morphotype-specific cannibalism exhibited by clone B campanulates would protect young clonemates of both the cruciform morphotype, which is associated with sexual reproduction and resting egg production (3), and the campanulate morphotype, which is induced by and probably designed for ingesting large food items, including conspecifics (1, 3, 5). Thus, the efficiency of the sexual process in the clone and the ability

Table 5.   Feeding behavior of starved, adult, clone B campanulate predators presented
with campanulate neonates from both clones B and C

| Behavior of predator after prey contact | Neonate prey | | | | | | | |
| | Clone B campanulates | | | | Clone C campanulates | | | |
	Exp. 1	Exp. 2	Exp. 3	Total	Exp. 1	Exp. 2	Exp. 3	Total
Response	0	8*	2	10	19	26*	10	55
No response	36	63	42	141	5	7	1	13
No. of contacts	36	71	44	151	24	33	11	68
No. of predators	5	9	6	20	5	9	6	20

* One of these responses led to prey capture and ingestion.

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Table 6.   Summary of predator-prey interactions within and between clones B and C of *Asplanchna sieboldi*.
Feeding responses of starved predators after contact with prey are given as attack probabilities*

Predator	Prey					
	Clone B saccates	Clone B cruciforms	Clone B campanulates	Clone C saccates	Clone C cruciforms	Clone C campanulates
Clone B campanulates	0.84	0.18	0.08	+	0.94	0.81
Clone C cruciforms	0.86	+	0.96	0.20	0.02	
Clone C campanulates	+	+	+	+	1.00	0.97

*The (+) sign indicates an unquantified but very high attack probability as judged from direct observations (Gilbert, unpublished).

of the clone to utilize large prey might be compromised less by a preferential feeding of campanulates on saccates.

Even in the absence of a morphotype-specific feeding response, the cruciform and campanulate morphotypes of clone B would be protected from cannibalistic clonemates by their body-wall outgrowths and large birth size, respectively. However, body-wall outgrowths are much less pronounced in clone B than in clone C cruciforms, and so the failure of campanulates to respond to such cruciforms may be especially adaptive. During evolutionary time, it is conceivable that the capability of a rapid transformation between saccate and campanulate morphotypes in clone B became associated with reduced body-wall outgrowths in the intermediate, cruciform morphotype and that there was subsequent selective pressure for the development of a defense mechanism based on a morphotype-specific recognition response. Clone C campanulates exhibit no morphotype-specific feeding responses and respond to contact with both cruciform and campanulate clonemates. However, the extreme development of body-wall outgrowths in clone C cruciforms, which are stable and self-reproducing, probably gives them complete protection from such predation.

There are several possible reasons why an immunity of saccates to campanulate predation may not have evolved in clone B. First, if a population were comprised primarily of one clone, campanulates that did not eat saccate clonemates might be less able to survive in the absence of alternative prey. Second, all saccates from this clone respond quite similarly to conditions inducing the cruciform and campanulate morphotypes (3); and so, as W. C. Kerfoot (personal communication) has suggested, the co-occurrence of saccate and campanulate clonemates might be rare. Hence, there may never have been sufficient selective pressure for the evolution of a defense mechanism. On the other hand, cruciforms and campanulates co-occur under a variety of dietary conditions (3).

Clone-specific feeding responses, such as those exhibited by clone B campanulate and clone C cruciform predators, are obviously adaptive. They would minimize intraclonal mortality, and they would increase the competitive ability of the clone by directly interfering with (cannibalizing) other competing clones. Natural populations of cannibalistic *A. sieboldi* probably consist of more than one clone, and so the reproductive success of each clone would be increased by a mechanism preventing feeding responses from being released after contact with a clonemate.

The experiments described in this study probably underestimate the specificity of cannibalism in natural populations. All of the predatory individuals observed were starved for relatively long periods of time and hence may have exhibited less than normal selectivity. Selectivity would vary inversely with the degree of satiation, and the morphotype- and clone-specific feeding responses reported here probably break down in very starved predators. In fact, some of the deviations from morphotype- and clone-specific feeding responses observed in the different experiments may have been the result of extreme starvation.

In conclusion, it is clear that cannibalism in *A. sieboldi* is extremely complex. Analysis of some of the predator-prey interactions between the morphotypes of two distinct clones demonstrates that various types of predators may respond very differently to contact with prey of different morphotypes and clones. Superimposed on this heterogeneous feeding behavior are sometimes dramatic differences in the sizes and shapes of predator and prey. Therefore, the ability of cannibals to utilize conspecific prey in a population probably varies tremendously depending on the clones and morphotypes present. The dynamics of cannibalism in other species may also be more complicated than suspected. Even if such species exhibit little or no polymorphism, specific recognition and feeding responses may have evolved to protect organisms from being cannibalized by members of their own clone, population, or race.

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