Reciprocation, Reproductive Success, and Safeguards Against Cheating in a Hermaphroditic Polychaete Worm, *Ophryotrocha diadema* Åkesson, 1976

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Abstract. *Ophryotrocha diadema,* a simultaneous hermaphroditic polychaete worm, forms pairs in which both partners regularly alternate sex roles and trade eggs. Since *O. diadema* has a protandrous phase, safeguards against cheating by a non-reciprocating partner, either male or hermaphrodite, have evolved. Results of a mate choice experiment indicate that protandrous males are generally discarded as mates because they are unable to reciprocate with eggs. Reproductive success (measured by estimating the mean number of egg masses per individual per day) of hermaphrodites paired with males was significantly lower than the reproductive success of hermaphrodites paired with hermaphrodites. This indicates that *O. diadema* is able to time spawning activity according to the sexual condition of its partner. On the other hand, oogenesis and the production of multiple batches of mature oocytes is independent of the presence of a partner. Worms did not discard mates with substantially fewer eggs. The small size of clutches and the short interval between successive spawnings could be considered a form of egg parcelling, which would prevent exploitation of hermaphroditic individuals by partners unable to reciprocate.

Introduction

Ophryotrocha diadema Åkesson, 1976, is a small (4 mm long) simultaneous hermaphrodite which was discovered in 1976 by Reish and Åkesson among the fouling fauna in Los Angeles Harbor. Larvae are released from the egg case at a body length of 4 setigerous segments. Its sexual life begins with a protandrous phase, which lasts two to three weeks. The simultaneous her-

maphroditic phase begins at the fourth week of life, at a body length of 14 setigers. Sperm are produced throughout life in the third and fourth setigers only, and eggs are produced from the fifth setiger onwards. Fecundity is age-dependent (Åkesson, 1976). In the first week of reproduction, cocoons contain 15–17 eggs. Maximum reproductive output (30–40 eggs/day) occurs four weeks after spawning begins and then slowly declines (Åkesson, 1982). Cocoons are released at intervals of about three days.

The main features of the mating system of *O. diadema* were described by Sella (1985) and can be summarized as follows:

(1) Pairs are formed preferentially between simultaneous hermaphrodites. Spawning synchronization is achieved by means of close mutual contact during a courtship lasting several hours.

(2) Partners repeatedly alternate sex roles with the same partner at intervals of about 30 hours. As one partner releases its eggs, the other fertilizes them. This alternate egg laying has been defined as egg trading by Fischer (1980) and Maynard Smith (1982, p. 160).

(3) Both partners care for the developing embryos.

This paper addresses the following question: does the mating system of *O. diadema* represent an evolutionarily stable strategy (ESS), *sensu* Maynard Smith (1982, p. 12) and Parker (1978), *i.e.,* a strategy such that, if all the members of a population adopt it, no mutant strategy is likely to invade it under the influence of natural selection? In the case of *O. diadema,* the mutant strategy would be reproducing only as a male. *O. diadema* eggs require greater nutritive resources than sperm (Sella, in prep.), so reproducing only as a female would probably be selected against.

A mating system involving reciprocity would not be stable if safeguards against non-reciprocating individuals had not evolved. Two partners have a common interest in reciprocating but there is a potential advantage in non-reciprocation. Since eggs require greater nutritive resources than sperm, a hermaphrodite could increase its fitness by fertilizing the eggs of other individuals on days when it has no mature eggs to give. For reciprocal spawning to be critical to reproductive success, there should be strong constraints on reproducing only as a male in both the protandrous and hermaphroditic phases. Therefore, reproductive success is expected to be significantly lower than average for those individuals that fail to reciprocate egg exchange. In *O. diadema* at each spawning, ovaries release all their mature eggs. Therefore, in order not to be a loser in egg trading, a hermaphrodite is expected to have evolved an ability to perceive how many eggs its partner has and, eventually, to discard a partner with substantially fewer eggs than it has.

To assess the evolutionary stability of the mating system of *O. diadema* I estimated the probability that subadults will succeed in mating with hermaphrodites, compared the reproductive success of a hermaphrodite mated to a male with that of a hermaphrodite paired to another hermaphrodite, and investigated whether worms can perceive egg loads of their partners and discard partners having fewer stored eggs.

Materials and Methods

Animals used in experiments were taken from laboratory populations originating from specimens of *O. diadema* collected in Los Angeles Harbor (Åkesson, 1976). Populations were reared according to the methods described by Åkesson (1976) and Sella (1985). They were placed in filtered sea water with a salinity of 34‰ at a constant temperature of 20°C and fed parboiled spinach.

A genetically determined yellow or white coloration of eggs permitted identification of the partners in a pair since both eggs and embryos are colored according to the mother's phenotype (Sella and Marzona, 1983). Therefore, color of eggs was used to infer which partner spawned eggs or fertilized them in a white and yellow worm pair. No difference in the mean number of eggs per cocoon was observed between yellow and white individuals (Åkesson, 1976; Sella, unpub.).

In all experiments, only virgin individuals of the same age were used, since both fecundity and fertility are age dependent in *O. diadema* (Åkesson, 1982). To obtain virgin individuals, individual males were reared in separate containers until they reached the length (14–15 setigers) at which oocyte production begins. Only when their first oocyte batch was mature, were they used in experiments. Therefore, the term "virgin worm" refers

to a simultaneous hermaphrodite which is ready to spawn its first batch of eggs. Since body walls are transparent and ripe oocytes measure approximately 180 × 200 μm, oocyte growth (as well as embryo development) could be easily observed with a stereomicroscope at low magnification.

Although "reproductive success" generally is defined as the number of progeny that survive to reproduce, this number is often impossible to measure. When pairs are formed between two simultaneous hermaphrodites, approximately 95% of the eggs are fertilized and develop (Sella, in prep.). The mean number of eggs per cocoon per week has a standard error never greater than 1 (Åkesson, 1976). Therefore, if individuals of the same age are paired, reproductive success can be indirectly estimated by counting the cocoons released in a given time interval. The term "young male" refers to subadults 7–8 setigers long which have not yet reached the simultaneous hermaphroditic phase and release only sperm.

The following three experiments were conducted.

Mate selection

To study the intensity of sexual selection against males, 44 bowls each containing a yellow virgin hermaphrodite, a white virgin hermaphrodite, and a male six setigers long, were set up. Each hermaphrodite could choose to pair either with the male or with the other hermaphrodite. Animals were observed until a pair was formed in each bowl and courtship terminated with egg spawning by one of the two partners.

Reproductive success and failure to reciprocate

If reciprocal spawning is critical to mating success, reproductive success of a hermaphrodite mated to a young male is expected to be significantly lower than that of a simultaneous hermaphrodite paired with another simultaneous hermaphrodite. The reproductive success (number of cocoons spawned in 10 days) of a set of 72 pairs of yellow and white hermaphrodites was compared to a set of 72 pairs of young males and either yellow or white simultaneous hermaphrodites. In the latter situation, the simultaneous hermaphrodites can act only as females, since alternation of sex roles and reciprocation of fertilization is precluded. Each pair was kept in a separate container.

Ability to perceive partner's eggs load

There are two ovaries per setiger in *O. diadema*. Each ovary produces one or two mature oocytes at a time. Eggs are spawned only if a partner able to fertilize them is present. Isolated individuals accumulate ripe eggs in the coelomic cavity because they do not spawn. The experi-

Table I

*Reproductive success (mean number of egg masses per individual) in
10-day interval (A) and mean day interval between two successive
spawnings (B) of simultaneous hermaphrodites paired with a
simultaneous hermaphrodite or with a young male*

		(A)		(B)
Kind of pairs	N_A	$\bar{x}$ egg masses/ individual	N_B	$\bar{x}$ days between successive spawnings
♀×♀	144	2.1 ± 0.02	54	2.97 ± 0.20
♀×♂	72	1.8 ± 0.07	34	5.24 ± 0.26

(A) One-way ANOVA, $F_{1,142} = 7.58; 0.005 < P < 0.01$

(B) One-way ANOVA, $F_{1,86} = 6.26; 0.02 < P < 0.01$

N_A = number of sampled hermaphrodites; N_B = number of individuals from sample N_A which spawned twice during the experiment.

ment takes advantage of these characteristics to determine if an individual is able to perceive the number of eggs held by its partner and whether it can modulate its spawning behavior accordingly. Worms are expected to discard potential partners with fewer stored eggs.

Three equal groups of hermaphrodites with a different number of eggs in their coelomic cavity were allowed to choose their partner according to the number of mature eggs it had. The experiment lasted 124 hours. To obtain virgin individuals with different numbers of ripe oocytes, 3 groups of 100 virgin worms were isolated in separate containers for: (A) two weeks, (B) one week, and (C) one day, respectively, from the day the first ripe oocytes were visible through their body walls.

The experimental set up for matching individuals from groups A, B, and C was the following: in each of 50 Petri dishes filled with 30 ml of seawater, 6 individuals (2 from each group) were put together. A cellulose triacetate disc of the same size as the dish, printed with a millimeter grid, was placed beneath each dish to record the location of each pair upon its formation.

Results

Mate selection

Pairs between males and hermaphrodites formed in 14 bowls (32%) and pairs between hermaphrodites formed in 30 bowls (68%) out of 44. These values are significantly different than those expected (29.2 and 14.6) if mating choice had been random. (G test = 39.54; $P < 0.001$).

When hermaphrodites could choose between a male and another hermaphrodite, they preferred to mate with the hermaphrodite, *i.e.*, with a partner able to reciprocate eggs.

Reproductive success and failure to reciprocate

The mean number of egg masses each partner spawned in the set of simultaneous hermaphrodite pairs was significantly greater than the mean number each hermaphrodite spawned in the set of pairs between hermaphrodites and young males (Table I). Moreover, the mean number of days between successive spawnings of a simultaneous hermaphrodite mated to another hermaphrodite is significantly fewer than the mean interval between successive spawnings of an adult mated with young males (one-way ANOVA on data transformed in a $\sqrt{x + 0.5}$ scale) (Table I).

Therefore, the reproductive success of a simultaneous hermaphrodite is significantly influenced by the sexual phenotype (either male or hermaphrodite) of its partner. The number of spawned cocoons by a simultaneous hermaphrodite decreases when it cannot reciprocate because it has a young male as a partner.

Ability to perceive partner's egg load

Isolation treatment led to a significantly different number of ripe eggs accumulated in the coelomic cavity of worms from groups A, B, and C. (Table II). *A posteriori* comparisons among mean numbers of eggs (SS-STP test, Sokal and Rohlf, 1981, p. 245) from individuals which spawned eggs in groups A, B, and C are highly significant (Table II), thus indicating that egg accumulation in the coelomic cavity is significantly affected by the length of the isolation period.

The first pairs formed at the end of the first day after the beginning of the experiment and the first egg spawnings were observed during the second day.

Although the aim of this experiment was not to study reciprocation, it is worth observing that at the end of the experiment 67 of 110 pairs had reciprocated and that the

TABLE II

*Mean number of eggs per cocoon laid by individuals isolated
for 2 weeks (A), 1 week (B), and 1 day (C),
after the first maturation of oocytes*

	A	B	C
$\bar{x} \pm$ S.E.	32.35 ± 1.51	24.46 ± 1.42	17.54 ± 1.03
N	60	53	46

One-way ANOVA, $F_{2,149} = 25.34; p < 0.001$
A posteriori comparisons among means, (SS-STP test):

comparisons	SS	
A *versus* B	1362.264	significant
B *versus* C	1227.5642	significant
C *versus* A	4986.718	significant
critical SS	591.43	

Table III

Frequency of pairs formed between individuals belonging to groups A, B, and C, in 6 days

Kinds of pairs	A × A	A × B	A × C	B × C	B × B	C × C	total
observed	9	33	33	30	3	2	110
expected	12.2	24.4	24.4	24.4	12.2	12.2	110

G-test, $G = 31.12$, $P < 0.01$

Numbers of individuals, out of 100, involved in pair formation either as males or as females from

group A = 84
group B = 69
group C = 67

Heterogeneity G-test, $G_2 = 9.3$, $P = 0.01$.

proportions of individuals acting only as fertilizers was not significantly different in all three groups (heterogeneity G test, $G_2 = 1.19$). Most fertilizers of pairs formed in the last two days of the experiment did not have the opportunity to reciprocate by spawning.

Expectations about pair formation were not fulfilled by the experimental data (Table III). Animals of group A, with the greatest number of oocytes, became involved in pair formation in a significantly greater number than animals of groups B and C, but they did not pair more frequently among themselves than with worms with a lower number of oocytes belonging to groups B and C. During the experiment no individual paired twice.

Pairing was not random. An excess of pairs were formed by individuals belonging to different groups and a deficiency of pairs were formed by individuals belonging to the same group compared with frequencies expected under the hypothesis of random mating.

Worms released all the eggs they currently had without parcelling them, irrespective of how many eggs their partners had, thus indicating that *O. diadema* does not select its mate by number of eggs.

Discussion

Simultaneous hermaphroditism with juvenile protandry would be an evolutionarily unstable reproductive strategy if males, competing for fertilization with hermaphrodites, had greater reproductive success than simultaneous hermaphrodites. This does not seem to happen in the mating system of *O. diadema* because there are strong constraints limiting reproductive success either as a non-reciprocating hermaphrodite or as a male. Hermaphrodites, which did not reciprocate because they mated with young males, laid fewer cocoons than reciprocating hermaphrodites. Males experience the following three selective constraints: (1) they are generally discarded as mates because they are unable to reciprocate; (2) if they are paired to a hermaphrodite, the number of egg masses laid by the hermaphrodite per day is less than

the number of egg masses laid by a hermaphrodite paired with another hermaphrodite; and (3) they do not fertilize eggs as efficiently as hermaphrodites (Sella, in prep.). Therefore, one would expect a very brief protandrous phase, or for such a phase to be completely absent in the early part of the life cycle of *O. diadema*, because of the selective constraints against it. Indeed, the protandrous phase lasts about ¼ of the most fertile period of the life of *O. diadema*. Testes are comparable in size to ovaries, but, since they are present only in the third and fourth setigers throughout life, investment in testicular tissue may be much less. The return of this investment in juveniles is small, because they do not have many opportunities to pair, compared to the return of the same investment during the hermaphroditic phase, where opportunities to fertilize eggs are regularly offered to reciprocating individuals.

Despite the constraints on reproducing only as a male, 32% of young males engaged in mating competition with hermaphrodites succeeded in pairing with hermaphrodites. In another mate choice experiment described by Sella (1985), males succeeded in being the first to pair with hermaphrodites in three out of sixteen bowls. The difference between frequencies of males paired with hermaphrodites in both experiments is not statistically significant (heterogeneity G test, $G_1 = 1.04$). The only apparent competitive advantage that young males might have over hermaphrodites is their greater mobility, which could help them in searching for and courting a partner if population density were low. Ghiselin (1974, p. 194) advanced a similar hypothesis to explain the existence of small males associated with hermaphrodites in many animal groups; when adults have a mode of life in which motility is restricted, small males, able to capitalize on their motility, tend to evolve. Hermaphrodites of *O. diadema* and adults of other *Ophryotrocha* species have a restricted motility since they spend much of their time in egg brooding. Ghiselin (1974, p. 210), Gould (1977, p. 330), and Charnov (1982, p. 250) observed that

selective pressures favoring progenesis in males concomitantly increase as population density decreases. A hermaphrodite living in a low density population would incur some metabolic cost in searching for a better mate, and, at a certain stage of oogenesis, might be hormonally programmed to mate with the first available mate. In general, interstitial fauna are present at low densities (Svedmark, 1964). Unfortunately, nothing is known about the population structure of *O. diadema* in the field, apart from the original observation in Los Angeles Harbor that only 2 individuals of *O. diadema* were collected with more than 300 individuals of *O. labronica pacifica*. Since then, no additional specimens have been found in the same locality (D. Reish, pers. comm.).

It is difficult not to expect some form of egg parcelling in *O. diadema*, since it would prevent exploitation of hermaphroditic individuals by partners with no eggs. Although the excess of matings between individuals with a different number of oocytes observed in the third experiment merits further investigation, the results indicate that in *O. diadema* each partner releases all of its ripe eggs. The higher frequency of pairing by group A individuals may be explained by the greater number of accumulated eggs compressing coelomic walls and causing release of all the eggs. Results of the second experiment indicate that there is, at least, a sort of temporal parcelling of cocoon production: (1) time intervals between acts of egg laying by a worm are briefer if immediately reciprocated by an egg laying partner; (2) conversely, longer intervals between acts of egg-laying occur in matings with partners that do not lay eggs. The small size of the clutches and the short intervals between them can be considered a form of egg parcelling. It is interesting to observe that in other simultaneous hermaphroditic species of *Ophryotrocha* [*i.e., O. hartmanni, O. maculata,* and *O. bacci* (Åkesson, 1973a, b)], egg masses contain only 20–40 eggs and in *O. gracilis* even fewer (Westheide, 1984). Intervals between successive spawnings in these species are not known. In contrast, in gonochoric and sequential hermaphroditic species of *Ophryotrocha,* egg masses contain a hundred eggs and are spawned only once every one or two weeks. Therefore, the previous doubt about the ability of *O. diadema* to parcel its clutches according to the reproductive behavior of its partner (Sella, 1985) needs to be corrected in light of these considerations.

In the simultaneous hermaphroditic coral reef fish *Hypoplectrus nigricans* a graded strategy of laying eggs in small batches, rather than all at once, has evolved to guard against cheating (Fischer, 1980). According to Maynard Smith (1982, p. 160), this form of egg parcelling can be regarded as a game of incomplete information, since each fish knows whether it has eggs to trade, but not if its partner has eggs. In *O. diadema,* sufficient

information on the sexual condition of conspecifics (males, simultaneous hermaphrodites, or hermaphrodites temporarily out of eggs) could be obtained through pheromonal signals. In *Ophryotrocha puerilis,* the existence of sex attractants emanating from females has been observed repeatedly (Pfannenstiel, 1977, 1984; Marchionni and Rolando, 1981; Franke and Pfannenstiel, 1984; Grothe and Pfannenstiel, 1986; Berglund, in prep.). In the gonochoric species *O. robusta* and *O. labronica* (Rolando, 1984), as well as in *O. macrovifera* (Sella, unpub. obs.), pheromones have been hypothetized as the most likely explanation for sexual induction phenomena.

As both Axelrod and Hamilton (1981) and Maynard Smith (1982, p. 169) observed, an ability to discriminate between other members of the same species may extend the range of stable cooperation. This discrimination allows individuals to handle interactions with many other conspecifics in different ways. In *O. diadema,* the ability to recognize the sexual condition of the partner, and to time spawning activity accordingly, could both greatly reduce the risk of being cheated and enhance the evolutionary stability of the mating system.

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