Life history and sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*: the role of sperm competition

M. Cristina Lorenzi,[1] Dáša Schleicherová and Gabriella Sella

Department of Animal and Human Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin, Italy

Synopsis Sex allocation theory predicts that, in hermaphroditic organisms, individuals allocate a fixed amount of resources divided among male and female functions to reproduction and that the proportion devoted to each sex depends on the mating group size. As the mating group size increases, hermaphrodites are predicted to allocate proportionally more resources to the male and less resources to the female function (approaching equal allocation to both sexes) to face increased sperm competition. Up to now little experimental evidence has been provided to support the theory in hermaphroditic animals. Facultative shift between male and female allocation in response to variation in local group size does occur in several taxa but not always in the expected direction and not with similar patterns. In the protandric and then simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema* reproductive resources are flexibly allocated in the protandrous and the hermaphroditic phase. The cost of male reproduction during adolescence is spread over the whole energy budget of the animal as shown by the shortening of lifespan and the lowering of growth rate in individuals with enhanced male expenditure during the protandrous phase. Moreover, in this species, short term sex allocation adjustments differ from those described in other taxa. Individuals regulate their reproductive output so that where reproductive competitors are present, the number of female gametes is strongly reduced but the number of male gametes (although it changes) is not significantly increased. Resources subtracted from the female function are not directly allocated to sperm production, but to expensive male behaviors that are likely to enhance male reproductive success. These results are discussed in the light of the relevance of sexual selection in large populations of hermaphrodites.

Introduction

In hermaphrodites, sex allocation theory predicts that individuals allocate to reproduction a fixed amount of resources divided among male and female functions and that the fraction devoted to each sex depends on the mating group size (Charnov 1982; Fischer 1984). In outcrossing hermaphrodites forming pairs, the mating group size is considered to be = 2. In this condition both partners are expected to allocate as few resources as possible to the male function. All remaining resources are expected to be devolved to egg production. To face ensuing sperm competition (Parker 1998), hermaphrodites are predicted to allocate proportionally more resources to the male function and fewer resources to the female function as mating group size increases.

Charnov (1982) and Fischer (1984) derived these theoretical predictions from the Hamilton's (1967) theory of Local Mate Competition (LMC). According to LMC theory, in separate sex organisms, when populations are structured in such a way that mating group sizes are small and related males compete for fertilization, females are expected to bias the sex ratio of their offspring towards daughters (that is, the sex which suffers less competition) and produce the minimum number of sons which can ensure fertilization of all their daughters. As the number of females in a patch increases, their progeny will mix. The LMC will diminish, additional sperm released by a male will be less likely to compete with sperm from related males and thus parental allocation to the number of sons can increase until it approaches a sex ratio of 0.5 (Hamilton 1967). Therefore, according to sex allocation and LMC theory, both gonochoric and hermaphroditic organisms should be able to adjust their sex allocation budget to the social conditions encountered.

While in several separate sex animals there is evidence in favor of this hypothesis (see review by Hardy

2002), little experimental evidence has been provided in hermaphroditic animals.

Experiments performed with snails (Doums and Jarne 1996; Locher and Baur 2000) and with a tapeworm (Schärer and Wedekind 2001) failed to show that these hermaphrodites are able to regulate their sex allocation flexibly in relation to population density. Tan and others (2004) documented that in the leech *Helobdella papillornata*, male, but not female allocation is adjusted to current mating group sizes. This is compatible with the results of Schärer and Ladurner (2003) with the flatworm *Macrostomum* sp., although in the latter a trade-off between sexual functions is revealed under specific conditions (Schärer and others 2005). In gastropods, evidence for a trade-off between sexes was found only after considerable experimental manipulations. De Visser and others (1994) observed that castrated animals had a larger female allocation than intact animals. Locher and Baur (2002) observed a shift in the same direction after *Arianta arbustorum* snails had experienced nutritional stress. In *Lymnaea stagnalis* reared in large groups, an unexpected increase in egg production was observed (compared to that of isolated individuals) and it occurred at the expense of body growth (Koene and ter Maat 2004). In the barnacle *Catomerus polymerus* (Raimondi and Martin 1991) and in the parasitic trematode *Echinostoma caproni* (Trouvé and others 1999) average male and female allocations were adjusted as a function of density (and hence presumably of mating group size). Recently, Lorenzi and others (2005) found that the polychaete worm *Ophryotrocha diadema* increases its female allocation as the social group size diminishes.

Although these findings confirm that hermaphrodites adjust their sex allocation flexibly in relation to population size, the direction and the amount of the adjustment follow different patterns in the different species studied. Trade-off patterns may be obscured by unknown aspects of each organism's biology.

Although both Charnov (1982) and Parker (1998) refer to sperm competition as an evolutionary force that selects for adaptations at different levels (in anatomical, physiological, behavioral, and life history traits), studies rarely take into account anything more than gametic investment when testing for sex allocation models. However, when reproductive competitors are present, investing in behaviors to compete with rivals can increase fitness returns. If investment in such behaviors is expensive, gametic investment is expected to decrease (for example, for separate sex organisms, Scaggiante and others 2005). The general trade-off pattern is complicated by the fact that resources devoted to reproduction vary among individuals of a population or between populations and multiple constraints may

limit the precision of adaptation (Schleicherová and others 2006). Moreover, complex trade-offs may occur between resources allocated to reproduction and those devoted to growth and maintenance. In a growing number of hermaphroditic species sex allocation is reported to be also size dependent (Petersen and Fischer 1996; Trouvè and others 1999; Schärer and others 2001; Angeloni 2003; Tan and others 2004). Many hermaphroditic species have a protandrous male phase that must be taken into consideration over a lifespan energy budget of sex allocation. Analyzing such constraints and the crucial aspects of an organism's biology as well as including them in the context of sex allocation theory remains a major challenge.

The polychaete worm *O. diadema* provides one of the best model systems through which to test the mechanisms underlying sex allocation adjustment in outcrossing hermaphrodites. In this species, sex allocation is not complicated by gender-specific supporting structures (such as reproductive organs, genitalia, etc.). Reciprocal egg fertilization occurs through regular alternation of sexual roles within a mating pair and each individual fertilizes as many eggs as it lays approximately every 2 days on average (Sella 1985). Thus, each *O. diadema* individual will have the option of choosing the proportion of resources allocated to each sex at each reproductive bout as often as every 2 days.

In the present article we examine our previously reported data on sex allocation patterns in *O. diadema* (Sella and Lorenzi 2003; Lorenzi and others 2005) and discuss the extent to which individuals are able to make their short-term sex allocation adjustments to varying social conditions. We then investigate which other male functions may weigh on sex allocation and show recent experimental data on lifetime consequences of current sex allocation choices. Finally we discuss our findings with respect to current hypotheses regarding sex allocation decisions.

Life cycle of the model organism *O. diadema*

All the *O. diadema* life cycle data (Åkesson 1976, 1982) and the information on the main features of its mating system (Sella 1985, 1988, 1990, 1991; Premoli and Sella 1995; Sella and Lorenzi 2000) have been obtained through laboratory observations. A 20-day long protandric phase (approximately 1/3 of their fertile life) precedes the simultaneously hermaphroditic phase and hermaphrodites mate significantly more often with other hermaphrodites than with adolescent males (Sella 1985, 1988). Paired partners take turns at

laying a cocoon of 20–25 eggs every 2 days on average after a long-lasting courtship involving mutual rubbing. In larger groups, hermaphrodites are not monogamous (Sella and Lorenzi 2000, 2003). Nine days after egg laying, offspring are released from the cocoon as small 4-segment individuals, soon ready to produce their first sperm (Sella 1990). The hermaphroditic phase is reached at a body length of 14–17 segments: sperm are produced in the fourth and fifth segment and eggs in the posterior segments (Åkesson 1976).

An advantage of the *O. diadema* model is that a neutral genetic marker is present in its populations (a biallelic locus determining a yellow or white egg coloration, Sella and Marzona 1983). Either yellow or white oocytes can be easily detected through the transparent body wall of mature worms. By means of this marker it is possible to identify focal hermaphrodites in a group and estimate their gamete output. In our experiments focal individuals are generally yellow egg individuals. During the experiments, individuals were reared in marine water (20°C, 33 psu) in 10 ml bowls and fed with spinach *ad libitum*.

Current sex allocation adjustments

To test the relationship between sex allocation and group size, Lorenzi and others (2005) set up an experiment where *O. diadema* hermaphrodites were reared in different social conditions, controlled for crowding and density by rearing worms in enclosures of 2 different sizes. Results showed that the density had no significant effect on female or male allocation while population size strongly influenced sex functions. As expected, compared to hermaphrodites reared in isolated pairs, hermaphrodites reared in large groups (that is, 12 individuals) produced significantly fewer cocoons. Moreover, the number of nonlaying hermaphrodites (exhibiting pure-male behavior) was significantly larger among hermaphrodites reared in large groups than in pairs. However, contrary to expectations, the number of sperm produced by focal hermaphrodites was not significantly affected either by group size or density. The trend of the shift between male and female allocation was the opposite of that of the previously cited hermaphroditic taxa on which phenotypic plasticity in sex allocation had been tested. Furthermore in *O. diadema* the female function was drastically reduced in large groups, while the male function was not increased to the same extent.

However, in *O. diadema*, resources subtracted from the female function and not allocated to sperm production are devoted to other aspects of the male function (see Experiment 1).

A major factor constraining the extent to which individuals adaptively adjust sex allocation is their ability to evaluate the size and/or composition of the local group, but this has rarely been investigated in hermaphroditic organisms.

Schleicherová and others (2006) documented that *O. diadema* hermaphrodites are able to assess the number of reproductive competitors by means of a water-borne species-specific chemical pheromone released by mature individuals. Paired individuals reared in water collected from large populations of mature hermaphrodites ('conditioned water') biased their sex allocation toward the male sex as if they were reared with competitors, indicating that they perceived some water-borne chemical acting as a cue for the size of the potential local mating group.

O. diadema hermaphrodites can regulate their female allocation not only in the presence of mature competing hermaphrodites but also if adolescent males are present. As reviewed before, adolescent males, even if generally rejected as partners, can compete with hermaphrodites for fertilizations (Sella and Lorenzi 2003). Pairs of hermaphrodites, each reared with 3 adolescent males, significantly reduced their female allocation compared to isolated pairs of hermaphrodites. The sex allocation adjustment occurred in the same direction as that of individuals reared in large populations of hermaphrodites (Fig. 1, D. Schleicherová, unpublished data), indicating that hermaphrodites perceive both adolescent males and mature hermaphrodites as reproductive competitors.
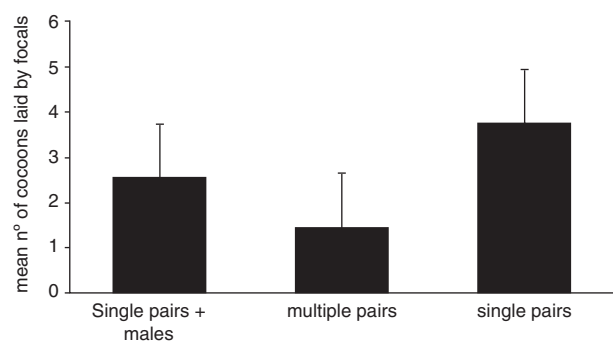

Fig. 1 Female allocation of virgin focal individuals kept in 3 different social conditions for 21 day. Each focal was reared either with a mature hermaphrodite and 3 adolescent males (32 replicates) or with 11 mature hermaphrodites (multiple pairs, $n = 35$ replicates) or with a mature hermaphrodite (single pairs, $n = 36$ replicates). Adolescent males were less than 10 segments long. The number of laid cocoons was significantly different in the 3 treatments (one-way ANOVA, $F_{2,100} = 25.02$, $P < 0.0001$; *post-hoc* Tukey test, $P < 0.01$, all comparisons) (D. Schleicherová unpublished data).

Experiments: properties of the sex allocation adjustments of the model organism

Study 1: Behavioral interactions can weigh on male allocation

Introduction

Mating may require resources to be invested not only in gamete production but also in behavior, for example, direct competition with reproductive rivals, be they adolescent males or mature hermaphrodites. Such behavioral interactions may be very expensive.

Sella and Lorenzi (2003) showed that 38% of *O. diadema* adolescent males that competed with mature hermaphrodites for egg fertilization disappeared within the 21-day period of the experiment. This cost was paid by only 5% of adolescent males when they were paired with only one hermaphrodite (*G*-test; $G = 25.50$; $P = 0.001$). The significant difference between these frequencies suggests that egg fertilization, that is not risky *per se,* becomes so when adolescent males are competing with mature hermaphrodites.

To discriminate between resources allocated to sperm production and those allocated to behavior, we performed qualitative and quantitative analyses of the behavioral interactions and gamete output of focal individuals, which were in either real or simulated conditions of reproductive competition. In real reproductive competition (large populations) individuals invested resources both in sperm production and in reproductive behaviors. In simulated reproductive competition, isolated pairs were manipulated so that they could not invest in competitive behaviors but only in sperm production.

Methods

Focal individuals were reared in groups with 11 other, white-phenotype hermaphrodites ('large groups', 31 replicated bowls) or in isolated pairs, each with a white-phenotype hermaphrodite ('treated pairs', 34 replicated bowls) for 9 days. Treated pairs received water collected from groups of 12 mature hermaphrodites daily ("conditioned water"). In these conditions, they behaved as if they lived in large populations, in the sense that the number of cocoons they produced was similar to that produced by hermaphrodites reared in large groups (Schleicherová and others 2006). Group-reared focals are expected to pay the costs of interacting with reproductive competitors while paired focals reared in conditioned water are not. We expected that focals from the large groups would be involved in interactions (and aggressions) more often than those from treated pairs. On days 6–8 from the beginning of the experiment, we scored each focal individual for its oocyte development on a 4-class scale (0 = no visible oocyte in the coelome, 1 = small oocytes, 2 = medium sized oocytes, 3 = ripe oocytes). Behavioral interactions were recorded on the same days. The behavior of each focal individual was observed at 120× magnification for a total of 30 min (in 2 non-consecutive 15-min long sessions). We noted whether focals were involved in one of the following 5 behaviors: contact (focal is touched by or touches another individual with any body part) rubbing behavior (focal is gently stroked or strokes its body against the body of another individual), biting, fast withdrawal (focal abruptly changes its movement direction after contacting another individual or it makes another individual change its movement direction), pursuing (focal is followed by or follows another individual; both crawl fast one below the other).

Bites, pursuits, and fast withdrawals were considered intolerant, contacts were considered neutral, and rubbing behaviors were assumed to be affinitive interactions (related to courtship).

In both experimental groups we considered the amount of resources allocated to behavioral interactions to be the number of acts and the gamete investment of focals to be the number of cocoons, eggs per cocoon and sperm produced respectively during or at the end of the experiment (see Lorenzi and others 2005 for method of sperm counting).

Results

The number of laid cocoons was significantly different between large groups and treated pairs (average ± SD: 0.16 ± 0.37 and 0.47 ± 0.61, respectively; Mann–Whitney *U*-test, $n_1 = 34$, $n_2 = 31$, $U = 664$, $P = 0.023$), while the number of sperm was not (Mann–Whitney *U* test, $n_1 = 28$, $n_2 = 31$, $U = 542$, $P = 0.101$) (Fig. 2). The number of cocoons and eggs is significantly correlated, Spearman correlation, rs = 0.98, $P < 0.0001$, therefore we report the number of cocoons only.

The significantly different number of cocoons produced by individuals from the 2 treatments suggests that, compared with individuals in treated pairs, hermaphrodites in large groups subtracted a significant amount of resources from the female investment, while they kept sperm allocation substantially unchanged.

The difference in the number of interactions in which individuals were involved in groups or in pairs was highly significant (Fig. 3). Focals in groups had significantly more contacts (ANOVA, $F_{1,66} = 10.97$, $P < 0.002$) and intolerant behaviors (ANOVA, $F_{1,66} = 35.44$, $P < 0.0001$) with potential partners or
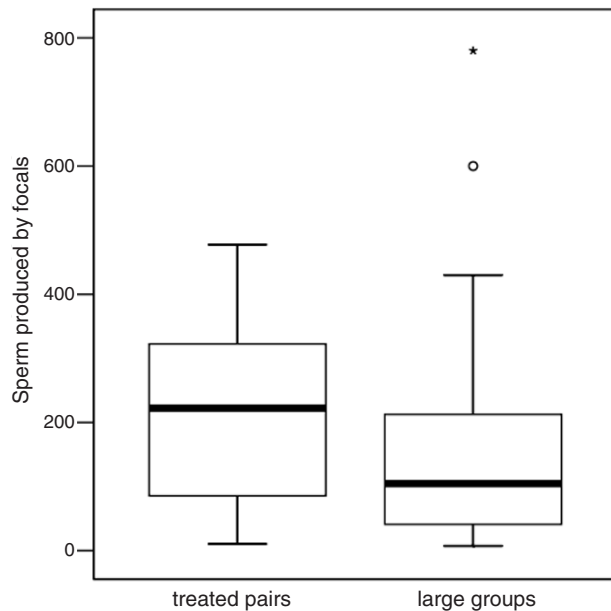
Fig. 2 Allocation to sperm in focals from treated pairs and large groups in the experiment on costs of behavioral interactions.
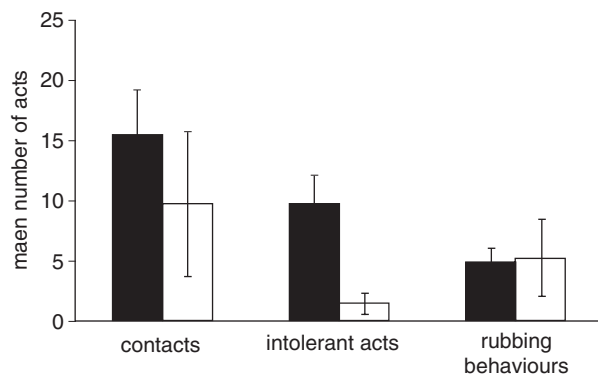

Fig. 3 Mean numbers of focals' interactions/30 min (contacts, intolerant acts and rubbing behaviors) and standard deviations. Focals' behavior was observed in treated pairs or in large groups (white bars: treated pairs, black bars: large groups).

competitors than those in pairs. Interestingly, focals were engaged in a similar number of rubbing behaviors irrespective of whether they belonged to treated pairs or to large groups (ANOVA, $F_{1,66} = 0.05$, $P = 0.82$). Oocyte development, included as a covariate, had no significant effect on the dependent variables.

Study 2: long-term consequences of current sex allocation choices

Introduction

Organisms have limited reproductive resources. In life history theory a well-established trade-off is that between somatic (growth and maintenance) and gametic investment. Increased allocation to reproduction may require deviation of resources from somatic growth and maintenance. Therefore, both growth and maintenance are expected to be reduced. There is little evidence of a relationship between reproduction and growth rate or life expectancy in hermaphrodites. In *Caenorhabditis elegans*, increased sperm production reduces lifespan (Van Voorhies 1992) and may lead to a delay in the onset of oogenesis. According to this author, in *C. elegans*, spermatogenesis, rather than oogenesis, is a major factor in reducing lifespan and causing an increase in minimum generation time and a decrease in population growth. A negative correlation was found in the colonial ascidian *Botryllus schlosseri*, between growth and sexual investment (Yund and others 1997) and in the terrestrial gastropod *Balea perversa* between growth and reproductive rate (Baur and Baur 2000). In *L. stagnalis*, experimental castration increases female allocation but does not modify body growth (De Visser and others 1994).

Protandry can be considered as a form of male allocation whose energy budget is expected to weigh on the lifetime balance of reproductive resources. Therefore, if during the protandrous phase there is an increase in male expenditure, a delay in reproduction is expected in the successive hermaphroditic phase, as documented by Barker (1992) in *C. elegans*.

In *O. diadema* the costs to growth rate of an increase in male expenditure during the protandrous phase were observed by Sella and Lorenzi (2003) in experiments where adolescent males had the possibility of fertilizing eggs of pairs of hermaphrodites. Adolescent males competed for egg fertilization with hermaphrodites and caused them fitness losses of 31.4%. Adolescent males that fertilized hermaphrodites' eggs had a significantly slower growth rate than adolescent males that did not have this possibility. This result indicates that when during the protandrous phase resources are allocated to reproduction, a trade-off occurs at the expense of somatic development.

Costs of allocation to the male sex during the protandrous phase can not only be traded against somatic growth but may also have long-term consequences on sex allocation and reproductive success. The larger the amount of resources individuals invest in reproduction in the protandrous phase, the smaller the amount they can allocate later in life (that is, during the hermaphroditic phase) to growth, maintenance, and reproduction. We tested this hypothesis by comparing lifetime female allocation, body growth, and survival in individuals that had or had not the opportunity of fertilizing eggs during their protandrous phase.

Methods

Ninety-six focal *O. diadema* individuals were followed from the newly hatched larval instar to the end of their life. When they were 8 days old (and 5 segments long), larvae that were to exhibit a yellow phenotype in the hermaphroditic phase were assigned to one of the following treatments:

(1) Lifelong-paired treatment (LP-treatment): each focal *O. diadema* larva was paired with a mature white-phenotype hermaphrodite from the beginning of the experiment (64 replicated bowls). In this way each focal had the opportunity to fertilize eggs from the very beginning of its sexual life.

(2) Hermaphrodite-paired treatment (HP-treatment): each focal *O. diadema* was isolated from its siblings as newly hatched larva and paired to a mature white-phenotype hermaphrodite only when it had its first ripe oocytes (32 replicated bowls). Since focals did not have the opportunity to fertilize eggs during the protandrous phase, they were expected to allocate the saved energy to growth and maintenance later in life.

To avoid pseudoreplications, neither individuals in the same treatment nor those in the same pair were siblings.

Each replicated bowl was observed twice a week (each third or fourth day) till the death of the focal individual, and each time we noted body growth (as number of segments), number of cocoons laid by focals, and number of eggs per cocoon. In the LP-treatment, we also noted the number of white cocoons (and eggs per cocoon) that focal males fertilized during the protandrous phase.

The date at which individuals reached the hermaphroditic phase was defined as the date when focals began to develop oocytes in their coelome. At this date, individuals from the HP-treatment were paired by adding a mature hermaphrodite in the bowls. During the hermaphroditic phase of focals, for each pair we noted the number of yellow laid cocoons and eggs/cocoon. To sum up, during adolescence, we measured a focal's male allocation as the number of cocoons and eggs it fertilized during the protandrous phase, and during the hermaphroditic phase, we measured its female allocation as the number of cocoons and eggs it produced.

At each check, cocoons were removed from the bowls to avoid variations in the social composition of the replicates.

In the experiment, the number of eggs laid was significantly correlated with the number of cocoons laid (Spearman correlation, LP-treatment: $r_s = 0.90$, $P < 0.0001$, HP-treatment: $r_s = 0.81$, $P < 0.0001$).

Thus, to analyze differences in focal individuals' lifetime reproductive success between the 2 treatments, we referred to the number of cocoons. To analyze the influence of the 2 treatments on female allocation, body growth, and survival, we used ANOVA, after testing that data were normally distributed (Kolmogorov–Smirnov test, n.s.). We used nonparametric tests if data were not normally distributed.

Results

Protandrous phase

As an average, during the adolescent phase, males from the LP-treatment fertilized 3.44 ± 1.50 cocoons laid by their partners (Fig. 4), while males from the HP-treatment could not fertilize because they were isolated. Adolescent males became hermaphrodites at a body length that was not significantly different in the 2 treatments (14.63 ± 1.10 segments on average for LP-treated hermaphrodites and 14.51 ± 0.92 for HP-treated hermaphrodites; Mann–Whitney *U*-test, $n_1 = 30$, $n_2 = 61$, $U = 844$, $P = 0.53$).

Female allocation, growth, and lifespan in the hermaphroditic phase

At the end of their life focals from the LP-treatment had, on average, a body size (18.5 ± 1.2 segments) lower than that of focals from the HP-treatment (19.8 ± 1.3 segments) (Mann–Whitney test, $n_1 = 30$, $n_2 = 61$, $U = 411.5$, $P < 0.0001$).

There is a complex relationship between the male allocation during the protandrous phase, the female allocation during the hermaphroditic phase and body size. Male allocation during adolescence is significantly and inversely correlated with final body size and marginally with female allocation in hermaphroditic phase. Female allocation in hermaphroditic phase is directly and significantly correlated with final body
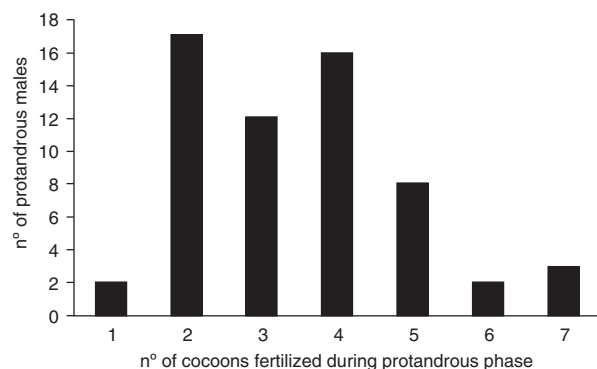

Fig. 4 Distribution of the number of cocoons fertilized by focals from the LP-treatment during the protandrous phase.
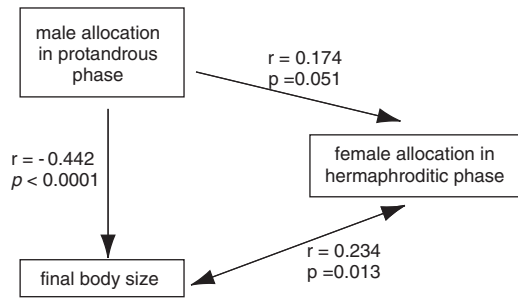
Fig. 5 Results of the relationships shown by partial correlations between male allocation in the protandrous phase, female allocation in the hermaphroditic phase and body size at the end of life (for each pair of variables, d.f. = 88, r and one-tailed probability P is reported; arrows indicate the supposed direction of the relation between variables).

size. Figure 5 shows the relationship between the 3 variables and the P-value of the 3 partial correlations (in each case keeping the other variable constant). The existence of significant correlations between each pair of variables indicates that none is a byproduct of the others.

Individuals that had fertilized more cocoons during the protandrous phase lived shorter lives (Pearson correlation tests, $r = -0.314$, $P = 0.002$). Since the longer individuals live, the more cocoons they lay ($r = 0.497$, $P < 0.0001$), individuals that had the opportunity to fertilize cocoons during the protandrous phase lowered their life expectancy and thus their fecundity expectancy during the hermaphroditic phase.

Discussion

The pattern of sex allocation of the outcrossing hermaphrodite *O. diadema* allowed us to identify some of the properties that participate in shaping the shifting of reproductive resources predicted by sex allocation theory for hermaphrodites.

In these worms, reproductive resources are flexibly adjusted between the male and the female function depending on social conditions (Lorenzi and others 2005) and individuals are able to make these adjustments both in large populations of hermaphrodites and in the presence of adolescent males. In large populations of hermaphrodites, each individual can meet both multiple partners and multiple rivals on the male function. However, in the experiment in which adolescent males, which lack the female function, competed with hermaphrodites for egg fertilization, hermaphrodites do bias their sex allocation in favor of the male sex. In large populations, sex allocation adjustments are mainly elicited by the presence of rivals for the male role rather than by multiple potential partners.

Phenotypic plasticity in sex allocation can be observed also between successive phases during the life of individuals. The amount of resources allocated to reproduction as males during the protandrous phase influences those devoted to the female allocation in the hermaphroditic phase (as shown by the inverse correlation between male allocation during the protandrous phase and female allocation during the hermaphroditic phase). The cost of reproduction during adolescence is paid for also by a shortened lifespan and a diminished growth rate. Therefore, the long-term consequences of adolescent male reproduction on growth and survival suggest that resource allocation is a multiple-way trade-off that involves reproduction, maintenance and growth (see also Yund and others 1997 and Koene and others 2004), thus influencing the whole energy budget of the organism.

One of the crucial aspects of sex allocation adjustment in *O. diadema* is that resources subtracted from the female function in favor of the male function with increasing group size are not reallocated directly to increasing sperm production. The number of sperm produced by hermaphrodites is never significantly affected by population size (Lorenzi and others 2005; this article, study 1), meaning that in this species, when group size varies, the shift of reproductive resources is more obvious when it is measured as female than as male allocation.

The difference in the pattern of variation of sex allocation between *O. diadema* and the other hermaphroditic model animals could be due to the fact that in *O. diadema* the male function requires proportionately more resources than are usual in a situation of sperm competition.

However, we believe that the amount of resources that hermaphrodites in groups subtract from the female function (compared to paired hermaphrodites) is allocated to behavioral interactions. In our experiment with behavioral observations, hermaphrodites belonging both to treated pairs and to large groups were engaged in a similar number of rubbing behaviors. This means that when they lived in groups, hermaphrodites were not involved in courtship more often than individuals in pairs, although multiple potential partners were available. It may be that in large groups hermaphrodites prefer to sneak copulations rather than engaging in courtship more frequently than hermaphrodites reared with single partners. However, hermaphrodites in groups were involved in intolerant acts more often than those in pairs, reinforcing the hypothesis that they perceived the other hermaphrodites more as competitors than as potential mates. Food was given daily and in excess, so focals were unlikely to perceive other individuals as competitors for food.

To sum up, results of this experiment suggest that hermaphrodites in group allocate part of their resources to direct competition with potential reproductive competitors. It is likely that such interactions are time and energy consuming and that such costs weigh on the amount of resources devoted to reproduction. Recently, studies in separate sex organisms have documented that behavioral strategies can be an alternative to sperm expenditure adjustments (see, for example, Candolin and Reynolds 2002; Scaggiante and others 2005).

The sex allocation pattern shown by *O. diadema* is different from that of the other hermaphroditic organisms hitherto tested, which generally showed an enhanced investment in sperm production as local population size increased, while the female function did not seem to vary.

Sexual selection operates in hermaphrodites (see Leonard, this volume) although it can be expected to be weaker as compared to gonochorists (Greeff and Michiels 1999). Here we show that sexual selection operates in large populations of *O. diadema* and imposes very high costs on hermaphrodites. In contrast, monogamous pairs of this outcrossing hermaphroditic species do not pay such costs (and have a higher reproductive output).

Acknowledgments

References

Åkesson B. 1976. Morphology and life cycle of *Ophryotrocha diadema*, a new polychaete species from California. Ophelia 15:25–35.

Åkesson B. 1982. A life table study on three genetic strains of *Ophryotrocha diadema* (Polychaeta, Dorvilleidae). Int J Invertebr Reprod 5:59–69.

Angeloni L. 2003. Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: body size, allocation to sexual roles and paternity. Anim Behav 66:417–26.

Barker DM. 1992. Evolution of sperm shortage in a selfing hermaphrodite. Evolution 46:1951–5.

Baur B, Baur A. 2000. Social facilitation affects longevity and lifetime reproductive success in a self-fertilizing land snail. Oikos 88:612–20.

Candolin U, Reynolds JD. 2002. Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (*Rhodeus sericeus*). Proc R Soc Lond B 269:1549–33.

Charnov EL. 1982. The theory of sex allocation. Princeton, New Jersey, Princeton University Press.

De Visser JAGM, ter Maat A, Zonneveld C. 1994. Energy budgets and reproductive allocation in the simultaneous hermaphrodite pond snail, *Lymnaea stagnalis* (L.): a trade-off between male and female function. Am Nat 144:861–7.

Doums C, Jarne P. 1996. The evolution of phally polymorphism in *Bulinus truncatus* (Gastropoda, Planorbidae): the cost of male function analysed through life-history traits and sex allocation. Oecologia 106:464–9.

Fischer EA. 1984. Local mate competition and sex allocation in simultaneous hermaphrodites. Am Nat 124:580–96.

Greeff JM, Michiels NK. 1999. Low potential for sexual selection in simultaneously hermaphroditic animals. Proc R Soc Lond B 266:1671–6.

Hamilton WD. 1967. Extraordinary sex ratios. Science 156:477–88.

Hardy ICW. 2002. Sex ratios: concepts and research methods. Cambridge: Cambridge University Press.

Koene JM, ter Maat A. 2004. Energy budget in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis*: a trade-off between growth and reproduction during development. Belg J Zool 134 (suppl. 1):41–5.

Locher R, Baur B. 2000. Sperm delivery and egg production of the simultaneously hermaphroditic land snail *Arianta arbustorum* exposed to an increased sperm competition risk. Invertebr Reprod Dev 38:53–60.

Locher R, Baur B. 2002. Nutritional stress changes sex-specific reproductive allocation in the simultaneously hermaphroditic land snail *Arianta arbustorum*. Funct Ecol 16:623–32.

Lorenzi MC, Sella G, Schleicherová D, Ramella L. 2005. Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. J Evol Biol 18:1341–7.

Parker GA. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: Birkhead TR, Møller AP, editors. Sperm competition and sexual selection. London, UK: Academic Press. p 3–54.

Petersen CW, Fischer EA. 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: the effect of individual size. Evolution 20:636–45.

Premoli MC, Sella G. 1995. Sex economy in benthic polychaetes. Ethol Ecol Evol 7:27–48.

Raimondi PT, Martin JE. 1991. Evidence that mating group size affects allocation of reproductive resources in a simultaneous hermaphrodite. Am Nat 138:1206–17.

Scaggiante M, Rasotto MB, Romualdi C, Pilastro A. 2005. Territorial male gobies respond aggressively to sneakers but do not adjust their sperm expenditure. Behav Ecol 16:1001–7.

Schärer L, Wedekind C. 2001. Social situation, sperm competition and sex allocation in a simultaneous hermaphrodite parasite, the cestode *Schistocephalus solidus*. J Evol Biol 14:942–53.

Schärer L, Ladurner P. 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proc R Soc Lond B 270:935–41.

Schärer L, Karlsson LM, Christen M, Wedekind C. 2001. Size-dependent sex allocation in a simultaneous hermaphrodite parasite. J Evol Biol 14:55–67.

Schärer L, Sandner P, Michiels NK. 2005. Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm *Macrostomum* sp. J Evol Biol 18:396–404.

Schleicherová D, Lorenzi MC, Sella G. 2006. How outcrossing hermaphrodites sense the presence of conspecifics and suppress female allocation. Behav Ecol 17:1–5.

Sella G. 1985. Reciprocal egg trading and brood care in a hermaphroditic polychaete worm. Anim Behav 33:938–44.

Sella G. 1988. Reciprocation, reproductive success and safeguards against cheating in the mating system of a hermaphroditic polychaete worm, *Ophryotrocha diadema*. Biol Bull 175:212–17.

Sella G. 1990. Sex allocation in the simultaneous hermaphroditic polychaete worm *Ophryotrocha diadema*. Ecology 71:27–32.

Sella G. 1991. Evolution of biparental care in the hermaphroditic polychaete worm *Ophryotrocha diadema*. Evolution 45:63–8.

Sella G, Lorenzi MC. 2000. Partner fidelity and egg reciprocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*. Behav Ecol 11:260–4.

Sella G, Lorenzi MC. 2003. Increased sperm allocation delays body growth in a protandrous simultaneous hermaphrodite. Biol J Linn Soc 78:149–54.

Sella G, Marzona M. 1983. Inheritance, maternal influence and biochemical analysis of an egg color polymorphism in *Ophryotrocha diadema*. Experientia 39:97–8.

Tan GN, Govedich FR, Burd M. 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, *Helobdella papillornata* (Euhirudinea: Glossiphoniidae). J Evol Biol 17:574–80.

Trouvé S, Jourdane J, Renaud F, Durand P, Morand S. 1999. Adaptive sex allocation in a simultaneous hermaphrodite. Evolution 53:1599–1604.

Van Voorhies WA. 1992. Production of sperm reduces nematode lifespan. Nature 360:456–8.

Yund PO, Marcum Y, Stewart-Sevage J. 1997. Life-history variation in a colonial ascidian: broad-sense heritabilities and tradeoffs in allocation to asexual growth and male and female reproduction. Biol Bull 192:290–9.