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SEX ALLOCATION AND MALE FITNESS GAIN IN A COLONIAL, HERMAPHRODITIC MARINE INVERTEBRATE

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Abstract.—While simultaneous hermaphroditism occurs in most animal phyla, theories for its adaptive significance remain untested. Sex allocation theory predicts that combined sexes are favored in sedentary and sessile organisms because localized gamete dispersal and local mate competition (LMC) among gametes promote decelerating fitness "gain curves" that relate male investment to reproductive success. Under this LMC model, males fertilize all locally available eggs at low sperm output, additional output leads to proportionally fewer fertilizations, and combined sexes with female-biased sex allocation are favored. Decelerating male gain curves have been found in hermaphroditic flowering plants, but the present paper reports the first analysis in an animal. The colonial hermaphroditic bryozoan *Celleporella hyalina* forms unisexual male and female zooids that can be counted to estimate absolute and relative gender allocations. I placed "sperm donor" colonies—each with different numbers of male zooids, and each homozygous for diagnostic allozyme alleles—among target maternal colonies on field mating arrays, and estimated donor fertilization success by scoring allozyme markers in target-colony progeny. Fertilization success increased with numbers of donor male zooids, but linear and not decelerating curves fit the data best. Mean sex allocation was not female biased, consistent with nondecelerating male gain. Sperm donors, moreover, did not monopolize matings as expected under high LMC, but rather shared paternity with rival colonies. Hence localized water-borne gamete dispersal alone may not yield decelerating male gain and favor the maintenance of hermaphroditism; relaxed sperm competition in low density populations might also be required. In free-spawning marine organisms, males cannot control access to fertilizations, intense sperm competition may be commonplace, and high male sex allocation may be selected to enhance siring success under competition.

Key words.—Bryozoan, fertilization success, hermaphroditism, male reproductive success, sex allocation, sperm competition.

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Simultaneous hermaphroditism (SH), where individuals bear both male and female gametes within the same breeding season, occurs in most animal phyla (Ghiselin 1969). SH is found in all species within some higher taxa (e.g., bryozoans and ascidians), as is separate sexes within others (e.g., brachiopods and echinoids), indicating that ancestry alone may account for which reproductive mode is present in some lineages. Yet in others, this is clearly not the case. Genera containing both hermaphroditic and gonochoric species, cases of derived SH scattered within predominantly separately sexed taxa, as well as secondarily evolved gonochorism in predominantly hermaphroditic groups (e.g., in the barnacles) are each patterns that suggest multiple evolutionary transitions and call for adaptive explanations. Furthermore, certain features of reproductive biology and of habitat are associated with SH (Ghiselin 1969, 1974). For example, in taxa containing species that brood young and others that broadcast planktonic larvae, the brooders often are hermaphroditic, while the broadcasters are gonochores (Heath 1977, 1979; Strathmann et al. 1984). Explanations for how natural selection may favor the unity or division of the sexes date to Darwin (1851, 1854, 1876) and abound in the literature, yet remain largely untested.

Two classes of models have explored the adaptive significance of SH. Earlier theories (e.g., Tomlinson 1966), termed "low density" models by Ghiselin (Ghiselin 1969), viewed SH as a means to assure complementarity between any two individuals mating at random when encounters are infrequent, such as may be the case with low population density. While mating assurance may help explain the prevalence of SH within deep-sea taxa and some parasitic groups, it fails to account for the many cases of SH among free-living, shallow-water species that aggregate or occur at high densities (Heath 1977).

More recently, sex allocation models (Charnov et al. 1976; Charnov 1979, 1982) have treated hermaphroditism not as a mating assurance mechanism, but instead as a resource allocation strategy evolved to prevent gamete wastage. These models invoke decelerating marginal fitness returns from increasing investment of resources into reproduction, which are described by saturating fitness "gain curves." At the allocation level where either the male or the female gain curve (or both) begin to decline, individuals are selected to shunt additional resources into reproduction as the other sex (Charnov et al. 1976; Charnov 1982), and combined sexes are favored. Linear or accelerating gain curves, on the other hand, are thought to exist where fitness return increases with reproductive allocation without bound, and individuals are selected to reproduce as unisexual males or females. Sex allocation theory assumes diminishing fitness gain to be the fundamental process promoting the evolutionary stability of the hermaphroditic state.

Saturating female gain curves may occur in plants and animals with poorly dispersed seeds or larvae, where competition among genetically related sibs causes female fitness to decline as investments increase (Maynard Smith 1978). Sib competition, however, is unlikely to account for SH in plants with well-dispersed seeds or in sedentary marine animals with planktonic larvae. On the other hand, many sessile and sedentary animals brood their young. Brooding could

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engender saturating female gain because resources for egg production and provisioning may increase with growth faster than the rate of expansion of brood space (Heath 1977, 1979; Strathmann et al. 1984). A single study of a brooding hermaphroditic sea star species has examined this brood space limitation hypothesis, and showed that larger individuals facing more severe brood space limitation did not increase their male gonadal investments (Strathmann et al. 1984). Hence, it is unclear how often saturating female gain favors the maintenance of SH.

Alternatively, saturating reproductive success as a male may occur when male gametes are transferred between sedentary mates, either via copulation, or through transport by an external vector. A form of local mate competition (LMC) among gametes might result, where male success reaches an upper limit due to localized gamete dispersal to a finite number of mates (Charnov 1979, 1982; Lloyd 1984), the approach to this limit decelerating with increasing investment in male reproduction. In animal-pollinated flowering plants, diminishing returns may occur whenever production surpasses the capacity of pollinators to carry pollen (Charnov 1979; Lloyd 1984). In either plants or animals, a similar result would be obtained if male gametes overwhelm available ova in neighboring females, such that male gametes released by single individuals compete among themselves for fertilizations (Maynard Smith 1978; Charnov 1979, 1982; Lloyd 1984). Diminishing fitness returns from male investment may therefore be found whenever mating is restricted to occur between neighboring individuals.

Recent empirical estimates of male fitness gain in hermaphroditic plants have fit both linear and saturating functions. Several studies in natural or experimental populations have used allozyme markers to assign paternity to fertilized ovules, then correlated paternal success with numbers of pollen-bearing structures produced. Siring success was shown to be a linear function of the number of male cones in pollen parents of white spruce, with decline in the curve evident, due to meager success of a single clone bearing numerous cones (Schoen and Stewart 1986). The number of seeds sired by milkweeds was found to be linearly related to the number of flowers borne on the paternal plant (Broyles and Wyatt 1990). Pollen removal from anthers of wild radish was shown to decelerate with increasing pollen production in two experiments, while data from a third experiment fit a linear function (Young and Stanton 1990). Another study of experimental wild radish populations used allozymes to establish paternity, and found a decelerating, negative quadratic relationship between flower number and male success in two years studied, but a third year's population showed a poor fit, either to a linear function or to a slightly decelerating one (Devlin et al. 1992). Finally, pollen color variants were used to track pollen delivery to stigmas in glacier lily populations, in which delivery was shown to be a decelerating function of the number of grains presented by pollen-bearing flowers (Thomson and Thomson 1989). It is therefore still unclear whether saturating male gain is a consistent feature of the pollination ecology of hermaphroditic flowering plants.

In contrast to the expanding list for angiosperms, not a single study has examined the form of a male gain curve in a hermaphroditic animal. This is unfortunate, because the reproductive ecology of sedentary and sessile animals make them likely candidates for showing LMC among gametes. Localized sperm dispersal has been demonstrated in several benthic marine species that shed sperm into the water column (Pennington 1985; Yund 1990; Grosberg 1991; Levitan 1991; Brazeau and Lasker 1992). Furthermore, hermaphroditic species often retain eggs inside the maternal parent, where free-spawned sperm are collected to fertilize eggs internally. In contrast to external fertilization, in which the number of eggs fertilized should be linearly related to sperm concentrations, an outcome favoring gonochorism (Charnov 1979), retention of eggs and internal fertilization should lessen the likelihood that sperm concentrations limit the proportion of eggs fertilized (Denny and Shibata 1989; Levitan and Petersen 1995). Internally fertilized eggs are also often brooded as embryos, so the number of eggs per clutch can be several magnitudes fewer than the number per spawn in external fertilizers. These combined features may allow sperm donors to fertilize eggs of neighboring mates at low levels of sperm output, with decelerating gain apparent as output increases. Therefore, while LMC theory for animal hermaphroditism has previously been applied only to sessile barnacles (Charnov 1980, 1982), where copulation enforces localized mating, it should also pertain to the many benthic species that release sperm and retain eggs.

Determination of male gain relations requires reliable measures of male reproductive investment and fertilization success. Reproductive investments can be conveniently estimated in plants because organs are external, modular, and can be counted. For animals where internal gonad is a consolidated mass of tissue, direct measurements might only be obtained destructively (e.g., by weighing dissected gonad). However, colonial marine species often produce gonads within iterated modules that can be counted to estimate investments noninvasively. In this paper, I use a hermaphroditic colonial bryozoan species that houses its gonads in unisexual modules, or zooids, to study the fitness consequences of male gonad allocation. I employ progeny analysis to estimate male fertilization success in experimental mating assemblages, using colonies with different amounts of male gonad as sperm donors. I then compare paternal success of these donors to their level of male allocation to test the local mate competition model.

Materials and Methods

Natural History and Reproductive Ecology of Celleporella hyalina

Celleporella hyalina L. is a cheilostome bryozoan that is circumglobally distributed in colder seas (Marcus 1938; Hastings 1979; Ryland 1979). In New England, it forms dense encrustations upon fronds of laminarian kelps (Osburn 1933; McCartney 1994). The roughly circular colonies are founded through settlement of a sexually produced lecithotrophic larva. Radial colony growth occurs through budding of sterile feeding units termed autozooids, which adhere to the substratum. Like all bryozoans, *C. hyalina* is a simultaneous hermaphrodite, but is unusual in that gonads are confined to specialized, morphologically distinguishable male and female zooids. These sexual zooids are produced through a

process known as frontal budding (Ryland and Gordon 1977; Cancino and Hughes 1988) such that they form a layer on top of the basal autozooid layer.

Male zooids do not feed, yet they maintain a modified lophophore (Marcus 1938; Cancino and Hughes 1988). Typical bryozoan zooids use this structure to capture food, but male zooids of *C. hyalina* do not feed. Instead, male zooid lophophores most likely serve to discharge sperm, which occurs through pores in feeding lophophoral tentacles in several other species (Silén 1972). Most of the internal volume of the male zooid is filled with spermatogenic tissue (Cancino and Hughes 1988). Female zooids consist of a zooid body, where eggs are ovulated, and a globular ovicell, to which a single egg at a time is transferred for brooding. Female zooids produce no lophophore and cannot feed. The site and timing of fertilization is unknown for *C. hyalina*, but occurs within the zooid body in other cheilostomes (Dyrynda and King 1983; Temkin 1991). No mechanism to prevent self-fertilization is known, and colonies reared in isolation in the laboratory produce viable progeny (Hunter and Hughes 1993; McCartney 1994). Eggs of *C. hyalina* undergo a 15-fold increase in volume during the approximately 16-d brooding cycle (Hughes 1987; McCartney 1994) in the ovicell, during which the embryo is provided with maternal nutrition (Hughes 1987). Embryo age can therefore be estimated from diameter measurements alone (Hughes 1987; pers. obs.).

In typical Maine colonies (McCartney 1994), the onset of male sexual maturity is marked by the appearance of male zooids at about four weeks after settlement, and female zooids first appear some seven to 10 days later. Through the remainder of a colony's life, male and female zooids are budded and function simultaneously. Male-first sex expression is typically followed by a steady increase in femaleness with age, and this ontogenic pattern complicates single-point estimates of sex allocation. Nevertheless, sexual zooids never change gender, and once formed remain functional through most of the remaining life of the colony. Hence the proportion of sexual zooids that are female (an estimate of sex allocation), counted when numbers of sexual zooids have first reached their peak, is closely correlated with cumulative sex allocation recorded over one breeding season (McCartney 1994). Since colonies on kelp do not survive to enter a second breeding season (Cancino 1986; McCartney 1994), this amounts to a colony's lifetime sex allocation.

A survey of several hundred colonies in Maine showed considerable among-colony variation in lifetime sex allocation (McCartney 1994). Previous studies of *C. hyalina* in Wales have also demonstrated variable sex allocation (Hughes and Hughes 1986; Hughes 1989). In Maine, hermaphroditic colonies ranged continuously from those in which nearly all sexual zooids were male through those showing the opposite bias. Lifetime peak number of male zooids, a measure of total male gonad volume, can range over greater than two orders of magnitude (McCartney 1994).

Field Fertilization Success Experiments

Overview

To estimate the male fitness return from investment in male gonad, I constructed mating arrays containing a small number of colonies clustered together. I used allozyme markers to distinguish adult colonies and to establish paternity of their brooded embryos. Siring success was estimated for a single distinctively marked sperm donor on each array. Several arrays were placed near *C. hyalina* natural populations to model mating under field conditions. Sperm donors selected, based solely upon allozyme genotype, produced variable numbers of male zooids, and so provided a range of variation in male gonad allocation to which siring success could be compared.

Methods of Collection and Laboratory Culture

Adult colonies used in mating experiments were settled from larvae by methods modified after Cancino and Hughes (1987). Near the laboratory dock of the University of Maine's Darling Marine Center in the Damariscotta River, Maine, I collected fronds of *Laminaria longicruris* that were heavily encrusted by *C. hyalina* colonies. Fronds were held in running sea water and total darkness for 16 h, then transferred to glass aquaria and illuminated with fluorescent light. Copious release of larvae ensued. Larvae were collected and placed in rectangular tanks containing glass microscope slides held in slide boxes. Circulating flow in these tanks was used to induce rapid larval settlement (McCartney 1994). Juvenile colonies on slides were reared in the laboratory in flowing sea water and fed every two days with an algal suspension consisting of equal volumes of *Tetraselmis* sp., *Isochrysis* sp., and *Rhodomonas* sp.

Survey for Electrophoretic Markers

At approximately three to four weeks of age, just prior to expression of sexual maturity under laboratory growth conditions, I screened colonies for allozyme markers. I removed small portions (5–10 zooids) and placed them into 10 μL of cold homogenization buffer (10 mL 0.1 M Tris-HCl pH 7.5, 10 μL Triton X-100 (Sigma), and 10 μL ß-mercaptoethanol) in a multiwell grinding plate. Samples were quickly homogenized and were held at 4°C prior to electrophoresis. Homogenates were electrophoresed on horizontal cellulose acetate membranes (Titan III membranes, Helena Laboratories, Beaumont, Texas). Membranes were run at room temperature for 1 h at 200 V, using 0.025 M Tris/0.192 M glycine pH 8.5 as a running buffer, then stained for the enzyme glucose-6-phosphate isomerase (GPI) using a method modified from Richardson et al. (1986). Colonies from the Damariscotta River are polymorphic for at least seven GPI alleles, four of which are rare (see results). Allele designations were expressed in units of percent mobility relative to the most common allele, which was designated GPI-100.

Design and Construction of Mating Arrays

Each array generated a fertilization success estimate for a single sperm donor, which could then be compared to its male gonad allocation. The donor, homozygous for a diagnostic GPI allele, was positioned at the center of the array and surrounded by four peripheral target colonies lacking this allele. Sperm donors were homozygous for diagnostic alleles GPI-80 or GPI-120. In 1991, target colonies were each 100/100 homozygotes, while in 1992, one of the four target col-

onies on each array was heterozygous for a rare allele (either GPI-30, -60, or -140), while the other three target colonies were each 100/100 homozygotes. The 1992 design included rare alleles to detect self-fertilizations, since previous field experiments estimated high selfing rates (Yund and McCartney 1994).

Sections of slides holding chosen colonies were excised using a glass cutter, centered on the surface of a square mount of black plexiglass, and attached using 100% silicon cement. Plexiglass mounts measured 2.5 cm^2, and were backed with a thin square of galvanized steel. The steel backing held the mounts in place against magnets imbedded at selected positions on a large plexiglass plate, which served as the mating array. In 1991, arrays measured 24 cm × 24 cm, while in 1992, their size was reduced to 15 cm × 15 cm to ease handling. The four peripheral colonies surrounded the center colony (sperm donor) in a cross pattern, each placed so that their centroids were 2.5 cm from the centroid of the sperm donor. In each year of the study, sperm donors on one-half of the arrays were GPI-80/80 homozygotes, while the remaining arrays used a GPI-120/120 sperm donor, so as to check for any differential fertility effects associated with these allozymes. Eight arrays were assembled in August 1991, and 12 in August 1992.

Study Site

The field experiments were performed at a subtidal site in the Damariscotta River, located about 100 m east of Hodgson's Island, and 4.5 km down river from the marine station. Salinities here are close to marine levels over most of the tidal cycle (McAlice 1979). Arrays were placed at 10-m depth on a smooth, featureless seabed consisting mostly of hard-packed sand and small cobblestones. The area is well-flushed due to its proximity to a major constriction in the river, and tidally driven currents often reach velocities of > 30 cm/s during ebb and flood (pers. obs.). Flow is not bidirectional at this site, as it is elsewhere in the estuary (Yund and McCartney 1994), and shows frequent and irregular directional fluctuations, especially during flooding tides (McCartney, pers. obs.). The nearest populations of *C. hyalina* are found in a small kelp bed about 50 m shoreward, and in a much larger bed located about 200 m down river. Colonies occur only infrequently on the bottom substrate within and outside of kelp beds.

A laboratory flume study conducted at representative unidirectional flow velocities previously demonstrated very similar vertical flow profiles at different positions on the plates (McCartney 1994). Velocities in that study were measured using a LASER-Doppler velocimeter, with the beam aimed 0.5–1.0 mm above the surface of the plates, so results should characterize flow conditions near the site of sperm discharge. It is therefore likely that colonies experienced similar fine-scale flow conditions regardless of their position on the mating array during periods of unidirectional flow. Effects of flow patterns on sperm transport that were present in the field and not anticipated from results of the flume study, moreover, could not have biased associations between sperm donor phenotype and fertilization success. Individual arrays were placed on the sea bed at haphazardly selected positions, and prior to the time when gender allocation of sperm donors was expressed.

Field Experiments

The mating arrays were bolted 5 cm above large concrete pads using threaded stainless steel rod. The surface of the plate to which colonies were attached was faced downward in 1991; in 1992, I turned them face-up to prevent losses to abrasion by crabs and snails that wedged themselves under the plates. The arrays were placed on a rectangular rope grid, with each array spaced 10 m from the next nearest one to prevent cross-contamination by sperm released on different arrays. The site was regularly patrolled by divers, who eliminated juvenile *C. hyalina* colonies that had settled on the arrays or anchoring pads, and removed drift kelp encrusted with adult colonies that had become entangled on the grid. These precautions succeeded in holding contamination from extraneous sperm sources to low levels (see results).

Colonies remained in the field for two to three weeks to permit growth and development of sexual zooids. Divers then removed the arrays, placed them in watertight containers, and returned them to the laboratory, where they were kept isolated from one another and held in flowing seawater. Using a camera lucida and an image-analysis system (Motion Analysis, Inc., Santa Rosa, CA), I traced the outline of each colony to obtain colony areas. I enumerated sexual zooids using a method adopted after Hughes and Hughes (1986). Triangular subsections of each colony were selected at random, and all sexual zooids contained within these subsections were counted and their gender noted. Next, I divided colony area by the proportion of this area that was subsampled for sexual zooids to yield an estimate of the total number of male and female zooids on the entire colony. Counts of functional male zooids (those packed with sperm) were used as a measure of male gonad investment, and counts of functional female zooids (those containing eggs and/or developing embryos) were used to estimate female fecundity. In no case did colonies being scored for size and reproductive characters remain in the laboratory for more than 24 h before being returned to the field.

Colonies were replaced on arrays with their positions unchanged, and arrays were returned to the site so as to preserve their original directional orientation. Arrays then remained at the field site for 16 d, which equals the average duration of the *C. hyalina* brooding cycle in Maine (McCartney 1994). Late-stage embryos present at the end of this period must then have been sired soon after replacement of the arrays at the field site. Sexual zooid counts and colony sizes should therefore approximate those present at fertilization.

Progeny Harvesting and Electrophoresis

Arrays were retrieved for a final time, returned to the laboratory, and handled as above. I harvested a subsample of 24 late-stage embryos from each colony that was brooding more, and the entire brood from colonies bearing fewer than this number. Harvested embryos were opaque white, filled the entire ovicell (150–200 μm in diameter), and possessed eyespots and ciliary activity; these features are present at 12–14 d of age (McCartney, pers. obs.).

Embryos were harvested by cracking the ovicell using a fine needle and forcing a stream of water over it to free the embryo. I collected embryos in 1 μL seawater and transferred each into 2 μL grinding buffer in the wells of an HLA plate held on ice, where embryos were homogenized using a fine, blunted needle. Electrophoresis and staining were conducted as described above. Heterozygous embryos from target colonies were assigned to sperm donor paternity if they possessed the donor's diagnostic allele. Target-colony progeny that were homozygous for target-colony alleles could not be unambiguously assigned. Embryos from sperm donors that were homozygous for the donor-colony allele were inferred to have developed from self-fertilized eggs. Progeny carrying rare alleles that were harvested from target colonies heterozygous for the same rare alleles were also assigned to selfing events. Finally, embryos carrying alleles not included on the array were assigned to exogenous sperm sources (contamination).

Estimates of Paternal Success and Correction for Contamination

Fertilization success of a sperm donor colony on a target colony was calculated as the proportion of target colony progeny that carried the donor marker allele. To calculate number of eggs fertilized, I multiplied these proportions by the number of female zooids counted on each target colony. Each ovicell broods one embryo per cycle, and cycle duration shows only minor variation (McCartney 1994). Some female zooids carry an egg in the zooid body at the same time that an embryo is being brooded in the ovicell, while in others, only an egg or an embryo is present (Hughes 1987; pers. obs.). Brooding cycles, moreover, are not synchronized across a colony's multiple female zooids; hence, an unknown proportion of them will contain a fertilizable egg at any given time. Therefore, counting all female zooids overestimates the number of eggs available, but the degree of error should not differ among different target colonies. Absolute paternity values were summed to yield the total number of progeny sired on the entire array. To calculate the proportion of all progeny on the array sired by the sperm donor, I divided the total number of embryos it sired by the total number of female zooids (eggs available) on the array.

Sperm donors chosen from natural populations were homozygous for common, but diagnostic, GPI alleles. In this manner, a set of sperm donors spanning a range of male gonad allocation could be collected without having to breed for rare-allele homozygotes (cf. Grosberg 1991). Use of common alleles presents another problem, however, since an appreciable number of colonies in adjacent natural populations will carry them. Sperm arriving from outside the arrays can contribute diagnostic alleles, and the "contaminant" fertilizations that result can be falsely assigned to sperm donors upon the arrays. To adjust for this problem, the following approach was adopted. The sperm donor used on each array was homozygous for either GPI-80 or GPI-120. Embryos collected from the array that carried the GPI allele of this pair that was not present on the array were assigned to contaminant sperm donors. Natural population frequencies of these two alternative alleles were very similar (see results). Therefore, their frequencies in pooled sperm spawned by naturally occurring colonies should also be similar; that is, if background contaminations result from a random sample of gametes from spawning adults in adjacent populations, which is the most parsimonious assumption.

Consider an array in which the sperm donor was GPI-80/80. The frequency of embryos collected from the array that carried the GPI-120 allele—those fathered by contaminant sperm—is calculated. This frequency should also estimate how often contaminant sperm contributed the diagnostic GPI-80 allele and thereby inflated estimates of paternity for the sperm donor, and this false paternity can be subtracted out using an adjustment factor. Such an approach assumes that the probability of fertilization by sperm carrying a given allele relates only to its frequency in a pooled spawn and not to its haploid genotype per se. A previous study of paternity in *C. hyalina* showed that fertilization success was not related to GPI genotype (Yund and McCartney 1994), so this final assumption appears valid.

I developed a simple formula to correct for the incidence of contamination. The frequency of occurrence of detected contaminant alleles was first calculated. The number of progeny (X_i) from target colony i that were found to carry the nonincluded allele was used as the index of contamination. This value allows calculation of the expected number of (undetected) contaminated progeny carrying the donor-colony allele ($E[X_{D_i}]$) and the target-colony allele ($E[X_{T_i}]$) by using population allele frequencies. For illustration, on an array in which the central colony was a GPI-80 homozygote, and in which three contaminant progeny (100/120 heterozygotes) were harvested from a target colony:

$$(E[X_{D_i}]) = X\left(\frac{r}{t}\right) = 3(0.261/0.202) = 3.8, \quad (1)$$

and similarly

$$(E[X_{T_i}]) = X\left(\frac{s}{t}\right) = 3(0.507/0.202) = 7.5, \quad (2)$$

where adult population allele frequencies of GPI-80 = r = 0.261, GPI-100 = s = 0.507, and GPI-120 = t = 0.202 (see results). Note that relative allele frequencies are each calculated with the frequency of the included allele in the numerator, and of the nonincluded contaminant allele in the denominator. These expected values are next subtracted from the actual values obtained via progeny analysis (N_{D_i} for progeny assigned to the donor colony, and N_{T_i} for those assigned to the target colonies) to yield the corrected paternal success of the central colony (P'_{D_i}) on target colony i:

$$P'_{D_i} = \frac{N_{D_i} - E[X_{D_i}]}{(N_{D_i} - E[X_{D_i}]) + (N_{T_i} - E[X_{T_i}])}. \quad (3)$$

This formula (3) excludes the tallies of contaminated progeny from the denominator, and hence estimates the proportional success of the donor colony had no contamination occurred. A second formula (4) includes contaminated progeny in the denominator:

$$P''_{D_i} = \frac{N_{D_i} - E[X_{D_i}]}{(N + X)_i} \quad (4)$$

where $(N + X)_i$ denotes the total number of progeny harvested from colony *i*. In effect, use of correction (3) explicitly limits the mating-group size to only those five colonies present on the array, while use of correction (4) allows fertilizations by an unknown number of "foreign" males to be included in the calculation of competitive fertilization success of the sperm donor.

Data Analysis

I first analyzed patterns of paternal success across individual mating combinations (i.e., among target-colony broods). Siring success showed high variance among the four target colonies within arrays (see results). Furthermore, distributions of both the proportion and the absolute number of embryos sired per brood over all arrays were strongly skewed, and could not be transformed to yield normality. Therefore, to judge the correlation between embryos sired per brood and male allocation of the sperm donor, I grouped sperm donors according to whether they produced greater than or fewer than the median number of male zooids (across sperm donors). I then compared the distributions of fertilization success between these two classes of colonies using nonparametric tests.

Values for sperm donor total fertilization success—calculated as the proportion of all embryos on each array that was sired by the sperm donor—were normally distributed and did not differ between years (see results). This permitted analysis of the functional relationship between siring success and male zooid number through parametric regression analyses. I used linear models to regress paternal success on number of male zooids, and *t*-tests to determine whether the y-intercept values differed significantly from zero (Sokal and Rohlf 1981). Nonlinear relationships between fertilization success and number of male zooids were evaluated by performing linear regression, after log-transforming both the dependent and independent variables. This fits male success (Y) to male zooid number (X) by the power function $Y = aX^b$, where a and b are constants derived from the linear equation $\log Y = \log a + b (\log X)$. Theoretical male gain relations in hermaphrodites are typically modeled using power functions (Charnov et al. 1976; Charnov 1979; Lloyd 1984). The presence of a decelerating function was evaluated by testing whether the exponent b was significantly less than one. Linear and power fits were performed on uncorrected paternal success, and on values corrected for contamination using equations (3) and (4) above.

All of the above analyses addressed fitness return from absolute investment into male reproduction, and not relative (male vs. female) investment, the usual currency of sex allocation theories. Sex allocation theory assumes that relative investment is tightly linked to relative transmission of genes through male versus female gametes (Charnov 1982), but this relationship has rarely been addressed (but see Broyles and Wyatt 1990). Relative female versus male "phenotypic gender" expressed by hermaphroditic plants is best represented as a continuous variable lying between male-only and female-only extremes (Lloyd 1980a,b; Lloyd and Bawa 1984; Devlin and Stephenson 1987; Broyles and Wyatt 1990). Recognizing that *C. hyalina* likewise shows continuous, quantitative gender variation (McCartney 1994), and adopting Lloyd's (1980a,b) convention, I computed the phenotypic gender (G_p) of *C. hyalina* colonies as the proportion of all sexual zooids (female + male) that were female. Next, I computed the proportion of reproductive success a colony gained through female function as:

$$G_{R_i} = \frac{f_i}{(f_i + m_i)} \tag{5}$$

where f_i denotes the number of embryos brooded by colony *i*, m_i denotes the total number of eggs that colony *i* fertilized, and G_{R_i} is its actual or realized gender. The Pearson correlation between realized gender and phenotypic gender values, both arcsine-transformed, was then computed.

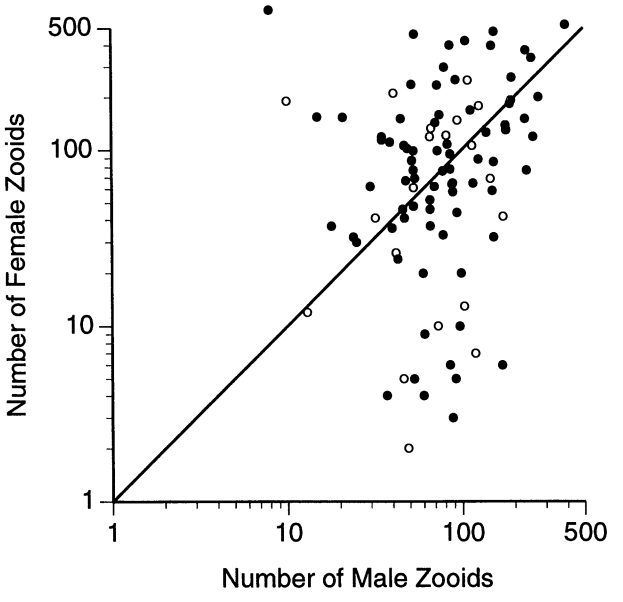

FIG. 1. Scatter plot of number of male zooids and number of female zooids on each experimental colony. Each point represents a single colony; open circles are sperm donors and closed circles are target colonies. The diagonal line marks equal numbers of male and female zooids; colonies above the line express female-biased and those below the line express male-biased sex allocation. Both x- and y-axes are on a logarithmic scale.

RESULTS

As found in a larger survey of natural populations of *C. hyalina,* colonies chosen for the arrays showed widely variable numbers of both male and female zooids (Fig. 1). Sex allocation variation among sperm donors was not atypical. Dispersion of female:male sex allocation estimates among sperm donors and among target colonies was similar and well balanced (Fig. 1): 10 donors showed male bias ($G_p < 0.5$) and 10 donors showed female bias ($G_p > 0.5$), while 41 target colonies showed male bias, 37 showed female bias, and two showed equal allocation. The mean and variance in male zooid numbers on sperm donors did not differ from that on target colonies (means similar: Wilcoxon two-sample test, $P > 0.4$; variances homogeneous: Brown-Forsythe (1974) test, $F = 1.18$, $P > 0.2$). Importantly, sperm donors were well-

TABLE 1. Allele frequencies at the glucose-6-phosphate isomerase (GPI) locus in *Celleporella hyalina* collected from two different locales and in two different years. N = number of colonies; colonies were adults removed from kelp fronds, or juveniles settled from larvae.

GPI allele	Source		
	1991 Hodgson's Island (adults)	1991 Lab Dock (adults)	1992 Lab Dock (juveniles)
30	—	—	.0005
40	—	—	.003
60	.029	.010	.005
80	.261	.219	.254
100	.507	.531	.509
120	.202	.229	.220
140	—	—	.008
N	69	48	1075

dispersed over a 17-fold range in male zooid counts, such that fertilization success could be assayed.

To minimize disturbance of test colonies, determinations of size and counts of sexual zooids were conducted only once, meaning that lifetime sex allocation could not be determined. Lifetime sex allocation of colonies from natural populations, however, has been shown to be accurately predicted by sex allocation at the age or size equal to or greater than the age or size when sexual zooid numbers first reach their peak, while allocation at earlier ages or smaller sizes is more male-biased (McCartney 1994). Compared to colonies from a nearby natural population at Hodgson's Island (HI) observed at peak sexual zooids (McCartney 1994), test colonies in the present study were similar in age (42 d [1991 arrays] and 56–70 d [1992 arrays] vs. 47 ± 1.2 d [$\bar{X}$ ± SE for the HI population]), were larger (752 ± 22 autozooids vs. 400 ± 20.4 autozooids for HI), and had more sexual zooids (215 ± 16 vs. 155 ± 13.1 sexual zooids for HI). Average sex allocation for test colonies was very similar to that for the natural population (G_p = 0.482 ± 0.024 vs. G_p = 0.488 ± 0.030 for HI). Therefore, lifetime sex allocation of test colonies was likely to be similar to the single-point value recorded in the present study.

Screening of adult colonies in 1991 and of juveniles settled from larvae in 1992 uncovered seven electromorphs at the GPI locus (Table 1), three of which were common enough to be regularly recovered from homozygotes. Genotypic frequencies calculated for the three most common alleles did not differ from Hardy-Weinberg expectations (Goodness-of-fit test: G = 3.877, df = 5, $P > 0.5$), and R × C contingency table analyses showed no allele frequency differences between years (likelihood ratio χ^2 = 5.776, df = 3, $P > 0.1$) or between sites sampled in 1991 (likelihood ratio χ^2 = 0.707, df = 2, $P > 0.7$). The enzyme stained reliably in extracts prepared from single larvae, and no mobility differences were found between larval and adult enzymes. Alleles used to mark sperm donors were commonly found in progeny harvested from target colonies, and foreign (contaminant) alleles were also found, but their frequency was low. Averaged across all (N = 100) maternal colonies, only about 4% of all embryos arose from contaminant fertililizations, and contamination was undetected in fully 50% of all broods.

Sperm donors were estimated to have sired from zero to 249 (mean = 34) embryos per target colony (N = 80 target colonies; self-fertilizations excluded). The proportion of each target colony's eggs fertilized by the sperm donor ranged from zero to 0.92. Distribution of both absolute and proportional paternity values showed a preponderance of low-success classes (Shapiro-Wilk test rejected normality: W = 0.645, $P < 0.001$ and W = 0.915, $P < 0.001$, respectively). Across the four target broods on any given array, siring success was far from uniform and instead showed great variation (Fig. 2). In 11 of 20 arrays, the sperm donor failed to fertilize eggs on at least one out of the four available target colonies. In only a minority of cases (14 of 80) did the sperm donor fertilize more than half the eggs of any target colony, and the proportion fertilized, totaled across the four target colonies, was less than 0.5 on every array (Fig. 2). Sperm donors did not monopolize fertilizations; instead, shared paternity was the rule.

Sperm donors were divided into two classes corresponding to whether they formed fewer than or greater than the median number (70) of male zooids. The distribution of absolute paternal success values for colonies with < 70 male zooids differed significantly from that for colonies with > 70 male zooids (Kolmogorov-Smirnov two-sample test: $P < 0.01$; Fig. 3), and colonies above sired more young than those below the median number of male zooids (Mann-Whitney U = 418, $P < 0.001$). This reproductive advantage was not due to differences in numbers of eggs available—the proportion of eggs sired per brood (data not shown) was also greater for high male-investment than for low male-investment colonies (Mann-Whitney U-test, $P < 0.01$).

On each of the arrays, I collected a few sperm-donor progeny that were homozygous for their GPI allele. These were treated as offspring of putative self-fertilization events (see Yund and McCartney [1994] for a more detailed discussion). In the present study, however, very few of all embryos (1–5 %; data for individual cases not shown) were assigned to selfing events, rates that were much lower on average than in Yund and McCartney (1994). No progeny with rare alleles were harvested from target colonies carrying diagnostic rare alleles, indicating a zero selfing rate for these colonies.

The total number of progeny sired by each sperm donor was calculated by summing over each of the five target colonies (including progeny sired through selfing). The distribution of these summed values, when square-root transformed, was not significantly different from normal (Shapiro-Wilk W = 0.920, $P > 0.05$). Total paternal success was an increasing function of the number of male zooids formed by sperm donors, and donors on 1992 arrays showed higher paternal success than they did in 1991 (data not shown). I performed an ANCOVA, using number of male zooids as a linear covariate, and year of study as the fixed treatment effect. Slopes of the regression lines estimated for each year were homogeneous (F = 0.09, $P > 0.5$). Results for the ANCOVA model (Table 2) showed significant differences between years (F = 11.210, $P < 0.01$), and number of male zooids was a significant covariate (F = 9.018, $P < 0.01$). Target colonies in 1992 formed on average many more female zooids (mean = 183) than they did in 1991 (mean = 49), and this difference was highly significant (Mann-Whitney U = 238.5, $P < 0.001$). Hence greater absolute paternal success

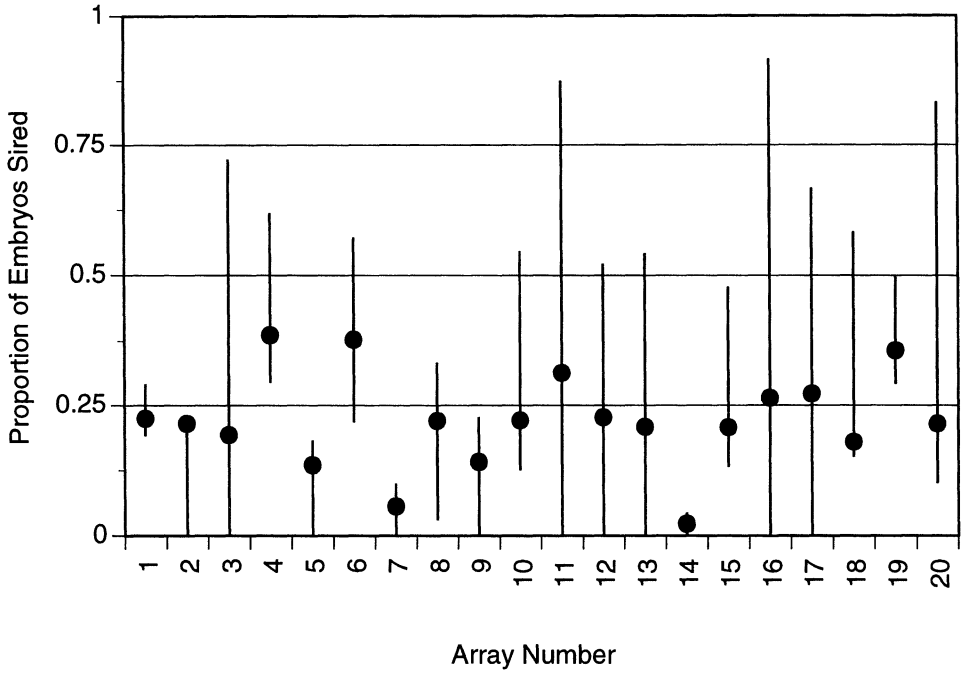

FIG. 2. Variation in siring success of sperm donors among target colonies within mating arrays. Numbers on the x-axis represent the array designation; those on the y-axis are the proportion of each target colony's brood that was sired by the sperm donor. Vertical bars extend from maximum to minimum per-brood values, and filled circles represent the array totals (proportion of embryos sired, totaled over all four target colonies on the array). Values are not corrected for contamination.

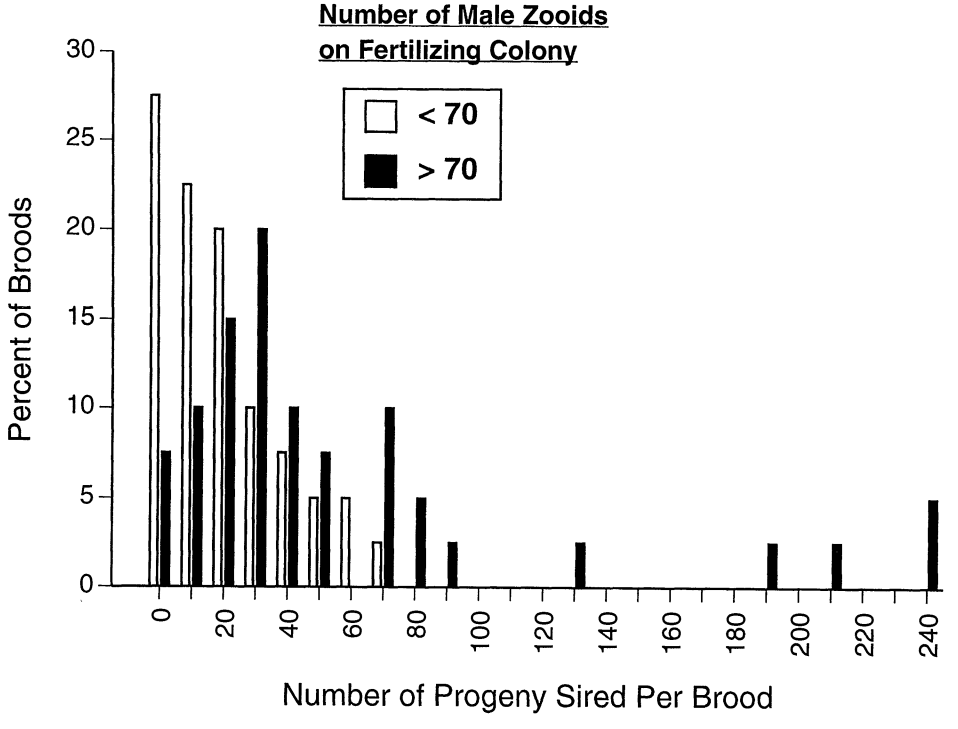

FIG. 3. Distributions of siring success per brood, grouped with respect to sperm donor male allocation. Values for number of progeny sired per target-colony brood are placed into classes limited by the upper class mark on the x-axis, and are grouped with respect to whether the sperm donor formed less than or more than the median number (70) of male zooids. The y-axis values are the percent of all harvested broods in which sperm donors sired the indicated number of progeny.

TABLE 2. Results of ANCOVA for effects on paternal fertilization success of year of study and of number of male zooids formed by the fertilizing colony. Dependent variable is square-root transformed total number of progeny sired on each array. The main effect is year of study, and the number of male zooids is the covariate.

Source	df	SS	4F	P
Year of study	1	130.08	11.21	0.004
Number of male zoiods	1	104.64	9.02	0.008
Error (dev. from regression)	17	197.26		

for sperm donors in 1992 was due to the fact that they mated with target colonies that carried larger broods than did target colonies in the previous year.

Values for the proportion of all eggs harvested from each array that were fertilized by the sperm donor were normally distributed (W = 0.944, $P > 0.30$), and did not differ between years (ANOVA: $F = 0.024$, $P > 0.5$). Since calculation of proportional paternal success removed the confounding effects of differences in numbers of available eggs, I therefore pooled results from both years to examine the gain curve relating male fertilization success to numbers of male zooids.

The pooled data showed a significant positive linear relationship ($F = 6.909$, $P = 0.017$) between the number of sperm donor male zooids and the proportion of eggs that they fertilized (Fig. 4). Log-transformation of both the dependent and independent variables prior to analysis was used to fit a power function to these data (see materials and methods). Linear regression of log proportional paternity on log number of male zooids was significant ($F = 7.899$, $P = 0.016$), and the estimated exponent of the power function (b = 0.484 ± 0.172 SE) was significantly less than one ($t_s = -2.996$, $P < 0.01$). The power function was decelerating (Fig. 4), but explained very little additional variation in paternal success ($r^2 = 0.305$) than did the linear function ($r^2 = 0.277$).

One interpretation of the y-intercept of the linear regression equation is as an estimate of the percent contribution from foreign sperm of sperm donor diagnostic alleles (undetected, "false positive" paternity assignment) since it corresponds to the percent occurrence of the marker alleles on a hypothetical array where the sperm donor produced zero male zooids (i.e., was incapable of producing sperm). The y-intercept was 0.134 ± 0.037 (SE), which is significantly greater than zero ($t_s = 3.560$, $P < 0.01$). I then applied corrections for contamination to the data from each target colony. A plot of corrected values (using equation [3]) against numbers of male zooids again revealed a positive relationship (Fig. 5), with a steeper slope than the plot of uncorrected values showed. Linear regression of the corrected values on numbers of male zooids was highly significant ($F = 14.698$, $P = 0.001$) and explained much more variation ($r^2 = 0.449$) than did regression on uncorrected values. The correction apparently did reduce contributions from contamination; the new equation yielded an intercept (0.061 ± 0.056 SE) not different from zero ($t_s = 1.091$, $P > 0.2$).

This time, the power (log-log) fit using corrected proportional paternity values was significant ($F = 5.055$, $P = 0.037$), but explained much less variation ($r^2 = 0.219$) than the linear fit to the corrected data. The exponent obtained from the power fit of corrected values (1.016 ± 0.452 SE) did not differ significantly from one ($t_s = 0.035$, $P > 0.9$). Use of the second correction equation gave similar results. The linear fit to values corrected using correction (4) was significant ($F = 9.499$, $P = 0.006$) and yielded an r^2 value

FIG. 4. Gain curves relating male fitness (fertilization success) to the number of male zooids produced by the sperm donor. Number of male zooids is on the x-axis; the y-axis shows the proportion of all embryos harvested from the array that were sired by the sperm donor colony. Open circles = 1991 data, filled circles = 1992 data. Data are not corrected for contamination. Lines show least-squares regressions. Linear equation: $Y = 0.135 + 0.0011\ X$; curvilinear equation: $Y = 0.026\ X^{0.484}$.

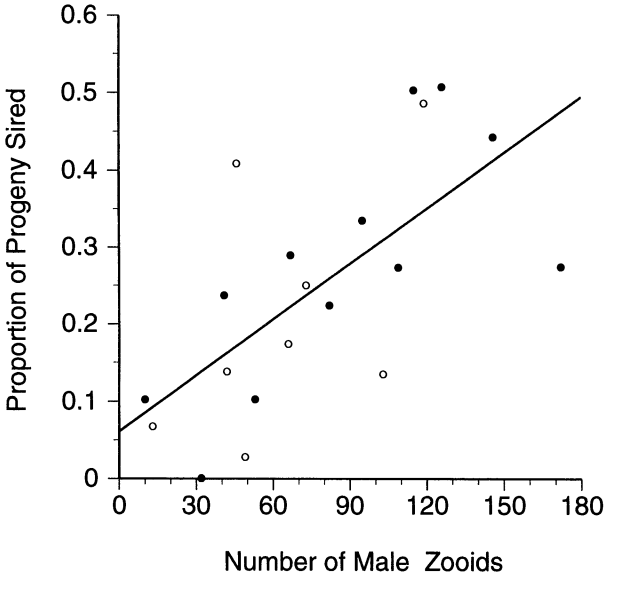

FIG. 5. Male gain curve fitted to data corrected for contamination by foreign sperm. Values were corrected using equation (3). The line depicts the linear equation $Y = 0.061 + 0.0024\ X$ from least-squares regression of the corrected array-total proportion of progeny sired (y-axis) on the number of male zooids formed by the fertilizing colony (x-axis). See Table 3 and text for power fit and tests of significance.

TABLE 3. Summary of results from regression analyses of the relationship between male zooid number and fertilization success. In all analyses, the dependent variable is the proportion of all embryos collected from the array that were sired by the sperm donor, and the independent variable is the number of sperm donor male zooids. Linear fits are from regressions on untransformed variables; power fits are of the form $Y = aX^b$, and are derived from linear regression with both the dependent and independent variables log-transformed. Intercept values are the y-intercepts from linear regression, and exponent refers to b in the above power function; for both parameters, ns = not significant, ** = $P < 0.01$. F (regression) refers to the F-test on the regression coefficient, P is its level of significance, and r^2 is the coefficient of determination of the regression model.

		Parameters		Significance tests		
Type of fit	Data	Intercept	Exponent	F (regression)	P	r^2
linear	uncorrected	0.135**	1	6.909	0.017	0.277
power	uncorrected	0	0.484**	7.899	0.016	0.305
linear	corr 3	0.061ns	1	14.698	0.001	0.449
power	corr 3	0	1.016ns	5.055	0.037	0.219
linear	corr 4	0.076ns	1	9.498	0.006	0.345
power	corr 4	0	0.953ns	4.481	0.049	0.199

that was intermediate between that from the two previous analyses (Table 3). Again, the intercept from this analysis did not differ from zero ($t_s = 1.702$, $P > 0.1$), and the exponent of the power function (0.953 ± 0.450 SE) was not different from one ($t_s = -0.104$, $P > 0.9$). Adjusting for contamination using both correction equations yielded a linear gain curve relating male fitness to increasing production of male sexual zooids. Results of all regression analyses of gain curves are summarized in Table 3.

Both phenotypic and realized gender values for sperm donor colonies were continuously distributed between nearly pure male and pure female extremes. All colonies gained reproductive success through both male and female function; no colonies functioned exclusively as males or as females (Fig. 6). Overall, phenotypic and realized gender were well correlated ($R^2 = 0.702$; Fig. 6). Hence, sex allocation estimated by male and female zooid counts was a good predictor of a colony's relative maternal versus paternal reproductive success.

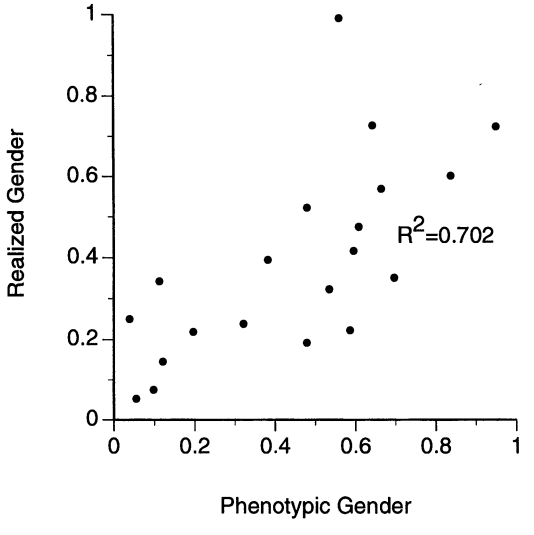

FIG. 6. The relationship between each focal sperm donor colony's phenotypic gender (number of female zooids/number of sexual zooids) and its realized gender (number of embryos brooded/[number of embryos brooded + number of embryos sired]).

DISCUSSION

This study is the first to demonstrate that male reproductive success in a marine invertebrate increases with male gonad allocation. The internal volume of male zooids varies little among *C. hyalina* colonies (Morris 1980; pers. obs.), so gonad volume will closely parallel male zooid number. Higher volume sperm production is therefore likely to underlie the fertilization success advantage of additional male zooids. Sperm in *C. hyalina* are shed externally, while eggs are retained and fertilized internally. Egg retention may allow accumulation of sperm from the water column, such that ambient sperm concentrations do not as severely limit the percent of eggs fertilized as when eggs are broadcast spawned (Denny and Shibata 1989; Yund and McCartney 1994; Levitan and Petersen 1995). In *C. hyalina* colonies reared in the field, an average per colony of less than 10% of eggs ovulated failed to develop (McCartney 1994), which indicates that most eggs are fertilized. Hence greater siring success of donors releasing more sperm may not derive from higher ratios of fertilized to unfertilized eggs. Instead, donors may gain fertilizations competitively—at the expense of those available to rival colonies. Consistent with this hypothesis, high levels of sperm competition have been detected in *C. hyalina* (Yund and McCartney 1994) under conditions similar to those used in the present experiments.

Colonies may gain an additional advantage from producing more male zooids. Like reproductive ramets in other modular organisms, *C. hyalina* sexual zooids are capable of autonomous function. Embryonic brooding cycles within the multiple female zooids of a *C. hyalina* colony are timed independently of one another (Hughes 1987; McCartney 1994); one zooid can begin to brood a recently fertilized egg while another is releasing a larva. Similarly, if cycles of sperm maturation and release in different male zooids within a colony are not synchronized, a colony with more male zooids could release sperm over a longer time period. This would benefit male success, because eggs in neighboring colonies do not become fertilizable all at once, but continuously.

While male success increases with investment in male zooids, female success clearly declines. Sexual zooids in *C. hyalina* colonies occur in a single layer, the area of which is maximally limited by the area of the underlying layer of

feeding zooids (Cancino and Hughes 1988). Space occupied by male zooids therefore detracts from space available for female zooids. Since one egg per cycle is brooded, and since the number of brooding cycles per female zooid is limited (Hughes 1987), fewer female zooids must imply lower female fecundity. A trade-off between male and female reproductive success is therefore an inevitable consequence of male versus female zooid allocation.

None of the colonies used as sperm donors escaped this trade-off—all were intermediate in realized gender, meaning that each transmitted some fraction of their genes through eggs and the remainder through sperm. Furthermore, female versus male fitness (realized gender) and female versus male allocation (phenotypic gender) were closely correlated. The existence of such a correlation is a fundamental assumption of sex allocation theory (Charnov 1982; Devlin and Stephenson 1987), and leads to the further prediction of slight variation in sex allocation at equilibrium (Brunet 1992). However, in both the present study and in a previous one where zooids were counted over the entire breeding season (McCartney 1994), sex allocation varied between extreme values of nearly pure maleness through pure femaleness. In neither study were frequency distributions clustered around a modal value, rather, colonies showing all levels of sex allocation bias were common. Facile adjustment to environmental conditions favoring female or male reproductive success might account for such broad variation. However, in a previous study, Hughes and Hughes (1986) split *C. hyalina* colonies into clonal fragments, then grew the fragments under different flow conditions (Hughes and Hughes 1986). While flow affected growth rates, clones retained similar values of sex allocation in the face of environmental variation. This calls for further studies of genetic and environmental contributions to sex allocation variation in *C. hyalina*.

While the causes of sex allocation variation remain to be explored, average sex allocation fits theoretical expectations. Analyses in this paper showed greater support for linear than for decelerating male gain. Linear fitness gain should select for equal male and female allocation, whereas decelerating male gain should select for female bias (Charlesworth and Charlesworth 1981; Charnov 1982; Lloyd 1984). For the 100 colonies used in these experiments, mean (± SE) phenotypic gender was 0.482 ± 0.024. Samples from two natural populations differ, but never show female bias (McCartney 1994). One population showed equal allocation (mean G_p = 0.488 ± 0.030; N = 129 colonies), while the other showed male bias (mean G_p = 0.236 ± 0.018; N = 281 colonies). Results showing nondecelerating male gain and lack of female bias in average sex allocation are consistent with one another.

Of course, the precise relationship between zooid gender ratios and resource sex allocation is unknown. Resource expenditure per male zooid per unit time will depend on that required to build the zooid, manufacture gonad, and on that lost through sperm release then reallocated to replenish gonad tissue. Similar quantities will apply to female zooids, with the added (and perhaps large) expenditure necessary to nourish an embryo for 16 days. Sex allocation estimates from zooid counts therefore only serve as crude estimates of the standing crop ratio of female:male gonad volume, and not of female versus male "productivity."

Despite these caveats, measures of female versus male gonad volume have been used to estimate sex allocation in other simultaneously hermaphroditic animal species, and results have qualitatively fit theoretical expectations. For example, pair-spawning in marine fishes is thought to produce rapidly diminishing male fitness returns, hermaphroditism, and female-biased sex allocation, because individuals are not selected to produce more sperm than is required to fertilize eggs of their single mating partner. Ovary volume far exceeds testis volume in several species of pair-spawning sea basses (Fischer 1981; Petersen 1991). Pair mating and highly female-biased gonad ratios occur together in the simultaneously hermaphroditic polychaete worm, *Ophryotrocha diadema* (Sella 1990). Barnacles can copulate with only a few neighboring individuals, and produce higher volumes of ovary than of testis (Raimondi and Martin 1991). Since average values of gonad allocation in *C. hyalina* are not female biased, male gain is not expected to strongly decelerate, and this is exactly what the present results indicate.

In local mate competition models applied to SH, male fitness is bounded by an upper limit on the number of mates that males have access to—the so-called mating group size (Charnov 1980, 1982; Fischer 1981, 1984; Lloyd and Bawa 1984). In mobile organisms like the pair-spawning fishes and polychaete worms mentioned above, this limit is enforced by the mating system. In immobile organisms that copulate, like barnacles, the size of the mating group is limited by the distance over which the penis can be extended. In sessile species that do not copulate but release sperm to the water column, like bryozoans, the size of the mating group will depend upon the spatial scale of water-borne sperm dispersal, as well as on the number and proximity of available mates. Measurements of sperm dispersal in other free-spawning marine invertebrate species have yielded estimates ranging over a scale of centimeters to a few meters (Pennington 1985; Yund 1990; Grosberg 1991; Levitan 1991; Brazeau and Lasker 1992).

While sperm dispersal distance, and hence mating group size, is unknown for *C. hyalina*, a reasonable range of values can be approximated. Density of colonies of *C. hyalina* varies widely among kelp fronds and among positions along frond surfaces (Cancino 1986; McCartney 1994), fronds censused in the summer in Maine harbored from one to as many as 50 colonies on a surface area of 25 cm^2. Hence, if sperm dispersal in *C. hyalina* were effective over just this area (corresponding to a radial distance just slightly greater than the distance over which fertilizations were detected in this study), a sperm donor could mate with anywhere from one to 50 colonies. At the low end, but not at the high end of this range of mating group sizes, male gain would saturate (Charnov 1980, 1982). With varying densities and spatial distributions of mates, mating group sizes would not be fixed but would vary, and the frequency distribution of group sizes could be used to predict the evolutionary consequences (Fischer 1984). Given considerable seasonal and spatial variation in population density of *C. hyalina* on kelp (pers. obs.), it is difficult to predict the outcome, but episodes of low density might be sufficient to promote local mate competition and favor hermaphroditism.

An important element of the local mate competition model

is the "saturability" of neighboring mates. Decelerating male gain results when donors can fertilize all eggs available, such that additional gametes released compete among themselves for fertilizations, and donors obtain proportionally fewer and fewer fertilizations (Fischer 1984; Lloyd and Bawa 1984; Petersen 1991). In the present study, I varied the gonad volume and potential sperm output of the donor, and asked whether, and at what level of gonad investment, sperm donors could saturate target colony eggs with sperm. Sperm donors fell far short of ever doing so. Colonies with the highest numbers of male zooids fertilized 50% or fewer of all eggs available. Rarely did a sperm donor sire even the majority of a single target colony's embryos: in over 80% of the harvested broods, the donor sired fewer than one-half of the progeny. In no case did a sperm donor sire the entire brood; instead, multiple paternity was the rule.

When fertilizations from foreign sperm are subtracted using the correction, it is clear that the four target colonies on the arrays, each of which formed male zooids and was capable of releasing sperm, acquired a considerable fraction of the fertilizations at the expense of the focal sperm donor. This is competition for fertilizations, and while appropriate controls to detect this effect (with rival colonies absent) were not performed in this study, they were included in previous experiments on *C. hyalina* (Yund and McCartney 1994). This earlier study showed sperm competition to be intense. When an additional (third) colony was present, sperm donors lost approximately 50% of the fertilizations they obtained when paired with single target colonies. Intensity of competition in the present study was likely to be even greater, since four colonies vied for the fertilization of any given target colony's eggs, and not two as in the previous study.

The effect of sperm competition between rival donors on male fitness gain in hermaphrodites has been modeled (Fischer 1981, 1984; Petersen 1991). When the number of individuals competing for fertilizations is low, sperm donors gain diminishing returns from increases in sperm output. Incremental increases in sperm output at some point are wasted because unfertilized eggs become exhausted, and eggs available per additional sperm released declines. However, as the number of sperm donors competing for fertilizations increases, these same increases in sperm output now allow displacement of sperm donated by competitors, permitting the donor access to an increased number of eggs. With low sperm competition, male gain is saturating, but becomes increasingly linear as the intensity of sperm competition rises (Fischer 1981, 1984; Petersen 1991). Therefore, incremental increases of sperm output by focal sperm donors in the present study may have increased displacement of rival-colony sperm from the site of fertilization. This scenario would be consistent with linear male gain.

An alternative explanation for the maintenance of hermaphroditism in *C. hyalina* might invoke nonlinear female gain due to the presence of embryonic brooding. One credible mechanism for this outcome has been applied to unitary organisms: as the maternal parent grows, resources captured for egg production and nutrition increase at a rate disproportionately higher than the rate of expansion of the brood space (Heath 1977, 1979; Strathmann et al. 1984). However, for colonial organisms that bud modular brood chambers more or less continuously as the colony grows, this mechanism seems unlikely to apply. In *Celleporella*, capacity to feed and to brood young should both expand as a function of colony area (proportional to the number of feeding zooids and female zooids, respectively).

Earlier anatomical studies suggested to many specialists that bryozoans were predominantly self-fertilizing animals, until Silén (1966, 1972) observed transport and capture of outcross sperm in several species. Progeny analyses (Yund and McCartney 1994; present study) provide direct evidence of primarily outcross mating in *C. hyalina*. Extensive population screening of the GPI locus yielded genotype frequencies conforming to Hardy-Weinberg expectations, again indicating a predominantly outcrossed mating system. Nevertheless, *C. hyalina* is self-compatible: adult colonies reared from larvae in isolation in the laboratory brooded viable young (Hunter and Hughes 1993; McCartney 1994). Furthermore, selfing rates for colonies in the field have been shown to decline as availability of outcross sperm increases (Yund and McCartney 1994). This suggests facultative selfing for reproductive assurance when outcross sperm is limiting.

In Yund and McCartney (1994), selfing rates ranged from 0–73% and averaged 28%, while in the present study, rates were estimated at 0–5%. Lowered selfing rates may have resulted from greater displacement of self by outcross sperm, as colonies in the present study were placed at higher density (five per similar-sized array previously holding two to three colonies) and formed more male zooids than in the previous study. Facultative selfing for reproductive assurance may help maintain hermaphroditism in animals (Strathmann et al. 1984; Charlesworth and Jarne 1993). Further studies of selfing in response to sperm limitation are necessary to evaluate this hypothesis in the case of *C. hyalina*.

This study indicates that sexual selection on male fertilization success may favor increased male gonad allocation in free-spawning marine invertebrates. Maximized sperm output may not be the only outcome, however. Mass synchronous spawns, greatly modified sperm and mechanisms of sperm transfer, and elaborate copulatory structures are familiar reproductive traits in marine species. Previously, these have been viewed as strategies evolved to counter dilution of gametes in the water column and enhance the percent yield of fertilized eggs (Giese and Kanatani 1987). But these traits may have been selected not only to increase efficiency of reproduction in the marine environment. Since males cannot monopolize access to females, competition for fertilizations may be widespread in invertebrates that release gametes externally (Brockmann et al. 1994; Yund and McCartney 1994), and sexual selection on male success in competition may play an important role in the evolution of reproductive traits in marine species.

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