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MALE SIZE, SPERM TRANSFER, AND COLONY FITNESS IN THE WESTERN HARVESTER ANT, *POGONOMYRMEX OCCIDENTALIS*

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Abstract.—Mating success in the western harvester ant, *Pogonomyrmex occidentalis,* increases with male size. We tested the hypothesis that increased mating success increases male fitness and the fitness of colonies that make large males by comparing the sperm content of males prior to and at the conclusion of the mating swarm. The number of sperm a male initially possesses is a function of male size, and large males transfer a greater proportion of their sperm than do small males. For colonies, the payoff per unit of investment is an increasing function of male size, and investment in large males is not equivalent to investing in a larger number of small males. Allocation ratios in species that show size variation in reproductives may need to be modified by the individual fitness functions.

Key words.—Ants, colony fitness, *Pogonomyrmex*, sex allocation.

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Fitness in social insects traditionally is measured as the genetic payoff to queens and workers from allocation to male and female reproductives. Usually the reproductive output of colonies is quantified by measuring investment, often presented as the total biomass, in males and queens (reviewed in Crozier and Pamilo 1996). Adjusting biomass measures to reflect energetic investment may be more accurate (Boomsma 1989). Uncorrected biomass measures may not accurately reflect the energetic investment in males and females (Boomsma 1989), and it may be preferable to correct the investment in males and females by the 0.75 power of the ratio of average male and female body weight to reflect the relatively greater cost of producing the smaller males (Boomsma and Isaaks 1985; Boomsma 1989). Regardless, both reproductive investment and colony fitness are measured by the number of grams of males and females produced.

The implicit assumption of equating the output of male and female biomass with colony fitness is that the partitioning of investment among males or among females is unimportant. The limitations of these assumptions have been addressed theoretically (Frank 1987a, b; Crozier and Pamilo 1993; Nonacs 1993). In particular, Macnair (1978) showed that when the size of reproductives affected the probability of mating or colony founding, the expectations of the allocation theory of Trivers and Hare (1976) need not be upheld.

Some empirical work suggests that individual reproductives are not always equivalent in fitness. Davidson (1982) found evidence of sexual selection in *Pogonomyrmex* harvester ants; larger males of both *P. desertorum* and *P. barbatus* had a higher probability of mating. In *P. occidentalis,* male size strongly influences mating success: Males collected in copula were significantly larger and differently shaped than randomly collected males from the mating aggregation (Wiernasz et al. 1995; Abell et al. 1999). Wiernasz et al. (1995) suggested the greater value of larger males should be taken into account when interpreting investment ratios and colony fitness because how greater investment in males (biomass or energetic investment) changes colony fitness will depend on how it is distributed among individuals. However, the linear fitness functions derived for male mating success (Abell et al. 1999) suggest that in *P. occidentalis* colonies may achieve equivalent fitness by partitioning allocation to a few large males or many smaller males.

The functions derived from comparisons of mated and random males indicate strong directional sexual selection on male size via mating success (Abell et al. 1999), however, copulation frequency may not be the most important component of individual fitness (Simmons and Siva-Jothy 1998). Males captured while mating may not transfer many sperm to females. For example, if large males are able to displace each other relatively easily, the time a male spends in copula with a female may be brief and male insemination success consequently low. Although insemination success is an incomplete measure of fitness, it should better approximate actual fitness. Studies of sperm competition in several other species of insects suggest that larger males are more likely to displace another male's sperm and are less easily displaced (McLain 1985; Lewis and Austad 1990; Simmons and Parker 1992; Ward 1993; Parker and Simmons 1994; Gwynne and Sneddon 1995).

A male *P. occidentalis* gains his entire insemination success through a single day of swarm mating. If the increased mating success of large males reflects higher fitness, then large males should transfer more sperm than small males. We tested the prediction that larger males have greater insemination success by quantifying sperm content in unmated males collected prior to the start of the mating flight and spent males collected at the conclusion of mating. A fitness advantage of large males will appear as a decrease in the slope of the relation between sperm count and body size in males before and after the mating swarm. We also examined whether colonies that allocate male investment among relatively few large males have payoffs equivalent to those that allocate investment among many smaller males.

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Materials and Methods

Study Organism

The western harvester ant, *P. occidentalis* Cresson, reproduces annually in large mating flights (Nagel and Rettenmeyer 1973; Hölldobler 1976). In western Colorado, the site of this study, reproduction is triggered by midsummer thunderstorms (Wiernasz et al. 1995). In this population, virtually all colonies consistently produce both males and queens (D. C. Wiernasz and B. J. Cole, unpubl. data), in contrast to species that have split sex ratios (e.g., Boomsma and Grafen 1990). Males aggregate on locally high hills and females fly to these aggregations. Mating flights typically begin in midafternoon (1500 h) and continue until approximately 1800 h. Male competition at the swarm is intense (Wiernasz et al. 1995; Abell et al. 1999), but females are actively involved in mating and can terminate copulations by biting (Hölldobler 1976; pers. obs.). Females mate multiply at the swarm (Cole and Wiernasz 1999) and then disperse to found colonies individually. Mortality of foundress queens is high; in most years fewer than 2% of newly founded colonies survive their first year (Wiernasz and Cole 1995; unpubl. data). Males remain on the hill until they die of desiccation the next day or are eaten by predators. Mating occurs on one day only.

Collection of Males

Males were collected from a population of *P. occidentalis* in western Colorado (Wiernasz and Cole 1995). Unmated males were collected directly from colonies; no more than five males were taken from a single colony. Males were collected individually as they emerged from the nest at the time of the flight. In colonies that had conspicuous male size variation, males at both size extremes were collected, otherwise the first five males to emerge were taken. Males from a subset of colonies were collected in reproductive traps prior to the mating flight. Reproductives were induced to emerge by watering the nest cone and were subsequently trapped (Cole and Wiernasz 2000). Although mating can occur inside the cages, it happens infrequently. The males collected from each experimental colony were sorted under refrigeration and five males were chosen for sperm analysis.

On 5 August 1997, we collected 260 mated males at a mating swarm adjacent to the main study area after the mating flight had finished (1830–1930 h). We attempted to collect over the size range present at the swarm. Although the mating status of these males could not be determined, all males had the opportunity to mate.

Males were stored individually in 1.6-ml microcentrifuge tubes, grouped in sealed plastic bags, and stored at 5°C in Colorado for 1–2 days before express shipment to the University of Houston. Before shipping, ants were checked to make sure they were still alive, and any dead ants were removed. Upon arrival in Houston ants were checked again, and dead ants were discarded. The remainder were stored at −80°C until dissected for sperm analysis.

Sperm Analysis

Our protocol is modified from that of Sakaluk and O'Day (1984) and uses fluorescent staining (Hoechst 33528) of DNA to visualize the sperm. For unmated males, one individual was arbitrarily selected from each colony. Ants were removed from the freezer, and the gaster was removed from the body at the suture between the postpetiole and the abdomen and placed dorsal surface upward in insect Ringers solution in a petri dish. The sperm-containing organs were extracted by grasping the claspers and the anterior edge of the gaster with fine forceps and pulling gently but firmly. The testes, seminal vesicles, and accessory glands were then separated from the rest of the genitalia and placed in a 1.6-ml microcentrifuge tube containing 75 μl of 0.5 M glucose and 75 μl of phosphate-buffered saline (PBS, pH 7.2). The tissue was homogenized with a form-fitting plastic pestle for approximately 2 min, and transferred to a 15-ml screw-top centrifuge tube containing 10 ml of PBT (PBS and 0.5% TWEEN-20). Preliminary analysis indicated that 5-ml and 10-ml dilutions produced virtually identical sperm counts; the larger dilution was chosen because it facilitated counting when the density of sperm was high. When the ant homogenate was added, the solutions were mixed by slowly inverting the 15-ml tube 10 times.

Ten males were dissected at a time. When all homogenates had been prepared, four drops of 3 μl each were transferred from each tube to a labeled 25 mm × 100 mm microscope slide that had first been cleaned with cleaning acid (Rogers 1973) and then coated with 0.5% polylysine. The drops were allowed to stand for 10 min, then the entire slide was recorded against a background of 1-mm graph paper using a Sony AVC-D7 CCD video camera connected to an Overlay Frame Grabber board (Imaging Technologies, Inc., Woburn, MA) housed in a 66-MHz 80486 computer. Captured images were stored on a 1-Gb optical disk, and drop area was quantified using Java® image analysis software (Jandell Scientific, Costa Madero, CA). After an additional 10 min, drops were dried evaporatively until nearly dry. Slides were fixed by immersion in a 1:3 solution of glacial acetic acid and methanol and then rinsed in PBS. Slides remained in PBS for 5–60 min until fixation; preliminary studies indicated that the amount of time in PBS postfixation did not affect the estimate of sperm number. Slides from five males were prepared at one time to ensure the consistency of individual preparations. After fixing and rinsing, slides were combined in a single rack for staining; staining in 5×10^{-7} M Hoechst (33258) was performed according to the protocol of Sakaluk and O'Day (1984).

Sperm nuclei were counted using an Nikon Diaphot photomicroscope equipped for epifluorescence microscopy. Sperm were visualized using a 10× fluorescence objective with a DAPI filter set (330-nm excitation filter with 80-nm bandwidth, 400-nm dichroic, and a 400-nm longpass emission filter; filters and dichroic from Omega Optical, Brattleboro, VT). Five fields of area 0.586 mm^2 were counted within each drop; fields from all four drops were counted from each male. The order of males scored was random with respect to male size or possible mating status. Sperm number was quantified as the average number per field within a drop; the average number was then divided by the field area and multiplied by the drop area to get the number of sperm per drop. The average number per 3-μl drop was multiplied by 3333.3

TABLE 1. Loadings of ln-transformed morphometric characters on principal component 1 (male size).

Character	Loading
Eye length	0.895
Head length	0.961
Head width	0.971
Mandible length	0.943
Mandible width	0.860
Thorax width	0.962
Thorax length	0.971
Pronotum length	0.966
Midtibia length	0.928

to give the estimated number of sperm per male. All sperm analyses were performed by a single individual.

Morphometric Analysis of Males

The remaining head-thorax of each male was measured for 15 morphometric characters (eye length, head length, head width, scape length, mandible width, mandible length, pronotum length, thorax width, thorax length, thorax depth, petiole length, midfemur length, hind femur length, midtibia length, hind tibia length; for a detailed description of these traits, see Abell et al. 1999). Previous multivariate analyses of male size in *P. occidentalis* suggested that these characters captured male size (PC1 representing 80% of the total variance; Abell et al. 1999) and were highly correlated with both wet and dry weight. Body parts were measured according to the methods of Abell et al. (1999). The identity of these males (pre- or postswarm) and their sperm counts were unknown to the individual doing the morphometry.

Statistical Analysis

The natural log (ln) transformation was applied to all morphometric variables to enhance the normality of the distribution and the homogeneity of the variances. Overall size (PC1) was estimated from a principal components analysis of the correlation matrix of nine ln-transformed variables (eye length, head length, head width, mandible width, mandible length, pronotum length, thorax width, thorax length, midtibia length; see Table 1 for loadings); five variables were dropped because of too many missing cases. PC1 represented 88.4% of the total variance. Because we cannot have data on dry weight for the males used in the sperm analysis, we estimated the relationship between PC1 and dry weight using another dataset with intact males (Abell et al. 1999): body size (PC1) = 1.02 (dry weight) − 4.137 (r^2 = 0.85, N = 325). Heterogeneity in sperm counts and drop areas of individual males were determined by mean/variance ratios; those males that had significant heterogeneity for either measure were dropped from the analysis.

We used analysis of covariance to test the hypothesis that large males lose more sperm than small ones. Total sperm number was regressed on PC1, with mating status (collected before or after the mating flight) as the classification variable.

RESULTS

We obtained sperm counts from 45 unmated males and 41 males that were collected at the mating swarm. We noted no qualitative variation in sperm length, either within a male or between males. Large males have more sperm than small males (Fig. 1, Table 2) although sperm number varies considerably. Unmated males had significantly more sperm than males collected at the swarm (Table 2), but males differ in the degree of sperm loss. Larger males have significantly greater sperm depletion than small males (Fig. 1; significantly different slopes indicated by the interaction term in Table 2). The regression for males collected prior to mating is unaffected by the removal of the outlier (sperm count = 1.607 [PC1] + 8.285, r^2 = 0.59, $P < 0.0001$). The regression for males collected after mating becomes nonsignificant with the removal of the extremely small male (sperm count = 0.379 [PC1] + 4.116, r^2 = 0.24, $P > 0.10$), reflecting the degree to which large males transfer sperm, but importantly, the ANCOVA results are not affected.

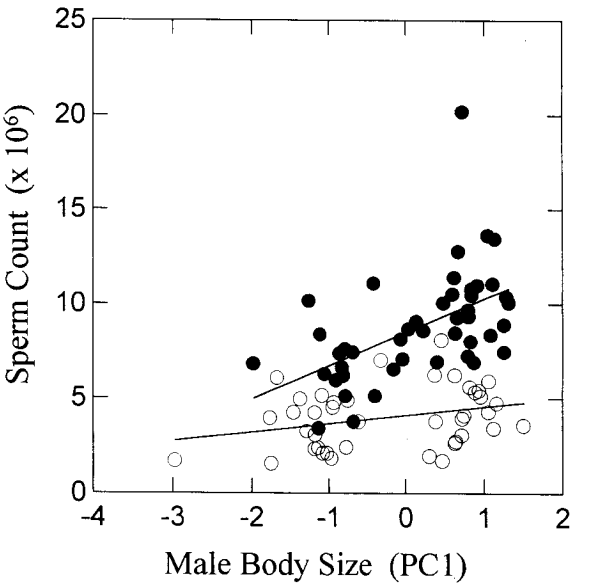

FIG. 1. The regression of sperm number (in millions) on body size for males collected prior to mating (solid circles) and after the mating swarm (open circles). Body size is the first principal component based on nine morphometric characters.

We estimated the selective advantage of male body size as the amount of sperm that a male transfers as a function of body size. The regression of sperm count on body size before mating is: sperm count ($\times 10^6$) = 1.887 (body size) + 8.493 (r^2 = 0.51, $P < 0.001$), where body size is PC1. After mating it is: sperm count = 0.448 (body size) + 4.105 (r^2 = 0.32, $P < 0.05$). The amount of sperm transferred by males as a function of body size is the difference between the two regression lines: sperm transferred = 1.439 (body

TABLE 2. Analysis of covariance for the interaction between male size (PC1) and sperm count.

Effect	df	Mean-square	*F*-ratio	*P*
Size (PC1)	1, 82	213.93	21.31	<0.0001
Mating status	1, 82	813.72	81.06	<0.0001
Size × mating	1, 82	79.01	7.87	0.0032[1]

[1] One-tailed probability that reflects the one-tailed hypothesis that larger males should lose greater amounts of sperm.

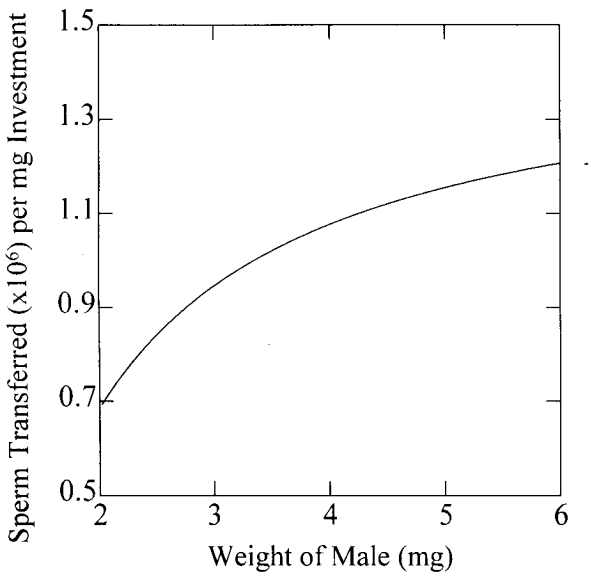

FIG. 2. Colony fitness per unit of investment (sperm transferred per milligram dry weight) as a function of male dry mass. (For derivation of the function represented by the curve, see the text.)

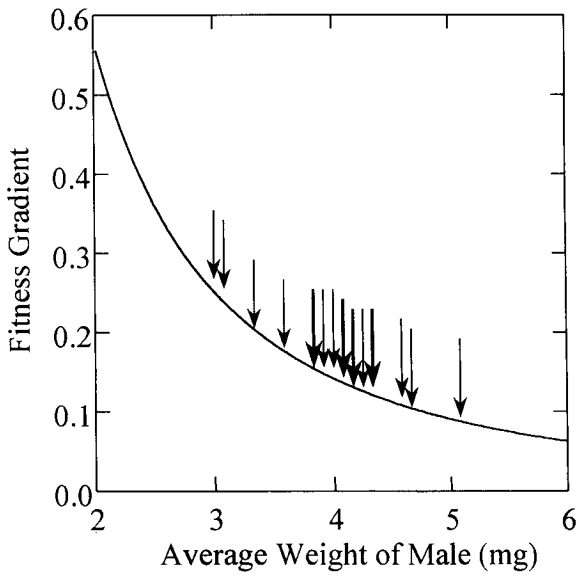

FIG. 3. The additional fitness gained by colonies that allocate a given amount of investment to incrementally larger males as a function of male size. Arrows indicate the average male dry mass from 31 colonies whose total reproductive output was collected in 1994 and that produced a minimum of 30 males. Heavier arrow shafts indicate multiple colonies.

size) + 4.388. The number of sperm that a male transfers during mating is an increasing function of body size. This relationship holds even when we recalculate the equation without the high sperm content outlier (sperm transferred = 1.159 [body size] + 4.180).

The relationship between sperm transfer and body size is a measure of absolute fitness. We calculated relative fitness by dividing this equation by mean absolute fitness ($W = 4.388 \times 10^6$), the number of sperm transferred by a male of mean size (PC1 = 0): relative sperm transferred = 0.328 (body size) + 1. Because our principal components are derived from the correlation matrix, the standard deviation of male size is one unit of PC1. The regression coefficient, 0.328, is the standardized selection differential, s^* (Lande and Arnold 1983).

The opportunity for selection, I, indicates the degree to which variance in fitness constrains trait evolution (Arnold and Wade 1984). Because our measure of relative fitness is a regression equation rather than data, we cannot calculate I directly. Instead we calculated the residuals of the regression of sperm remaining after mating on body size and used the standard deviation of these residuals (2.289×10^6) as the measure of variability in sperm transfer. Increasing the male body size one standard deviation increases sperm transfer by 0.912 standard deviations of sperm numbers.

The effect of individual selection for increased male size on colony fitness may be mitigated by a trade-off between male size and number. If a colony can apportion a given amount of investment in males in equivalent ways, colony fitness may be unaffected by sexual selection on individual males. We used the regression for sperm transfer (above) to calculate the colony fitness function for investment in individual males. We treat colony fitness as the payoff per unit investment in males of different sizes to determine whether colonies benefit disproportionately by investing in larger males. Sperm transfer as a function of dry weight (as the metric of male size) is then: sperm transfer = 1.468 (dry weight) − 1.565. Again, the shape of the equation is not affected by the removal of the high sperm content outlier (sperm transfer = 1.182 [dry weight] − 0.615). Finally, the value per unit of dry weight (mg) to male fitness: is Sperm Transfer/dry weight = 1.468 − (1.565/dry weight). The payoff to the colony in sperm transferred per unit of investment is greater for larger males (Fig. 2). For a given amount of investment in males, colonies that produce many small males will have lower fitness than a colony that produces fewer but larger males. This effect is more extreme if the cost of making a male is proportional to dry weight to the 0.75 power (Boomsma 1989). If small males are relatively more expensive than the estimate based solely on biomass, larger males are not as costly as we estimate.

Although an individual male's fitness increases linearly with body size, the payoff per unit of investment to colonies is asymptotic (see the previous expression). The incremental fitness gain with male body mass decelerates with the square of body mass. One can visualize this gradient as the additional fitness advantage to the colony for making incrementally larger males (derivative of fitness with respect to body mass, Fig. 3). The arrows show the average size of males that were produced by 41 colonies in 1994 (Cole and Wiernasz 2000; unpubl. data). Colonies tend to occur in the region where small changes in male size result in only marginal changes in fitness per unit of investment.

DISCUSSION

We have shown that large males transfer a disproportionate fraction of their sperm compared to small males; by this measure, individual fitness increases as a function of size. Our study did not measure fertilization success, but studies

of sperm competition in several other insect species have shown that large males have greater sperm transfer and higher fertilization success (Simmons and Parker 1992; Ward 1993; Parker and Simmons 1994). If the sperm of small males are of higher average quality than those of small males we may have overestimated the fitness advantage of large males. Although this possibility cannot be ruled out, there is no obvious length difference between the sperm of large and small males. The question of more subtle chemical competition among sperm of different males (e.g., Clark et al. 1995) is beyond the scope of this study.

Females of *P. occidentalis* are highly polyandrous (Cole and Wiernasz 1999), and it is likely that some males of this species also mate multiply. The male mating frequency of most species of ants is unknown. Although it has been suggested that male ants are incapable of mating multiply, this inference is based on data from two species (Ball and Vinson 1983; Hölldobler and Bartz 1985) that do not have a swarm mating system, and may not be general. Multiple mating by males does occur in the swarm-mating *Acromyrmex versicolor* (Reichardt and Wheeler 1996). Large males may benefit both by greater sperm transfer to any given female, which increases the probability that his sperm contribute to reproductives rather than workers (Hölldobler and Wilson 1990), as well as by transferring sperm to multiple queens. Given the individually low probability of foundress survival, ability to inseminate more than one female may significantly affect male fitness.

We can consider selection on male size from the point of view of either the individual male or the colony. Individual males benefit substantially from increased size. The typical large male (6 mg) transfers more than five times as many sperm to queens as the typical small male (2 mg). The standardized selection differential, 0.33, is substantial and larger than the sexual selection gradient estimated solely on the morphology of males collected while mating (Abell et al. 1999). Over the size range of males that we measured in this study (dry weight range estimated as 2.1–5.5 mg from PC1), the fitness advantage of larger males increases without asymptote. This may select for males within a colony to compete for access to resources, because any advantage that a male can gain in monopolizing resources within the nest will give him an advantage at the mating swarm.

From the colony's perspective, it is also advantageous to produce large males. The payoff per unit of investment for the largest males (6 mg) is nearly twice as great as that for the smallest males (2 mg). Colonies cannot compensate for the mating disadvantage of smaller males by using their lower cost of production to produce more of them; there is no tradeoff between male size and number for a given amount of investment in males. Although colony fitness increases with increasing male size, the average size of males in real colonies is clustered, suggesting that male size is limited at some point. This limit may result from some as yet unidentified component of selection or may be nonadaptive, the result of limited development time. Because colony fitness gain through male function is maximized when all males are as large as possible, colonies gain no apparent advantage by producing males that vary in body size. Although we cannot rule out some advantage of small males, such as competitively superior sperm or an alternative mating strategy, we have no evidence of such an advantage. In contrast, individual males are favored to become large at the expense of other males, particularly if the colony produces more males than can be made the maximal size. This conflict may be responsible for the variation in male size observed within individual colonies.

These results have important implications for the analysis of sexual investment ratios. We have shown that the amount of investment in male function does not accurately indicate the fitness obtained through male function. Under the simplest assumptions, a colony of *P. occidentalis* investing the same amount of resources in 6-mg males (the largest typical size) versus 2-mg males (the smallest typical size) has nearly double the fitness. When males differ in individual fitness (because of differences in size, etc.), it is misleading to quantify investment in males as total male biomass produced by the colony. Intercolonial size variation in males occurs in several species of ants (Davidson 1982; Ward 1983; Herbers 1990; Backus 1993; Fjerdingstad and Boomsma 1997). If reproductive size varies within or among colonies, it will be necessary to determine the fitness consequences of this variation for the colony. This will demand detailed studies of the relation of colony investment to reproductive fitness.

The predictions of sex ratio theory assume that fitness payoffs do not accrue differently to males of different sizes. One rationale for looking at the total investment (total biomass or energetic expenditure) of males and females is that sexual investment theory suggests that the numerical sex ratio is not important. The relative number of males produced matters less than the relative investment in males. Selection should balance allocation of investment in the sexes to maximize total fitness (Charnov 1982). For increased allocation to males to be favored, the proportionate gain in fitness through male function must be larger than the proportionate loss in fitness through female function (Fisher 1930; Shaw and Mohler 1953; MacArthur 1965; Charnov 1982; Charlesworth 1989). Standard theoretical formulations of allocation assume that the values of offspring increase linearly with investment and that investment, and therefore fitness, is interconvertible between the sexes: Increased investment in females takes away a proportional amount of fitness that could have been obtained through males (Pamilo 1991). We have shown that fitness may be gained, or lost, through male function without any corresponding change in fitness through female function. In *P. occidentalis,* colonies with identical investment in males need not gain equivalent fitness through male function; their fitness depends on the size distribution of males. Reproductive allocation ratios in other species that show size variation may be misleading unless modified by the individual fitness functions.

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