

Social control of primary sex differentiation in the Midas cichlid

(sex determination/growth/social dominance/teleost)

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Communicated by Peter Marler, August 9, 1993

ABSTRACT Sexual differentiation in teleost fishes is characteristically labile. The most dramatic form of sexual lability is postmaturational sex change, which is common among teleosts although rare or absent in other vertebrate taxa. In many cases this process is regulated by social cues, particularly dominance interactions. Here we show that in the Midas cichlid, *Cichlasoma citrinellum*, these same sorts of social interactions affect much earlier stages of sexual differentiation. In this species, males are larger than females. By manipulating relative size in juveniles, we show that this sex-based size difference does not arise from endogenous factors associated with sex. Rather, sex is determined by relative size as a juvenile. We argue that this mode of sex determination, which may be common among teleosts, is a heterochronic variant of postmaturational sex change, one in which some individuals are deflected from a default female trajectory before maturation, as a result of social signals. The size-advantage model, which specifies the optimal size for sex change in hermaphroditic species, can be extended to account for the decision whether to mature as a male or a female in the Midas cichlid.

In birds and mammals sex is determined at conception by a gene or genes residing on distinct sex chromosomes. Much research on other vertebrate taxa has been guided by the assumption that sex differentiation is initiated by similar genetic factors. An increased number of cases of environmental sex determination have been documented (1, 2), however, especially among reptiles (3–5). To date, studies of environmental sex determination in vertebrates have focused on the physical environment. In teleost fishes the social environment also plays an important role in sex differentiation (6), most notably in many sequential hermaphrodites in which size-mediated dominance interactions regulate sex change (7–9).

The size-advantage model (10, 11) provides a functional explanation for this labile form of sex differentiation, based on a consideration of factors that could maximize lifetime fecundity. This model is particularly effective in accounting for postmaturational, protogynous sex change among teleosts in which a few large males can monopolize mating within a population. The model is less clear in its predictions about monogamous species. In this type of mating system, for reasons first elucidated by Fisher (12), we would expect selection for sex-determining mechanisms that would tend to generate 1:1 sex ratios. The surest mechanism would be a two-factor genetic system. However, postzygotic means of achieving this are also possible. Such a mechanism would be desirable if two conditions are met: (i) the size-fitness trajectories of the two sexes are sufficiently divergent; and (ii) size differences result from factors other than gender itself. We provide evidence for a postzygotic mechanism of sex determination in a monogamous fish, one in which social interactions play an important role.

The Midas cichlid, *Cichlasoma citrinellum*, is monogamous and biparental (13). Within a cohort of Midas cichlids, as in many teleosts, pronounced variation in growth rate is apparent from an early age. The larger fish differentiate as males, and the smaller fish differentiate as females (14). A longitudinal study demonstrated that size ranks within a group are stable from an early juvenile stage through sexual maturity (14). Moreover, size ranges of males and females reared together overlap little. Because adult sex ratios in this species approximate 1.0 both in nature (13) and in the laboratory, the sex of a Midas cichlid can be reliably predicted from its relative size as a juvenile.

In a typical group of siblings, juveniles above the median size rank differentiate as males; those below the median size rank differentiate as females. The naive assumption is that this predictability of adult sex, from relative size in juveniles, arises because the sexes differ in growth rates. Another possibility, however, is that relative size of juveniles determines their sex. The first assumption implies that large relative size results from maleness, and the second assumption implies that maleness results from large relative size.

To decide between these alternatives, we manipulated relative size. A simple procedure for altering relative size within a single brood has been described, in which small fish are separated from larger fish as juveniles (15). Removal of the larger fish results in enhanced growth in the smaller fish such that within a few months of separation the size distributions are essentially coincident.

If sex is determined genetically, fish above the median size in the original group should become males, and those below the median size should become females, despite the alteration in relative sizes. If, however, sex is determined by relative size, at a stage subsequent to separation, such manipulations should result in size-assorted sexes of equal numbers within each group.

MATERIALS AND METHODS

Seventy-four 6-month-old juveniles from a single brood of Midas cichlids were randomly assigned to one of two groups according to size. Those above the median standard length (52.2 mm) were assigned to group L (large), and those below the median standard length were assigned to group S (small). The two groups were then reared separately under identical conditions in (100 × 60 × 60 cm) mesh enclosures placed in an outdoor pond for 6 months. When the fish were 1 yr old, at which time they could all be expected to be sexually mature, the fish were again weighed and measured. At this point, an initial sexual assessment was made through examination of the genital papillae, which are sexually dimorphic in this species (13). The fish were then sacrificed, and the gonad of each individual was inspected to confirm the initial diagnosis.

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Table 1. Size data for groups L and S at termination of study

	Weight, g		Standard length, mm	
	Mean ± SEM	Range	Mean ± SEM	Range
Group S	122.3 ± 7.5	78.1–210.5	130.9 ± 2.6	105.0–161.6
Group L	95.4 ± 5.4	39.7–164.3	123.6 ± 2.0	96.1–151.6

RESULTS

After 6 months, group S fish were of a larger average size than group L fish [Table 1; median standard length = 125.3 mm and 118.9 mm, respectively] indicating a growth potential in group S fish that had been suppressed by the presence of group L fish and that was released once the larger fish were removed.

If the initial size distribution reflected endogenous differences in growth rates resulting from factors (e.g., hormonal) associated with sexual differentiation, the size differences between group L and group S should have persisted when the two groups were separated. The alteration in the size distributions resulting from this simple manipulation indicates that the stable size ranks within intact groups are not maintained by endogenous factors. Rather, these size ranks result from a contingent nexus of social interactions determined largely by the original size ranks.

The sex ratios of both groups were moderately biased toward females, more so in group L (Table 2); the deviation from 1:1, however, was not significant in either case. The sex ratios of the two groups did not differ significantly ($\chi^2 = 0.169, P > 0.05$).

In both groups males were larger than females (Table 3). In group L three males were within the female size range (Fig. 1), but those fish above the median size were overwhelmingly male ($\chi^2 = 9.9, P < 0.001$); in group S the size distributions of the sexes did not overlap (Fig. 2; $\chi^2 = 29.2, P < 0.0001$). When we compare sizes of the males from group L with the females of group S, the size ranks of the two sexes do not differ significantly (Fig. 3; $\chi^2 = 0.286, P > 0.05$). This result further indicates that sex-based size differences are not caused by endogenous factors but are group-specific.

DISCUSSION

Sex in the Midas cichlid, therefore, is determined by relative size in juveniles sometime after they reach 6 months of age or a median size of 52 mm. Without detailed histological analyses, though, we cannot determine whether this represents the social determination of primary sex or of prematuration sex change of the sort described in other teleosts (16, 17). This distinction, though, may be arbitrary.

Sexual differentiation in teleosts is protracted and essentially protogynous (6, 18). Males of many species, even apparent gonochorists, may be derived secondarily as a result of a developmental switch that deflects them from the default female trajectory. Even in protandrous species, initial gonadal differentiation is female, although individuals mature first as males (19). The point during gonadal differentiation at which this switch is activated may vary between and within species, and its activation may be influenced by both endogenous and exogenous factors. If size-mediated dominance

Table 2. Sex ratios at termination of study

	Male, no.	Female, no.	Sex ratio	χ^2
Group S	15	18	0.83	0.133
Group L	15	22	0.68	1.010

Four fish from group S died during the course of the study. If all four were females, the sex ratios for the two groups would be identical. Whatever the sex of these fish, the results are not notably altered.

Table 3. Size data by sex for experimental groups

	Weight, g		Standard length, mm	
	Mean ± SEM	Range	Mean ± SEM	Range
Group S				
Male	159.1 ± 9.2	106.1–210.5	144.8 ± 2.5	128.6–154.9
Female	91.2 ± 3.0	71.9–105.1	119.5 ± 1.3	105.0–127.8
Group L				
Male	123.1 ± 8.6	72.6–162.2	134.5 ± 2.7	116.3–143.3
Female	76.5 ± 3.1	39.7–101.1	116.2 ± 1.3	96.1–123.0

interactions, for example, trigger the switch in the early stages of gonadal differentiation, it will be interpreted as the social control of primary sex. This may be the case in the Midas cichlid and perhaps the so-called “primary males” of diandric coral-reef species (20, 21). If the same interactions activate the switch later, it will be interpreted as socially controlled sex change.

Ghiselin’s (10) size-advantage model predicts protogynous sex change when females have higher fecundity than males at small sizes and males have higher fecundity at large sizes. Protandry is predicted when the reverse conditions apply. Refined versions of this model, which account for cost of sex change itself (22–24), have been used successfully to predict the timing and direction of adult sex change in a number of animal species (22, 23, 25). We propose that the logic of the model can be extended to prematuration life stages.

Viewed in this way, the size-advantage model should predict when the decision to deflect from a female trajectory is made along the time continuum extending from zygote formation. Size itself is not the critical parameter here, but rather, size at a given age for a given life-expectancy. The timing of the decision should be sensitive to the degree to which the slopes of the size/fecundity trajectories diverge and the cost of a mistake increases. Assume that the cost of conversion increases as sexual differentiation proceeds but that the risk of a sex-allocation error decreases during development as a result of increasing reliability of available information. Then, the less the divergence in the size/fecundity trajectories and, hence, the lower the cost of a sex-allocation error, the earlier the decision should be made. As the cost of a sex-allocation error approaches zero, we would expect increasingly canalized sex differentiation. Conversely, the greater the divergence in the size/fecundity trajectories and, hence, the greater the cost of a sex-allocation error, the later in development the switch should be triggered to reduce the risk of such an error.

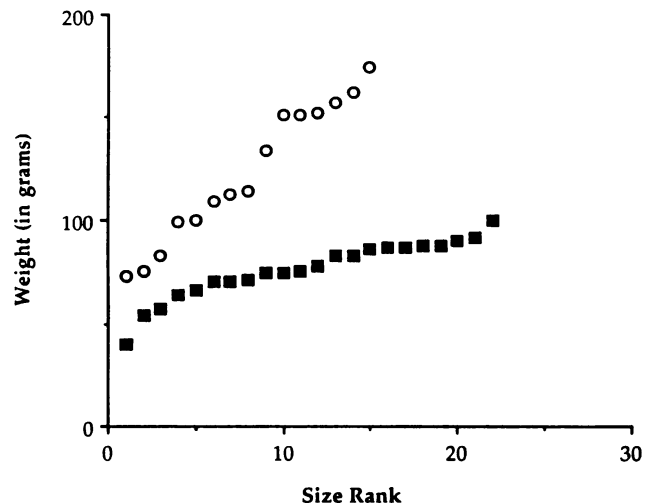


FIG. 1. Size ranges of males (○) and females (■) of group L arranged in order of size rank.

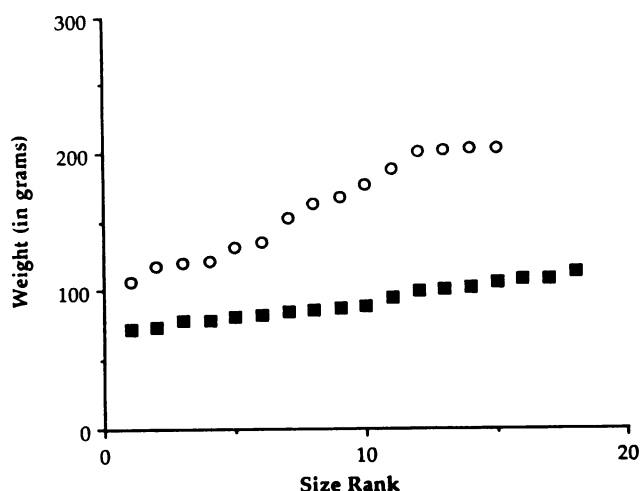


FIG. 2. Size ranges of males (○) and females (■) of group S arranged in order of size rank.

In the Midas cichlid this decision is made before maturation, suggesting that the size/fecundity trajectories for males and females do not diverge to the extent that they do in many coral-reef species. If, in the Midas cichlid, relative size as a juvenile is the signal whether to activate the developmental switch, it should be a good predictor of adult relative size, at least among a given age cohort, and it is. We would also expect that the juveniles assort roughly by age during this developmental period.

Given the monogamous breeding habit characteristic of this species, the question arises as to whether the size/fecundity trajectories of the two sexes differ sufficiently to warrant the size-mediated sex determination observed here. But whereas in a monogamous species the variance in male and female fitness is roughly equal, the sexes can differ substantially in the way fecundity varies with size. In *Amphiprion*, for example, female fecundity increases linearly with size, whereas the male size/fecundity trajectory is much flatter. Hence, members of this highly monogamous genus are protandrous. In the Midas cichlid and other monogamous members of this family, males are significantly larger than females. Female fecundity should increase as a function of size in this species as well. Hence, there must be even stronger selection for large size in males.

Within a breeding pair there is a division of labor, with the males having the primary role of defending the breeding territory against conspecific interlopers (26). Large size would be advantageous to males for this reason. Mate-choice experiments in the laboratory (26) and field observations (27) suggest strong female preference for large males. The male size-advantage may also indicate a greater opportunity for multiple mating in males than in females, but the details of the size-fitness trajectories for the two sexes remain to be established.

This mode of sexual differentiation, which may be common in teleosts, is another manifestation of a generally labile pattern of sexual development among bony fishes (6). This lability is a prerequisite for the adaptive patterns of sex differentiation explained by the size-advantage model.

The source of this lability may be in the developmental polarity of the brain-pituitary-gonadal axis. In mammals sex differentiation is initiated in the gonads, and gonadal products (e.g., steroid hormones) induce a cascade of events resulting in the sexual differentiation of the soma, including the brain (but see ref. 28). In contrast, the brain has been proposed as the initial site of sexual differentiation in teleost fishes, and

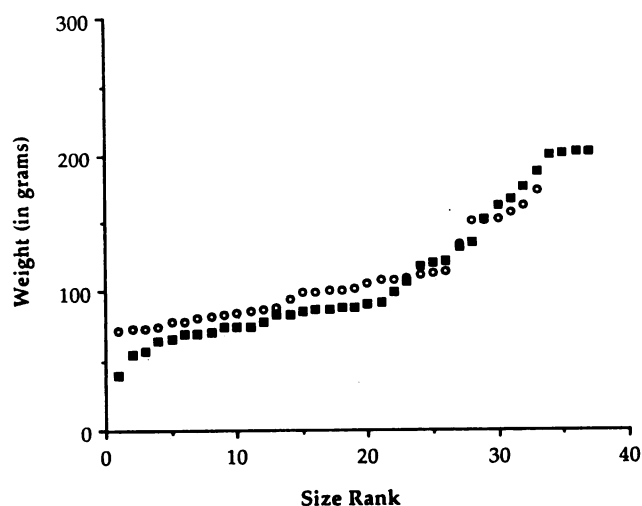


FIG. 3. Size ranges of males (■) and females (○) for both groups arranged in order of size rank.

the pattern of sexual differentiation in the teleost brain determines the fate of the gonads (6). This influence would help explain both the characteristic lability of teleost sexual development and its susceptibility to social influences.

We thank P. Godfrey-Smith and three anonymous reviewers for their helpful comments on the manuscript. This research was supported by a National Research Service Award fellowship to R.C.F. (MH41206-01) from the National Institute of Mental Health and by Grant HD18496 from National Institute of Child Health and Human Development to G.W.B.

1. Conover, D. O. & Heins, S. W. (1987) *Nature (London)* **326**, 496-498.
2. Rubin, D. A. (1985) *Copeia* **1985**, 233-235.
3. Ferguson, M. J. W. & Joanen, T. (1982) *Nature (London)* **296**, 850-853.
4. Bull, J. J. (1983) *Evolution of Sex Determining Mechanisms* (Benjamin/Cummings, Menlo Park, CA).
5. Bull, J. J. (1980) *Quart. Rev. Biol.* **55**, 3-21.
6. Francis, R. C. (1992) *Quart. Rev. Biol.* **67**, 1-18.
7. Robertson, D. R. (1972) *Science* **177**, 1007-1009.
8. Fricke, H. W. & Fricke, S. (1977) *Nature (London)* **266**, 830-832.
9. Ross, R. M., Losey, G. S. & Diamond, M. (1983) *Science* **221**, 574-575.
10. Ghiselin, M. T. (1969) *Quart. Rev. Biol.* **44**, 189-208.
11. Warner, R. R. (1975) *Am. Nat.* **109**, 61-82.
12. Fisher, R. A. (1930) *The Genetical Theory of Natural Selection* (Oxford Univ. Press, Oxford).
13. Barlow, G. W. (1976) in *Investigations of the Ichthyofauna of Nicaragua Lakes*, ed. Thorson, T. B. (Univ. of Nebraska Press, Lincoln), pp. 333-358.
14. Francis, R. C. (1990) *Ethology* **86**, 311-325.
15. Francis, R. C. (1988) *Anim. Behav.* **36**, 1844-1845.
16. Takahashi, H. & Shimizu, M. (1983) *Bull. Fac. Fish. Hokkaido Univ.* **34**, 69-78.
17. Francis, R. C. (1984) *Behaviour* **90**, 25-45.
18. Shapiro, D. Y. (1992) *J. Exp. Zool.* **261**, 194-203.
19. Reinboth, R. (1988) *Environ. Biol. Fish.* **22**, 249-259.
20. Warner, R. R. & Robertson, D. R. (1978) *Smithson. Contrib. Zool.* **254**, 1-27.
21. Robertson, D. R. & Warner, R. R. (1978) *Smithson. Contrib. Zool.* **255**, 1-26.
22. Charnov, E. L. (1982) *The Theory of Sex Allocation* (Princeton Univ. Press, Princeton, NJ).
23. Warner, R. R. (1988) *TREE* **3**, 133-136.
24. Iwasa, Y. (1990) *Behav. Ecol.* **2**, 56-68.
25. Wright, W. G. (1988) *TREE* **3**, 137-140.
26. Rogers, W. & Barlow, G. W. (1991) *Ethology* **87**, 249-261.
27. McKaye, K. R. (1977) *Ecology* **58**, 293-302.
28. Reisert, I. & Pilgrim, C. (1991) *Trends NeuroSci.* **14**, 468-473.