Evolution of a balanced sex ratio by frequency-dependent selection in a fish.

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Balanced (1 to 1) sex ratios are thought to evolve by a process known as frequency-dependent selection of the minority sex. Five populations of a fish with genetically based variation in temperature-dependent sex determination were maintained for 5 to 6 years in artificial constant-temperature environments that initially caused the sex ratio to be highly skewed. Increases in the proportion of the minority sex occurred in subsequent generations until a balanced sex ratio was established, thus confirming a central premise underlying the theory of sex-ratio evolution.

FISHER WAS THE FIRST TO EXPLAIN why many species tend to invest equally in the production of sons and daughters [1]. Because every offspring has one mother and father, the contribution of genes from each sex to succeeding generations must be equal. If one sex is less numerous, its per capita contribution is higher and genes that overproduce the minority sex should therefore increase until the primary sex ratio is balanced. Fisher's model hinges on frequency-dependent selection: an evolutionary process where the fitness of a phenotype is dependent on the relative frequency of other phenotypes in the population. Fisher's theory has gained widde acceptance: it potentially accounts for the widespread occurrence of sex-determining mechanisms, such as heterogamety, that ensure the production of 1:1 sex ratios and it is the foundation for virtually all adaptive sex ratio theory [2].

Serious doubts have arisen because balanced sex ratios could also simply be a nonadaptive consequence of Mendelian segregation of sex chromosomes [3]. Moreover, the only direct evidence is the same observation that originally generated the model: the ubiquity of species that have balanced sex ratios in nature [4]. The dilemma has been that the sex-determining mechanisms of many of these species contain little (if any) genetic variation and, therefore, may not be capable of evolving. Is frequency-dependent selection such a potent evolutionary process that dioecious species capable of producing unbalanced primary sex ratios are rare?

A species ideally suited for directly testing Fisher's theory is the Atlantic silverside, Menidia menidia. In this fish, sex is determined by the joint effects of temperature and major sex-determining genes during a specific period of larval development [5]. The Atlantic silverside is an annual fish that breeds during the spring and summer in bays and estuaries along the east coast of North America. Offspring produced early in the breeding season experience low temperatures that cause most larvae to differentiate into females; high temperatures that prevail during the late breeding season cause most offspring produced then to become male. Previous experiments have conclusively shown that temperature exerts a direct influence on primary sex differentiation rather than causing sex-specific mortality [5]. The influence of major genes on sex determination is indicated by large nonadditive effects of parentage on the response of sex ratio to temperature within families [6]. Moreover, the degree of genetic control differs greatly with latitude [7]. In South Carolina fish, the sex ratio changes by as much as 70% with temperature. The sex ratio of Nova Scotia fish, however, is insensitive to temperature, suggesting complete genetic control. Fish from New York show an intermediate sex ratio response to temperature.

In nature, the normal pattern of seasonal change in temperature usually results in a sex ratio close to 0.5 [5]. When progeny from a natural population are transferred to a constant-temperature environment, however, the resulting sex ratio can be highly skewed. We established five separate laboratory populations of silversides that constantly experienced either a high or low temperature environment during development each generation. Two populations were started in 1984 by randomly subdividing a common stock of several thousand newly hatched larvae that were collected as embryos from South Carolina [8]. One such population was reared during the temperature-sensitive period at 28 [degrees] C (SC-H) and the other at 17 [degrees] C (SC-L). This produced sex ratios (F/F+M) that were skewed in the initial generation toward opposite extremes: 0.18 in SC-H, 0.70 in SC-L. Fish in each line were reared to maturity and allowed to spawn en masse in laboratory tanks [9]. Their progeny were reared at the same high or low temperature during the sensitive period of larval development as were their parents. After the sensitive period, juvenile fish were subsampled to estimate the sex ratio [10] and about 100 remaining fish were reared to sexual maturity [11]. Subsequent generations were treated to a like manner. Two other populations were established in 1985 with field-collected New York embryos and maintained as above, but in these lines the initial sex ratios at 28 [degrees] C (NY-H) and 17 [degrees] C (NY-L) were 0.05 and 0.29, respectively. The fifth population was founded in 1985 with embryos from Nova Scotia, where the sex ratio is close to 0.5 at all temperatures. This laboratory

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population (NS-H) was reared at 28 [degrees] C each generation [12].

There are five possible general outcomes to this experiment. (i) No changes in sex ratio among generations. (ii) Random changes in sex ratio as might be caused by the effects of genetic drift. (iii) Shifts in sex ratio depending directly on thermal environment (that is, all high temperature lines change in a like manner, all low lines in another). (iv) Increases in the proportion of the majority sex, perhaps leading to the loss of one sex. (v) Increases in the proportion of the minority sex, with convergence of the sex ratio to 0.5. Only alternative (v) would support Fisher's theory. Note that we can distinguish between alternatives (iii) and (v) because the SC-L line begins with a female excess while the NY-L line begins with a male excess. Moreover, the NS-H population serves a control because its sex ratio starts at 0.5 and is therefore not expected to change.

Changes in sex ratio closely followed the predictions of Fisher's theory. Increase in the minority sex occurred in each of the four populations that started with skewed sex ratios (Fig. 1). In three populations (SC-H, NY-H, and NY-L), the sex ratio leveled off at 0.5. More generations are needed to verify that the sex ratio in SC-L is going to stabilize at 0.5. As expected, the control population, NS-H, maintained a sex ratio near 0.5 throughout the experiment.

The trajectory by which the sex ratio of each population approached 0.5 differed greatly among the populations (Fig. 1). One population reached 0.5 in generation one (NY-H), another approached 0.5 more gradually (SC-H), and still another initially overshot 0.5 by a considerable margin (SC-L), and then gradually approached 0.5. These differences in trajectory deserve further study, but they are not particularly surprising given that the genetic control of sex determination in fish from South Carolina, New York, and Nova Scotia differs substantially [7].

Significant deviations away from 0.5 occurred in several cases after a balanced sex ratio had become established (for example, generations two, five, and seven of the NS-H line, Fig. 1E). This result is not unexpected, nor is it at odds with Fisher's theory. The sex ratio is selectively neutral at the equilibrium level of 0.5 [2]. Given that our laboratory populations are small [11] and that sex determination within populations is genetically variable [6], shifts away from 0.5 can occur when the sex ratio is balanced, due to the random effects of genetic drift. Whenever departures from 0.5 occur, however, frequency-dependent selection has an opportunity to operate.

An alternative way of elucidating the effect of frequency-dependent selection on the laboratory populations is to examine the direction of sex ratio change following each generation where the sex ratio was skewed. Summed over all five populations, there were 19 generations where the sex ratio differed significantly from 0.5. In 18 of these instances, the proportion of the minority sex increased in the next generation (the exception was over generations two to three in the SC-L line). Genetic drift acting alone would have produced random changes in sex ratio. Our results, therefore, provided persuasive evidence of the effect of frequency-dependent selection on sex ratio evolution.

We have also explored whether frequency-dependent selection has altered the level of environmental (ESD) as opposed to genetic sex determination (GSD) within each of the laboratory populations. Several theoretical analyses have shown that ESD is adaptive if, and only if, the environment that offspring enter varies in a manner that effects the fitness of males and female differently [13]. Otherwise, the skewed sex ratios caused by environmental variability would result in frequency-dependent selection for strict GSD [14]. Because, in our experiment, ESD cannot be adaptive (all offspring within a population enter the same constant environment) and because the sex ratio is initially skewed, we would expect the level of ESD within each line to decline across generations. We measured changes in the level of ESD by rearing some offspring from each generation of each population at the temperature extreme opposite to that of their parental line. Results so far have been promising: the level of ESD has declined initially in three of the four lab populations with ESD [15], but additional generations are required to confirm this conclusion.

This study provides, to our knowledge, the first direct confirmation of Fisher's sex ratio principle [1]. Because our model system involves a species with ESD, these findings also have implications for the debate over the impact (if any) of rapid changes in climate on species with ESD [16]. Our results show that given genetic variation, sex-determining mechanisms are capable of evolving so as to ensure continued production of a balanced sex ratio, even after substantial perturbations.

REFERENCES AND NOTES

[1]R. A. Fisher, The Genetical Theory of Natural Selection (Oxford Univ. Press, Oxford, 1930). [2]J. J. Bull and E. L. Charnov, in Oxford Surveys in Evolutionary Biology, P. H. Harvey and L. Partridge, Eds. (Oxford Univ. Press, Oxford, 1988), vol. 5. [3]G. C. Williams, Proc. R. Soc. London Ser. B 205, 567 (1979). [4]Much indirect support for Fisher's

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theory comes from species with extraordinary sex ratios, most of which can be explained as cases where the model's assumptions do not hold. See review in [2] and W. D. Hamilton, Science 156, 477 (1967). [5]D. O. Conover and B. E. Kynard, Science 213, 577 (1981). [6]D. O. Conover and S. W. Heins, Copeia 1987, 732 (1987). [7]__, Nature 326, 496 (1987). [8]Egg collection procedures and specific localities are as described in [7]. Rearing techniques are described in [6, 7, 10]. [9]Maturity was induced by photoperiod manipulations. Silversides spawn in large groups and do so daily in laboratory tanks [D. P. Middaugh, Copeia 1981, 766 (1981); D. O Conover and B. E. Kynard, Environ. Biol. Fishes 11, 161 (1984)]. Eggs are deposited on filamentous substrates. We placed small mops of yarn in the spawning tanks and removed these every few days after several hundred embryos had accumulated. Embryos were reared in temperature-controlled seawater baths until beyond the temperature-sensitive period of sex determination. To ensure that the fish in each generation were a representative sample of progeny produced by the parental generation, at least 3 to 5 such batches of offspring were combined. Afterward, juveniles were transferred to the spawning tanks and reared at 20 [degrees] to 25 [degrees] C to maturity. [10]All fish examined for sex were either juveniles ([is greater than or equal to] 20 to 25 mm in total length) or adults, both of which can be sexed by dissection and direct examination of the gross morphology of gonads under a microscope with techniques described by D. O. Conover and M. Fleischer [Can. J. Fish. Aquat. Sci. 43, 514 (1986)]. Conover and Fleisher also show that once sex is determined it is irreversible and that the sex ratio of juveniles is not affected by temperature. Sex ratios of juveniles and adults within each generation of each line generally reported here agreed closely. No hermaphrodites were found. [11]The mean number (and range) of breeding adults per generation of each population was: SC-H, 129 (60-271); SC-L, 108 (48-220); NY-H, 131 (41-179); NY-L, 125 (42-198); and NS-H, 139 (50-247). However, the effective population sizes ([n.sub.e]) were generally less because some populations started with highly unbalanced sex ratios. Harmonic mean [n.sub.e] for each population over the course of the experiment was: SC-H, 69; SC-L, 53; NY-H, 39; NY-L, 80; and NS-H, 100. These estimates of [n.sub.e] assume that all fish exposed to photoperiod manipulations [9] become mature. Direct observations of groups spawning in the tanks and of the enlarged size of gonads in preserved specimens examined after spawning suggest that this assumption is correct. [12]There was one exception. Because of an insufficient number of fish available at 28 [degrees] C, the initial generation of the NS-H line came from fish reared at 19 [degrees] C. This should have little

bearing on the outcome of the experiment because the sex ratio at all temperatures in Nova Scotia fish is 0.5 [7]. All subsequent generations were reared at 28 [degrees] C. [13]E. L. Charnov and J. J. Bull, Nature 266, 828 (1977); J. J. Bull, The Evolution of Sex Determining Mechanisms (Benjamin/Cummings, Menlo Park, California, 1983). The adaptiveness of ESD in silversides is discussed in D. O. Conover [Am. Nat. 123, 297 (1984)]. [14]J. J. Bull, Heredity 46, 9 (1981); M. G. Bulmer and J. J. Bull , Evolution 36, 13 (1982). [15]D. O. Conover, unpublished data. [16]M. W. J. Ferguson and T. Joanen, Nature 296, 850 (1982); N. Mrosovsky, P. H. Dutton, C. P. Whitmore, Can. J. Zool. 62, 2227 (1984); E. A. Standora and J. R. Spotila, Copeia 1985, 711 (1985); G. Head, R. M. May, L. Pendleton, Nature 329, 198 (1987). [17]We thank S. Heins, R. McBridge, R. Nyman, L. Chiarella, M. Meade, M. Green, T. Present, I. Lagomarsino, F. Juanes, R. Marks, R. G. Rowland, K. Sosebee, and J. Schreer for assistance in rearing the fish, and G. C. Williams for reviewing the manuscript. Supported by NSF grant nos. BSR84-15878 and BSR87-17315 to D.O.C.