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# A polygenic hypothesis for sex determination in the European sea bass - *Dicentrarchus labrax*

Vandeputte M<sup>1,3,\*</sup>, Dupont-Nivet M<sup>1</sup>, Chavanne H<sup>2</sup>, Chatain B<sup>3</sup>.

<sup>1</sup>INRA, UR544 Génétique des Poissons, F-78350 Jouy en Josas, France

<sup>2</sup>Panittica Pugliese, I-72010 Torre Canne di Fasano, Italy

<sup>3</sup>IFREMER, F-34250 Palavas les Flots, France

\*: Corresponding author : Vandeputte M., email address : [marc.vandeputte@jouy.inra.fr](mailto:marc.vandeputte@jouy.inra.fr)

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### Abstract:

Polygenic sex determination, although suspected in several species, is thought to be evolutionarily unstable and has been proven in very few cases. In the European sea bass, temperature is known to influence the sex ratio. We set up a factorial mating, producing 5.893 individuals from 253 full-sib families, all reared in a single batch to avoid any between-families environmental effects. The proportion of females in the offspring was 18.3%, with a large variation between families. Interpreting sex as a threshold trait, the heritability estimate was  $0.62 \pm 0.12$ . The observed distribution of family sex ratios was in accordance with a polygenic model or with a four-sex-factors system with environmental variance and could not be explained by any genetic model without environmental variance. We showed that there was a positive genetic correlation between weight and sex ( $r_A = 0.50 \pm 0.09$ ), apart from the phenotypic sex dimorphism in favor of females. This supports the hypothesis that a minimum size is required for sea bass juveniles to differentiate as females. An evolution of sex ratio by frequency-dependent selection is expected during the domestication process of *Dicentrarchus labrax* populations, raising concern about the release of such fish in the wild.

**Keywords:** sex ratio, heritability, growth, fish, *Dicentrarchus labrax*

## INTRODUCTION

In gonochoric species with genetically determined sex, a one to one sex ratio is known to be optimal in an infinite population of diploid individuals with random mating and Mendelian segregation (Fisher 1930; Charnov 1975). The observation of skewed sex ratios may imply, among others, non-Mendelian segregation like in *Drosophila* (Vaz & Carvalho 2004), non-random mating (Hamilton 1967) or environmental sex determination (ESD - (Bull 1985)). In the latter case, the sex of an individual is not fixed at conception, but is influenced by environmental conditions during its early life. ESD is expected to be favored when the offspring lives in patchy environments, which may confer advantages to being male or female, and neither the offspring nor the parent have control and/or predictive ability on the type of patch the offspring will live in (Charnov & Bull 1977). Temperature (e.g. (Bull & Vogt 1979; Baroiller & D'Cotta 2001)) seems to be the main environmental factor implied, but density (Ellenby 1954) and social status (Francis & Barlow 1993) have been shown to be possible sex-determining environmental factors. In species with ESD, in many cases there is also a genetic variation (Bull *et al.* 1982; Conover & Heins 1987a; Janzen 1992; Baroiller & D'Cotta 2001), which in some cases has been described as polygenic (Bull *et al.* 1982; Janzen 1992). Polygenic sex determination, however, is considered to be evolutionary unstable (Rice 1986) and its maintenance is still poorly understood. It is thought by some authors to be the ancestral type of sex determinism in fish (Kirpichnikov 1981), but organisms where it is accepted that sex has a polygenic component are indeed very few: the parasitic wasp *Nasonia vitripennis* (Orzack & Gladstone 1994), the turtles *Graptemys ouachitensis* (Bull *et al.* 1982) and *Chelydra serpentina* (Janzen 1992), and probably the swordtail *Xiphophorus helleri* (Kosswig 1964).

The European sea bass (*Dicentrarchus labrax*) is a gonochoristic teleost fish distributed in the North-Eastern Atlantic, the Mediterranean and the Black Sea (Pickett & Pawson 1994). They live in shallow, coastal waters, estuaries, lagoons and harbours, moving to deeper waters (up to 100 m deep) as they grow. Although they can live in waters under 5°C, they seek temperatures above 10°C, and even 15°C in their first year (Kelley 1988). They spawn in open waters in late winter-early spring, depending on the latitude. The eggs hatch in 4-9 days, and the young fish move inshore in their first month, towards the warmest waters, especially in estuaries (Pickett & Pawson 1994). Sex remains undifferentiated for a long period: differentiation occurs between 128 and 250 days post-fertilisation - dpf (Saillant *et al.* 2003a). Records of sea bass sex ratio in wild populations are scarce. They show balanced sex ratios (Saillant *et al.* 2003a), excess of males (Menu, pers.comm.) or excess of females (Arias 1980), but as a whole do not contradict the hypothesis of balanced sex ratios in the wild. The sea bass is an important species in Mediterranean aquaculture, and it appears that in all aquaculture populations, sex ratios are strongly biased towards males (75 to 95%, e.g. (Blazquez *et al.* 1998; Saillant *et al.* 2002; Saillant *et al.* 2003a)), which is a problem for farmers as males mature earlier and grow less than females. Temperature has been shown to have a major effect on sex determination in sea bass (Blazquez *et al.* 1998; Pavlidis *et al.* 2000; Saillant *et al.* 2002; Mylonas *et al.* 2005). The effect of temperature is not fully understood, as two studies show an increased proportion of males with cold temperature (15°C: (Blazquez *et al.* 1998), 13°C: (Saillant *et al.* 2002)) while the other two show an increased proportion of females at 13 and 15°C (Pavlidis *et al.* 2000; Mylonas *et al.* 2005). The current hypothesis is that low temperatures early in development (before 100 dpf) may favor female sex differentiation, but that long-lasting low temperatures,

through a negative effect on growth, may preclude female differentiation and result in an increased proportion of males (Piferrer *et al.* 2005). Thus, the excess of males observed in culture would be due to the use of temperatures higher than in nature, for productivity reasons. From the genetic point of view, in addition to the environmental effect on sex, simple female homogamety can be excluded, as the sex ratios of normal diploid and gynogenetic offspring are equivalent (Felip *et al.* 2002; Peruzzi *et al.* 2004). The sex ratio of the offspring from masculinized females is not female biased and would rule out both XX-XY (female homogamety) and ZW-ZZ (male homogamety) systems (Blazquez *et al.* 1999). In this latter study however, the possible male bias induced by high rearing temperatures (22.5°C), and the impossibility to ascertain the genetic sex of the sex-reversed parents makes the demonstration a little weak. Therefore, male homogamety with environmentally male-biased sex ratios would still be a possibility. Additionally, parental influence on the sex ratio of progenies has also been demonstrated, however with very limited experimental settings (Saillant *et al.* 2002; Gorshkov *et al.* 2003), showing that there is a genetic component of progeny sex ratio. Although it is clear that the sex of sea bass is determined both by genetic factors and by the environment (mostly temperature), the sex determination system of this species remains basically unknown (Piferrer *et al.* 2005).

In this study, our aim was to describe the genetic component influencing sex ratio in sea bass, using a large number of families in classical aquaculture conditions, and to determine which genetic models could describe it best. We described sex using a threshold model with an underlying variable (sex tendency), as this type of model integrates both genetic and environmental effects, which are known to influence sex ratio in the sea bass.

## MATERIAL AND METHODS

**A Partly Factorial Mating Design:** The brood fish used were from a group of 33 males and 51 females of wild Atlantic origin, collected in 2000 on the French coasts of Brittany. Each brood fish was individually tagged and fin clipped for DNA extraction. The sperm of males was cryopreserved in 250  $\mu$ l straws (Fauvel *et al.* 1998). In January 2001, 51 females were injected with 10  $\mu$ g/kg LHRH (SIGMA, D-TRP6-LHRH), and eggs were stripped 72 hours later. Twenty-three females gave a sufficient quantity of good quality oocytes. From these spawns, we produced a mating design combining 33 males and 23 females in 3 full factorial sets of 11 $\sigma$  x 9 $\phi$ , 11 $\sigma$  x 7 $\phi$  and 11 $\sigma$  x 7 $\phi$ , for a total of 253 families. All full-sib families were fertilized individually, then eggs were grouped by female for incubation (48 hours at 13°C), after which 2 ml of viable eggs per female (approx. 2.000 eggs/female) were collected to create one batch containing all families. Standard rearing conditions were used, with temperature gradually increasing from 13 to 18°C in the first 64 days. Temperature was then kept at 18°C until 238 days (mean length of fish 117 mm, mean weight 23.6 g), then lowered to 14°C in order to slow down growth until the time scheduled for tagging. Although late low temperatures are suspected to masculinize the progenies (Piferrer *et al.* 2005), this does not apply at 238 days, as it was shown before that lowering temperature from 20 to 13°C at 149 days (mean length 81 mm) had no impact on the sex ratio (Saillant *et al.* 2002).

**Recording of Traits and Parentage Assignment:** At 370 days, the fish had reached a mean weight of 35 g. Seven thousand of them were randomly selected, on which individual weight and length were measured. Each fish was individually tagged and fin clipped for DNA extraction. The fish were then sent to four different sites (1.750/site), where they were reared until approx. 400 g mean weight. This rearing in

different sites was designed for estimating genetic parameters and genotype-environment interactions for growth and quality traits, in parallel of the present study. Still, it was not expected to have any impact on the sex ratio, as the differentiation period is over well before 370 days. At 400 g, the remaining fish (5.988) were slaughtered and sex was recorded by visual observation of the gonads after dissection, and 5.960 of those had an identifiable sex phenotype. In all sites, the difference between males and females was straightforward (female gonads were orange, and male gonads pink/white), and only 28 fish in total could not be determined with certainty. Parentage assignment was done by Landcatch Natural Selection (Alloa, UK) using 6 microsatellite markers on both parents and offspring. Out of the 5.960 offspring with a sex phenotype, 5.896 (98.9%) could be assigned to a single parental pair.

**Statistical Methods:** Sire 23 (set 3) gave only 3 offspring, probably due to bad sperm quality. It was removed from the analysis, as it created a major disequilibrium in the data, thus reducing the number of families studied from 253 to 246. Then, the base data set comprised 5.893 offspring from 246 families. Apart from sire 23, 245 of 246 possible families had offspring. No offspring were found in the sire 9xdam 2 family (set 1), probably due to a bad quality straw of cryopreserved sperm, as both male 9 and female 2 gave satisfactory results in all other crosses. In order to avoid computational problems, these missing data were replaced by simulated data corresponding to the expected numbers of male and female offspring in this family (19 males and 3 females, which are the average numbers of males and females per family produced by male 9 and female 2)

In a first analysis, the number of females was calculated in each paternal or maternal half-sib family, and compared to the expected number of females with a uniform

proportion corresponding to the observed proportion of females in the whole sample, using chi-square tests, to test for the existence of significant genetic variation in progeny sex ratio. In a second step, the family sex ratios in each of the three full-factorial sets was analyzed by logistic regression using SAS® proc Logistic, where the proportion of females was explained by a sire and dam effect. The model fit was tested using the Hosmer and Lemeshow test (Hosmer & Lemeshow 1989). In a third step, sex was considered as a threshold trait with a polygenic basis (Bulmer & Bull 1982). Sex was analyzed using a single trait model including additive random effects for sire and dam, and a residual error. Restricted maximum likelihood (REML) estimates of variance components for the random effects in the model were obtained on the underlying liability scale using the ASREML software (Gilmour *et al.* 2002). Both sire and dam heritabilities were estimated, using standard formulae (Becker 1984). Genetic correlations between sex and growth were estimated with a trivariate (sex, weight and length at 370 days) animal model, with sex coded on the observed scale (0 or 1), using the VCE5.0 software (Kovac & Groeneveld 2003). This model included an animal additive genetic effect for all traits, and for length and weight, a fixed effect of sex, which was necessary due to sex dimorphism on length and weight (females are larger than males). We used the observed scale since it produces unbiased genetic correlations as long as the threshold trait does not have both low heritability and low incidence (Olausson & Ronningen 1975). We also estimated the heritability of sex dimorphism for growth using classic formulae of (co)variance for a difference (Chapuis *et al.* 1996).

Then, we used the estimates generated to simulate samples of 5.893 fish from 23 dams and 32 males in three sets of 9x11, 7x10 and 7x11, using a heritability of sex of 0.62 on the underlying scale, and a mean proportion of females of 18.3%. We also

generated simulated samples of the same size using a threshold model where the underlying sex tendency  $m$  would be determined, instead of polygenes, by one or two bi-allelic loci with effect size  $f$ , such that genotype  $aa$  had a genetic effect of  $-f$  on  $m$ , genotype  $Aa$  had a genetic effect of 0 and genotype  $AA$  had a genetic effect of  $+f$ . The allelic frequencies were 0.5 for each allele, except for the one locus case where allelic frequencies 0.2, 0.4, 0.5, 0.6 and 0.8 were tested. The effect size  $f$  was tuned so that the proportion of genetic variance over phenotypic variance was 0.62, and a random residual effect representing 38% of the total phenotypic variance was added. This model is an extension to skewed population sex ratios of the two-factor model with environmental variance (Bull 1983). Purely genetic threshold models were also tested, either polygenic with  $h^2=1$ , or with one to five bi-allelic loci, but no residual environmental variance. For each model, we generated 10.000 samples, and compared the simulated distribution of full and half-sib family sex ratios with the observed one, to test for the coherence of the model with the observed dataset.

## RESULTS

The overall proportion of females in the population was 18.3%, and there were no sex ratio differences between fish from each of the four growing sites (17.4%-19.4%,  $\chi^2=4.62$ , 3 d.f.,  $P>0.20$ ). Family sex ratios are given in Appendix 1. Comparison of observed values to expected values under the hypothesis of equal sex ratio in all families shows a strong inequality of contributions between half-sib families ( $\chi^2=350.6$ , 31 d.f.,  $P<0.0001$  for paternal half-sib families,  $\chi^2=327.6$ , 22 d.f.,  $P<0.0001$  for maternal half-sib families). Proportions of females ranged from 4.7% to 46.3% in paternal half-sib families, and from 0.5% to 40.3% in maternal half-sib families. The logistic regression analysis showed that both sire and dam had a highly significant effect on the progeny sex ratio ( $P<0.0001$ ), and that this model without interaction was enough to explain the observed dataset, the Hosmer and Lemeshow chi-square tests being far from significance ( $\chi^2=6.04$ , 8 d.f.,  $P>0.6$  in set 1,  $\chi^2=1.94$ , 8 d.f.,  $P>0.9$  in set 2,  $\chi^2=6.04$ , 8 d.f.,  $P>0.6$  in set 3). The estimated heritability of sex on the underlying scale was  $0.52 \pm 0.13$  (sire heritability),  $0.72 \pm 0.20$  (dam heritability), or  $0.62 \pm 0.12$  (sire+dam heritability). The maternal effect ratio (non genetic maternal variance/phenotypic variance) that would explain the difference between sire and dam heritability is  $m^2=0.05 \pm 0.06$ , which is clearly non significant. The heritability of growth sex dimorphism was low (0.15 for weight and 0.09 for length at 370 days). The estimated genetic correlation was  $0.50 \pm 0.09$  between sex and weight, and  $0.48 \pm 0.09$  between sex and length. Some graphical illustration of this can be seen on Figure 1, which shows that apart from the fixed effect of sex on weight (females being on average 40.8% heavier than males at 370 days), larger fish tended to be found in the families with the highest proportion of females. The distribution of proportions of females among the 55 half-sib families scored (32

paternal half-sib families, 23 maternal half-sib families) is plotted on Figure 2, and compared with simulated distributions in the same experimental setting. Detailed information about models fit can be found in Table 1. Additive polygenes with  $h^2=0.62$  could explain the data with excellent fit. For a single locus with environmental variance, the best fit was observed with allelic frequencies of 0.6 for the male-orienting allele and 0.4 for the female-orienting allele, but this could not account for the observed half-sib data. On the contrary, a two-locus system with environmental variance could fit both half-sib and full-sib observed data. No purely genetic model (without environmental variance) could explain the observed data, whatever the number of loci implied (from monogenic to polygenic).

## DISCUSSION

**The genetic influence on sex in sea bass:** Our results are fully in accordance with a polygenic model, as described by Bulmer and Bull (1982), where the sex of an offspring is determined by the fact that an underlying sex tendency (determined both by polygenes and environmental effects) is greater or less than a threshold value. In our experiment, both sires and dams have an effect on the sex ratio of their progenies, and the effects are similar in size, pleading for an additive genetic effect on sex ratio. The heritability is high ( $h^2_{s+d}=0.62 \pm 0.12$ ), and clearly different from zero. It may not be biased by non additive genetic factors, as the mating design is factorial and all fish were in the same environment at the time of sex determination. Moreover, we verified that each dam ( $\chi^2=73$ , 66 d.f.,  $P>0.2$ ) and each sire ( $\chi^2=104$ , 93 d.f.,  $P>0.2$ ) contributed the same proportion of offspring in all sites, and that the same sex ratios were observed in all sites, so even unexpected late actions of the environment on the sex-ratio would not bias the between-family data. The “classical” vertebrate chromosomal sex determination model is expected to give 50% males and 50% females, and clearly cannot explain the data obtained, even with eventual sex-reversal of genetic females to males due to temperature. This holds as long as sex reversal is homogeneous among families (i.e. not genetically determined). If sex reversal was not homogeneous among families, this would imply the existence of a secondary genetic component of sex ratio, in addition to the chromosomal system. Purely environmental sex determination (ESD) can also be excluded, as it is not supposed to yield any between families differences in sex ratios. This was already suggested by previous results (Blazquez *et al.* 1999; Saillant *et al.* 2002; Gorshkov *et al.* 2003; Peruzzi *et al.* 2004). As it is clear that environment can influence sex ratio in sea bass (Piferrer *et al.* 2005), the genetic component we evidence can be either

considered as a genetic variation of the primary sex ratio, or as a genetic sensitivity to the environmental effects. As we tested only one environmental condition however, we cannot distinguish between both. The observed occurrence of intratesticular oocytes in many (up to 62%) young sea bass males (Gorshkov *et al.* 1999; Saillant *et al.* 2003a) could be in favor of a polygenic primary sex ratio, with sex reversal occurring as a consequence of environmental effects. However, the existence of genetic by environment interactions, which would favor the second hypothesis, has already been evidenced (Saillant *et al.* 2002). Nevertheless, we show that whatever its true nature, there is a genetic effect which leads to a continuous distribution of family sex ratios in this species, at least in one (masculinizing) environmental condition. We also show that, in addition to the global effect of temperature, which certainly skewed sex ratio towards males in the present experiment, it is necessary to include environmental variance within this global environment to explain the observed distribution of sex ratio, meaning that a purely genetic model where individual sex would be uniformly influenced by the environment could not explain our data. Even with environmental variance allowed, a two-factor system can be excluded, but a four-factor system (two bi-allelic loci) can explain the observed data. Similarly, in the apple snail, it was concluded that a continuous variation in family sex ratios was most likely due to at least four sex factors (Yusa 2007). Unlike what was seen in the silverside fish *Menidia menidia* (Conover & Heins 1987b), we could not observe a multimodal distribution of family sex ratios, which was considered an indication of the existence of only a few sex factors in this species. Indeed, as pointed out by Bull (1983), it is extremely difficult to ascertain the polygenic nature of a sex determining system, when compared to a system with only a few factors and some environmental variance. Nevertheless, both

systems are expected to behave in very similar manner, and may be described by the Bulmer and Bull threshold model, provided sex factors have individually weak effects (Bull 1983). Finally, we showed there was considerable genetic variation for sex ratio in a given environment, as half-sib family sex ratios range from 0.5% to 46.3% of females (the proportions range from 0 to 75% in full-sib families, but their small size – on average 24 individuals – makes it less significant). We can compare this range of variation in female proportions to that produced by temperature treatments: 0-27% (Blazquez *et al.* 1998), 18-66% (Koumoundouros *et al.* 2002), 24-74% (Pavlidis *et al.* 2000) and 11-32% (Saillant *et al.* 2002). This shows that in this species, the genetic and environmental components of sex determinism are of comparable magnitude, which confirms there are no fundamental barriers between ESD and GSD, as proposed by Bull (1983).

**The genetic relationship between size and sex:** An interesting feature of our results is the relatively strong genetic correlation between sex and size ( $r_A=0.50 \pm 0.09$ ), which means that some of the genes acting on sex determination and growth are the same, or at least are strongly linked in our sample. This is in accordance with previous experimental evidence showing that females are larger at the time of sex differentiation (Blazquez *et al.* 1999; Saillant *et al.* 2001). Still, we have shown that once corrected for this sex dimorphism, there was still a size advantage in families with high proportions of females (Figure 1). This strengthens a lot the connection that was established between growth and sex. Indeed, as sea bass is a group spawner, it is clear that the size of females should have a strong impact on their fitness, through their absolute fertility, whereas it may be less important for males. Therefore, the idea that a minimum size is needed at the time of sex differentiation to be able to differentiate as female is plausible, and strengthened by the fact that the

size advantage of females is never as large as at the time of sex differentiation (+41% weight at 1 year, +20% at 2 years in the present study, +67% at 10 months and +25% from 2 years in (Saillant *et al.* 2001)). This type of determinism is observed in nematodes (Ellenby 1954) and eels (Roncarati *et al.* 1997), where high density (hence limited resources for growth) favors male differentiation. However, density had no effect on sex ratio in sea bass (Saillant *et al.* 2003b), but in this latter case the densities and rearing conditions were chosen to avoid any impact on growth, in order not to confound effects of growth and density *per se*.

**Evolutionary consequences of polygenic sex in seabass:** Our heritability estimates for sex ratio are high (0.62), as in turtles, where high estimates (in the range of 0.5-0.8) have also been found (Bull *et al.* 1982; Janzen 1992). However, the estimates in turtles were likely inflated by maternal and/or dominance effects, due to the use of full-sib designs, which is not the case in our experiment. Moreover, in turtles, the impact of high heritability estimates was considerably lowered in natural conditions by the high variance between nests temperature, which, combined with the very narrow temperature range for complete sex change, reduces a lot the potential for selection on sensitivity to ESD (Bull *et al.* 1982). In our case, although temperature has a large effect on sex ratio, it cannot produce 100% female progenies, and there is a wide spectrum (13-25°C) having an impact on sex determination. Still, the temperature of the coastal waters during the first year of life of the sea bass may be quite variable, and thus reduce the effectiveness of natural selection on polygenic sex ratio in this species. As our experimental growing conditions are different from natural ones (fish are 13.5 cm at 1 year, *vs.* 7-10 cm in the wild in the Atlantic - Pickett & Pawson 1994), and with the suspected genotype-environment interactions for sex ratio in sea bass (Saillant *et al.* 2002), variation which may be hidden in wild

(low temperature) conditions may be expressed in experimental or farm (warm) conditions. This may be even accentuated by the growth-sex genetic relationship, as growth also has a high heritability in this experiment ( $0.54 \pm 0.08$  for weight), and is expected to have a higher heritability in fast-growing conditions than in slow-growing conditions.

Considering the high heritability of sex ratio observed in our conditions, we could calculate the effects of frequency-dependent selection on sex using the Bulmer and Bull (1982) model (Figure 3). It shows that equilibrium sex ratio should be reached in 7-8 generations. This shift towards females, which are more interesting for aquaculture, could even be accelerated through artificial selection of female-producing families. If artificial selection on growth is also practiced, as is the case in several hatcheries, the shift in sex ratios in aquaculture populations should be even faster, and may lead to predominantly female populations. As aquaculture of sea bass expands in the Mediterranean area, the impact of aquaculture escapees, with modified sex determinism, will have to be carefully evaluated, as sex ratio is doubtlessly a major determinant of fitness, and may therefore have an important impact on the fitness of natural populations of this species (Lynch & O'Hely 2001).

**Conclusion:** We have demonstrated that sex determinism in the sea bass is not monogenic, is sensitive to within-tank variations in the environment, and that the genetic component is essentially additive, is linked to the growth capacity of the fish, is of the same magnitude as the environmental component controlled by temperature, and can be precisely described using a polygenic threshold model with  $h^2=0.62$  on the underlying scale.

Selective breeding experiments are under way to explore the effective response to sex ratio selection and the correlated sex ratio response to selection for growth. They

will also provide material for QTL search in the coming years, hopefully allowing us by that time to determine more precisely if sex determinism in this species is effectively polygenic or only oligogenic.

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## Appendices

Appendix 1. Numbers of males and females (M:F) in 246 European sea bass families, representing 5.893 fish from three full-factorial matings

A) Set 1: 11 sires x 9 dams

Sires	Dams									Mean proportion of females	Total offspring number
	D34	D35	D36	D37	D38	D39	D40	D41	D42		
S01	19:2	21:4	20:5	20:12	20:11	19:12	30:9	19:8	30:5	0.256	266
S02	17:0	13:0	16:0	14:3	35:4	26:6	33:3	33:5	21:0	0.092	229
S03	16:1	22:0	25:1	14:3	25:1	36:2	30:9	26:8	31:2	0.107	252
S04	7:3	15:0	12:3	10:7	27:9	17:12	36:11	17:7	18:3	0.257	214
S05	14:4	21:2	19:5	5:5	11:7	15:6	20:4	27:7	13:3	0.229	188
S06	16:0	9:0	21:2	10:2	18:3	33:4	22:4	27:8	18:1	0.121	198
S07	14:8	18:3	24:10	12:8	22:8	12:17	29:15	15:10	7:3	0.349	235
S08	9:1	11:1	11:2	6:0	22:0	18:0	31:0	6:2	7:0	0.047	127

S09	16:2	<sup>a</sup>	14:0	21:1	37:4	24:3	22:4	15:5	17:0	0.103	185
S10	3:7	14:3	8:3	4:12	14:7	12:14	18:16	15:12	13:13	0.463	188
S11	11:8	24:0	20:5	20:21	17:11	19:16	28:13	22:14	30:6	0.330	285
Mean proportion of females	0.202	0.072	0.159	0.352	0.208	0.285	0.227	0.279	0.149		
Total offspring number	178	181	226	210	313	323	387	308	241		

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<sup>a</sup>no offspring observed in this family

## B) Set 2: 11 sires x 7 dams

Sires	Dams							Mean proportion of females	Total offspring number
	D43	D44	D45	D46	D47	D48	D49		
S12	29:4	17:3	15:0	20:1	19:0	27:0	12:0	0.054	147
S13	15:8	34:8	30:4	47:2	29:4	35:1	11:6	0.141	234
S14	11:17	27:2	12:0	28:0	26:4	26:2	7:3	0.170	165
S15	18:3	28:3	18:1	19:0	24:0	18:0	16:0	0.047	148
S16	9:16	21:11	26:4	28:3	38:12	20:4	13:6	0.265	211
S17	25:18	34:15	27:1	37:1	39:6	34:1	25:2	0.166	265
S18	11:10	29:5	31:1	26:1	41:1	35:2	9:1	0.103	203
S19	11:10	22:2	13:1	21:0	26:3	27:1	18:2	0.121	157
S20	19:10	24:9	14:1	28:1	29:1	31:1	14:1	0.131	183
S21	18:10	11:5	11:2	25:1	18:1	26:3	14:2	0.163	147

S22	16:17	11:15	24:8	22:1	14:8	16:3	11:5	0.333	171
Mean proportion of females	0.403	0.232	0.094	0.035	0.117	0.058	0.157		
Total offspring number	305	336	244	312	343	313	178		

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## C) Set 3: 10 sires x 7 dams

Sires	Dams							Mean proportion of females	Total offspring number
	D50	D51	D52	D53	D54	D55	D56		
S24	3:1	4:0	5:0	5:0	5:1	7:0	13:2	0.087	46
S25	13:3	24:9	12:2	29:0	24:9	18:1	43:20	0.213	207
S26	34:9	9:5	10:1	32:0	12:2	11:0	15:7	0.163	147
S27	22:4	17:6	9:0	27:0	22:2	6:0	21:4	0.114	140
S28	44:4	19:2	8:0	23:0	24:4	9:0	18:2	0.076	157
S29	13:8	9:9	4:3	10:1	6:4	5:1	6:9	0.398	88
S30	37:10	21:9	17:2	33:0	38:7	16:0	20:7	0.161	217
S31	33:6	20:9	14:1	25:0	18:11	14:1	19:15	0.231	186
S32	29:3	31:4	16:0	18:0	28:2	6:0	30:0	0.054	167
S33	26:0	27:3	9:0	12:0	22:1	11:0	24:5	0.064	140

Mean proportion of females	0.159	0.236	0.080	0.005	0.178	0.028	0.254
Total offspring number	302	237	113	215	242	106	280

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**Tables**

Table 1: Comparison of family sex-ratio distributions observed in the present study with simulated distributions from various sex-determination models. P-value <0.05 shows incompatibility between simulated and observed data

Model	Half-sib families		Full-sib families	
	sex ratio distribution		sex ratio distribution	
	$d.f.=6$		$d.f.=11$	
	$\chi^2$	P-value	$\chi^2$	P-value
Polygenic, $h^2=0.62$	2.07	>0.9	10.9	>0.4
1 locus with envir. variance, $p=0.2$ <sup>a</sup>	58.1	<0.001	171	<0.001
“ , $p=0.4$	59.6	<0.001	18.4	>0.05
“ , $p=0.5$	22.9	<0.001	6.2	>0.8
“ , $p=0.6$	14.8	<0.05	10.2	>0.5
“ , $p=0.8$	18.2	<0.01	28.1	<0.01
2 loci with envir. variance, $p=0.5$	1.42	>0.9	12.6	>0.3
1 locus without envir. variance	383	<0.001	865	<0.001
2 loci without envir. variance	23.9	<0.001	139	<0.001
3 loci without envir. variance	12.8	<0.05	63.3	<0.001
5 loci without envir. variance	6.33	>0.4	38.2	<0.001
Polygenic without envir. variance ( $h^2=1$ )	8.04	>0.2	40.1	<0.001

<sup>a</sup>  $p$ = frequency of the male-orienting allele

### Figure Legends

Figure 1. Relationship between arcsine transformed proportion of females in sire half-sib families of European sea bass and mean weight at 370 days of male and female offspring in the same families.

Figure 2. Observed frequencies of females in 55 half-sib families of European sea bass, and expected frequencies under a threshold model with 18.3% females in the offspring and 38% environmental variance, where the genetic component of the underlying variable is: a) polygenic; b) 1 bi-allelic locus with  $p=0.6$  for the masculinizing allele,  $q=0.4$  for the feminizing allele; c) 2 bi-allelic loci with  $p=q=0.5$ .

Figure 3. Expected evolution of sea bass sex ratio along generations of random mating in constant environmental conditions, using the frequency-dependent model of Bulmer and Bull (1982), with heritability 0.62 and initial sex ratio 18.3% females (this study's estimates)

Figures

Figure 1

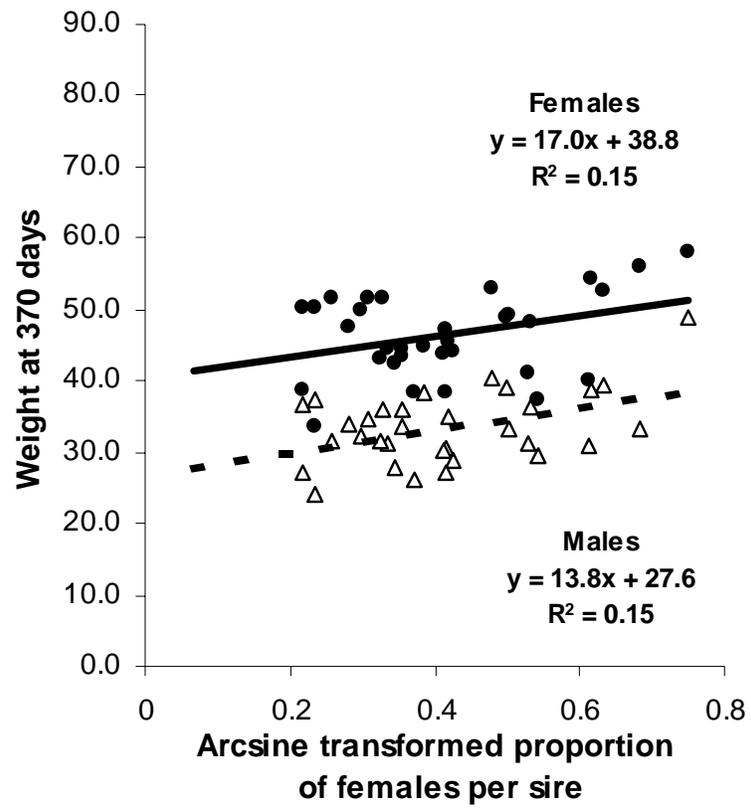


Figure 2 (a,b)

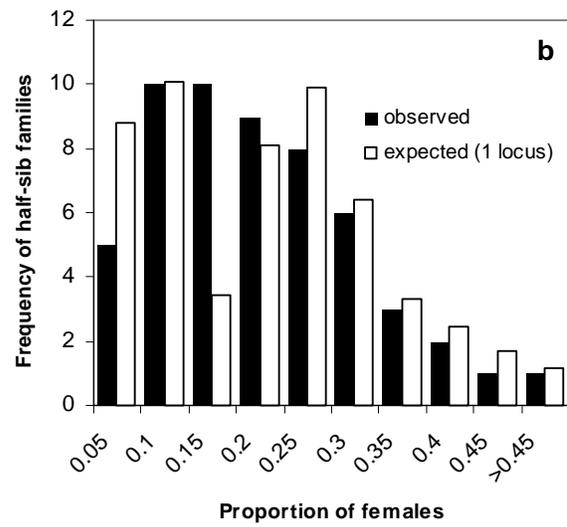
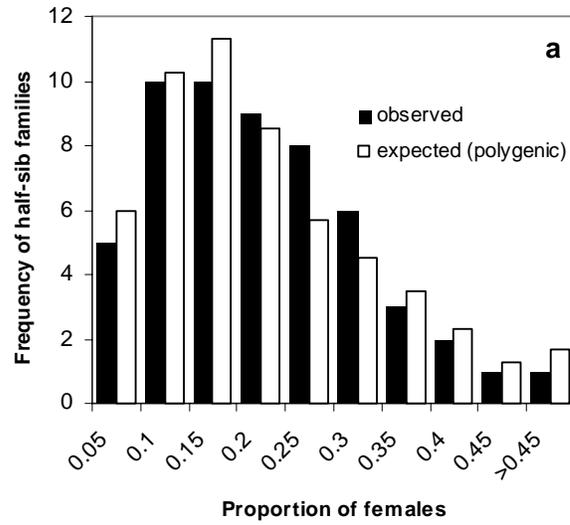


Figure 2 (c)

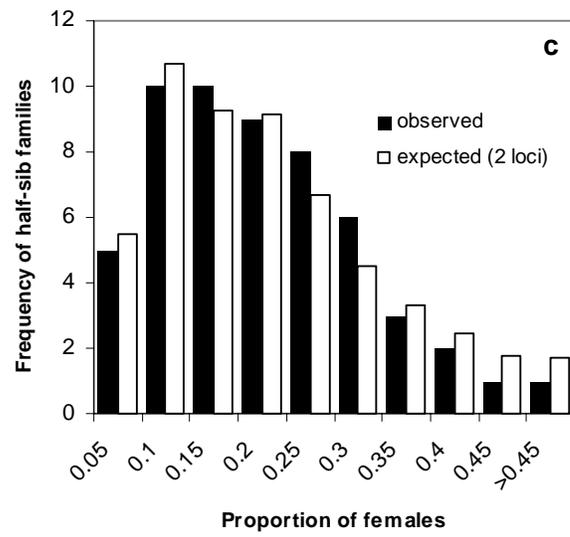


Figure 3

