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Sex Determination: Genetic Models for Oysters

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Abstract

In oysters, sex is determined partly by environment, but previous studies employing controlled crosses suggest that genetic factors are also important. Sex ratios in both full- and half-sib families of the Pacific oyster show paternal control of sex ratio and suggest that a single major gene with 2 genotypes controls sex in the Pacific oyster, with *FM* oysters being male and *FF* oysters maturing as male or female. Here, we show that such a model does indeed produce a stable polymorphism for either single or multiple age-class populations, though under limited ranges of *f*, the probability that an *FF* individual matures as a female. However, this 2-genotype model cannot explain observed heterogeneity of sex ratios among progeny from different dams within half-sib families. We propose an alternative 3-genotype model that also produces a stable polymorphism, for either single or multiple age-class populations, but over all values of *f* between zero and one. This model accounts for sex ratio heterogeneity among male half-sib families because it features 2 types of females, a protandric *FM* and a fixed female *FF*. Furthermore, the 3-genotype model, accounts for the frequencies of mating types inferred from the observed sex ratios of families more closely than the 2-genotype model. Although the mechanism of sex determination may ultimately prove more complex, simple genetic mechanisms can account for the broad features of sexual maturation in oyster families and the stability of sex ratios in populations.

Key words: *age classes, Crassostrea gigas, oyster, polymorphism, sex chromosomes, sex determination*

Genetic mechanisms of sex determination have long been of interest to evolutionary biologists. Sex determination in many animal species is achieved by means of differentiated sex chromosomes, with either male heterogamy and female homogamy (♂ XY/♀ XX) or male homogamy and female heterogamy (♂ ZZ/♀ ZW) (Bull 1983). Still, sex determination mechanisms vary widely from environmental sex determination to various forms of genetic sex determination, and the precise mechanism for genetic control of sex has not been determined experimentally in many animals outside of a limited group of model species (see Bull 1983). Investigations into sex determination among invertebrate animals that apparently lack strict control over sex ratios or a typical XY/XX or ZZ/ZW chromosomal system may shed light on the potential origins of sex determination in higher animals (Yusa 2007).

Cupped oysters of the genus *Crassostrea* are dioecious, sequential hermaphrodites (Amemiya 1929; Coe 1932, 1943; Galtsoff 1964; Quayle 1988). Sex is thought to be partially determined environmentally because factors that promote rapid growth, primarily temperature and food, favor early maturation as male (Coe 1936). In the eastern oyster *Crassostrea virginica*, for example, populations in warmer locations have higher proportions of yearling (age 1+) females, particularly late in the second summer, than do populations in cooler locations (Coe 1936). Furthermore, cupped oysters are capable of sex reversal, both within and between summer spawning seasons, although simultaneous hermaphrodites are rare (Amemiya 1929; Coe 1936) and not all individuals undergo sex reversal. For the Pacific oyster *C. gigas*, Guo et al. (1998) found that 37.1% of 1-year-old oysters, 54.9% of 2-year-old oysters, and 75.0% of 3-year-old oysters were female, respectively. The progressive increase of females with age and size is concordant with sex allocation theory (Charnov 1982) but does not rule out a primary genetic sex-determining mechanism.

Controlled crosses of eastern and Pacific oysters suggest that sex in cupped oysters is also under genetic control. Haley (1977, 1979) reported sex ratios in 5 full-sib families and proposed an oligogenic model for sex determination in the eastern oyster *C. virginica*. In this model, a pair of additive alleles, *m*, causing maleness, and *f*, causing femaleness, exists at each of 3 diallelic loci (6 alleles total). Sex is then determined as a ratio of *m* to *f* alleles so that *m:f* genetic ratios in excess of 3:3 represent "true" or fixed genetic males, ratios of 2:4 and 1:5 cause the development of

protandric (individuals that begin life as males and then may change into females) females, and a ratio of 0:6 results in true genetic females.

In a large experimental study, Guo et al. (1998) examined sex ratios in 86 full- and half-sib families of the Pacific oyster. They found paternal control of sex ratio variation in families and a clumped distribution in family sex ratios suggestive of 2 types of males, as noted earlier by Coe (1932). On the basis of these observations, Guo et al. (1998) proposed that a single major gene controls sex in the Pacific oyster, with a dominant *M* allele for male maturation and a protandric *F* allele. *FM* oysters in this model are fixed males; *FF* oysters may mature as males or females, depending either on other genetic or on environmental factors. To account for the increase in the proportion of females with age, Guo et al. (1998) hypothesized that the probability of an *FF* oyster maturing as a female was 50% in the first year. In each following year, they assumed that the probability of the remaining *FF* males becoming female was 50% and that once *FF* oysters become females they remain female for the rest of their lives.

Cupped oysters lack heteromorphic sex chromosomes (Ahmed and Sparks 1967; Longwell et al. 1967). Although a major sex-determining gene in *Crassostrea* was not located in a genome-wide mapping attempt, using amplified fragment-length polymorphisms (Li and Guo 2004), multiple major quantitative trait locus (QTL), several of them clustered on linkage group III (Hubert and Hedgecock 2004), have been found in an F_2 population of Pacific oysters (Hedgecock D, Perry GML, Voigt M-L, in preparation).

Here, we examine the 2-genotype model of Guo et al. (1998) and show that it can lead to a stable polymorphism. Although this model can explain the observed data of increasing female proportions with age, it cannot explain heterogeneity of sex ratios in half-sib families with a single male parent and different female parents. Therefore, we propose an alternative 3-genotype model that also produces a stable equilibrium, produces an increase in proportion of females with age similar to what Guo et al. (1998) reported, and can account for sex ratio heterogeneity among male half-sib families. Our purpose is neither to account for all facts about sex ratios in oysters nor to produce a comprehensive model of sex determination in a cupped oyster but to demonstrate that simple genetic mechanisms yield stable polymorphisms and may account for the broad features of sexual maturation and sex ratios in cupped oyster families and populations.

Family Data of Guo et al. (1998)

Guo et al. (1998) found extensive variation among experimental crosses in the proportion of females, both among families and at different ages of progeny. We note that the sample sizes for the progeny from these crosses were large (averaging 22.5 progeny for the 167 different families and approximately 3 times that for the half-sib families with a common male parent). In other words, sample sizes were large enough that differences in the proportion of females among families were not due to chance. For comparison to our model predictions below, Table 1 summarizes their data for 3 crosses, one assessed at age 1 and 2 at age 2. Notice, for example, that for cross W2E, the proportion of females at age 1 for families sired by different males ranged from 16.3% (male 8) to 60.4% (male 2). The 3 female half-sib families for male 8 had very similar female progeny proportions of 16.7%, 12.5%, and 19.6%, whereas the 3 female families for male 2 had female progeny proportions of 71.0%, 44.2%, and 65.9%.

For cross H2E, the proportion of females at age 2 for families sired by different males ranged from 38.1% (male 3) to 72.3% (male 4). The 3 female families for male 3 had female progeny proportions of 52.4%, 26.1%, and 35.7%, whereas the 3 female families for male 4 had very similar progeny proportions of 75%, 70%, and 72%. Below, we will see if the predictions from our models are consistent with these observations.

Model

Two-Genotype Model of Guo et al. (1998)

Single Age-Class Model

Guo et al. (1998) proposed that sex was determined by 2 genotypes at a single locus with *FM* individuals always being males and *FF* individuals being either males or females. Therefore, there are 2 mating types that have different males, *FM* (male) × *FF* (female) and *FF* (male) × *FF* (female), as given in Table 2. Assume that a proportion f of *FF* progeny mature as females and a proportion $1-f$ mature as males and that the frequencies of *FM* males, *FF* males, and *FF* females are H, P_m, and P_f, respectively ($H + P_m = 1$). The proportions of the different genotypes in the progeny are then

Table 1 The proportion of females (sample sizes in parentheses) in 3 family groups fathered by 8 different males, age 1 for family group W2E and age 2 for family groups H2E and D1E (Guo et al. 1998)

	Age (family group)		
Male	1 (W2E)	2 (H2E)	2 (D1E)
1	0.178 (153)	0.597 (72)	0.677 (43)
2	0.604 (115)[a]	0.660 (66)	0.622 (43)
3	0.568 (123)	0.381 (72)[b]	0.435 (66)[b]
4	0.508 (175)	0.723 (73)[a]	0.737 (80)
5	0.313 (129)	0.619 (71)	0.603 (80)
6	0.266 (76)	0.547 (73)	0.795 (67)[a]
7	0.342 (105)	0.400 (62)	—
8	0.163 (95)[b]	0.611 (79)	—
Mean (*N*)	0.379 (971)	0.569 (568)	0.660 (379)

Note that although the same numbers are used here for the male parents in the 3 different family groups, they are in fact different males, for example, male 1 is different for each of the 3 family groups.

[a] Indicates largest proportions of females for each family group.

[b] Indicates smallest proportions of females for each family group.

Table 2 For the 2-genotype model of Guo et al. (1998), the 2 possible mating (or family) types, their frequency, and the expected segregation proportions in their progeny

	Mating			Progeny genotype		
Type	Male	Female	Frequency	*FF* male	*FF* female	*FM*
1	*FM*	*FF*	HP_f	$(1-f)HP_f/2$	$fHP_f/2$	$HP_f/2$
2	*FF*	*FF*	P_fP_m	$(1-f)P_fP_m$	fP_fP_m	—

$$H' = \frac{HP_f}{2W_m} \tag{1a}$$

$$P_m' = \frac{(1-f)P_f(H+2P_m)}{2W_m} \tag{1b}$$

$$P_f' = \frac{fP_f(H+2P_m)}{2W_f} \tag{1c}$$

where W_m and W_f are the proportions of male and female offspring, and are

$$W_m = \tfrac{1}{2}HP_f(2-f) + P_fP_m(1-f)$$

and

$$W_f = fP_f(H/2 + P_m)$$

Multiple Age-Class Model

Now let us assume that individuals live for more than one year and that, as individuals age, each year a constant proportion f of the *FF* males becomes female and that these individuals remain female for the rest of their lives. If we assume that there are 3 adult age classes (because we want to compare our findings with those of Guo et al. 1998), then the frequency of male parents that are *FF* in year t is the weighted frequency of male *FF* individuals from the previous 3 years (i.e., *FF* males that are now 1, 2, and 3 years old) or

$$P_{m(t)} = P_{m(t-1)}\frac{1}{\varkappa} + P_{m(t-2)}'\frac{1-f}{\varkappa} + P_{m(t-3)}'\frac{(1-f)^2}{\varkappa} \tag{2a}$$

where

$$\begin{aligned}
\varkappa &= 1 + 1 - f + (1-f)^2 = 3 - 3f + f^2\\
P_{m(t-2)}' &= P_{m(t-2)}(1-f)/W_{m(t-2)}\\
W_{m(t-2)} &= P_{m(t-2)}(1-f) + H_{t-2}\\
P_{m(t-3)}' &= P_{m(t-3)}(1-f)^2/W_{m(t-3)}\\
W_{m(t-3)} &= P_{m(t-3)}(1-f)^2 + H_{t-3}
\end{aligned}$$

Notice that the frequency of *FF* males is a weighted value that reflects the reduced proportion of males that are *FF* as individuals age over 3 years. The frequency of male parents that are *FM* in year t is then

$$H_t = 1 - P_{m(t)} \tag{2b}$$

As we discussed above, there are only 2 different mating or family types for this model. For each of these family types, we can predict the proportion of female progeny within the family types for different aged progeny. As above, assume that f of the *FF* progeny are initially female and remain female throughout their life. The remainder of the *FF* progeny that are male may change each year to females with a probability f. In other words, the expected proportion of females in the progeny for the 2 mating types in Table 2 at age t are

$$\begin{aligned}
X_{t(1)} &= \tfrac{1}{2}f\sum_{i=0}^{t-1}(1-f)^i\\
X_{t(2)} &= f\sum_{i=0}^{t-1}(1-f)^i
\end{aligned} \tag{3}$$

Three-Genotype Model

Single Age-Class Model

Assume that there is a sex-determining locus with alleles F and M, *FF* individuals are females, *MM* individuals are males and that a proportion f of *FM* individuals mature as females and a proportion $1-f$ mature as males. (Note that we are again using f to indicate the proportion of individuals that mature as females but for genotype *FM* in this model, not genotype *FF* as in the 2-genotype model.) The frequencies of genotypes *FF* and *MM* are P and Q, respectively, and the frequencies of *FM* individuals that are females and males are H_f and H_m, respectively ($H_f + H_m = H$).

Because there are 2 types of females and 2 types of males, there are the 4 mating types as given in Table 3. If we assume random mating and normal segregation, then the expected frequencies of the 4 genotypes in the progeny are

$$\begin{aligned}
P' &= \frac{H_m(1-\frac{1}{2}H_f)}{2W_f}\\
H_f' &= \frac{f(1+PQ)}{2W_f}\\
H_m' &= \frac{(1-f)(1+PQ)}{2W_m}\\
Q' &= \frac{H_f(1-\frac{1}{2}H_m)}{2W_m}
\end{aligned} \tag{4}$$

where W_m and W_f are the proportions of male and female offspring, and are

$$\begin{aligned}
W_f &= \tfrac{1}{2}[H_m(1-\tfrac{1}{2}H_f) + f(1+PQ)]\\
W_m &= \tfrac{1}{2}[H_f(1-\tfrac{1}{2}H_m) + (1-f)(1+PQ)]
\end{aligned}$$

The frequencies of the F allele in males (p_m) and females (p_f) are

$$p_m = \tfrac{1}{2}H_m$$

and

$$p_f = P + \tfrac{1}{2}H_f$$

Multiple Age-Class Model

Let us again assume that individuals live for more than one year and that, as individuals age, each year a constant proportion f of the *FM* males becomes female and that these remain female for the rest of their lives. If we assume that there are 3 age classes, then the frequency of male parents that are *FM* in year t is the weighted frequency of *FM* individuals from the past 3 years that remained males or

Table 3 For the 3-genotype model, the 4 possible mating (or family) types, their frequency, and the expected segregation proportions in their progeny

Mating				Progeny genotype			
Type	Male	Female	Frequency	*FF*	*FM* female	*FM* male	*MM*
1	*MM*	*FF*	PQ	—	fPQ	$(1-f)PQ$	—
2	*FM*	*FF*	H_mP	$H_mP/2$	$fH_mP/2$	$(1-f)\ H_mP/2$	—
3	*MM*	*FM*	H_fQ	—	$fH_fQ/2$	$(1-f)H_fQ/2$	$H_fQ/2$
4	*FM*	*FM*	H_fH_m	$H_fH_m/4$	$fH_fH_m/2$	$(1-f)H_fH_m/2$	$H_fH_m/4$

$$H_{m(t)} = H_{m(t-1)}\frac{1}{y} + H'_{m(t-2)}\frac{1-f}{y} + H'_{m(t-3)}\frac{(1-f)^2}{y} \tag{5a}$$

where

$$y = 1 + (1-f) + (1-f)^2 = 3 - 3f + f^2$$
$$H'_{m(t-2)} = H_{m(t-2)}(1-f)/W_{m(t-2)}$$
$$W_{m(t-2)} = H_{m(t-2)}(1-f) + Q_{t-2}$$
$$H'_{m(t-3)} = H_{m(t-3)}(1-f)^2/W_{m(t-3)}$$
$$W_{m(t-3)} = H_{m(t-3)}(1-f)^2 + Q_{t-3}$$

The frequency of female *FM* parents in year t is the weighted frequency of *FM* individuals from the past 3 years that remained or became females or

$$H_{f(t)} = H_{f(t-1)}\frac{1}{x} + H'_{f(t-2)}\frac{1+f}{x} + H'_{f(t-3)}\frac{1+f(2-f)}{x} \tag{5b}$$

where

$$x = 1 + 1 + f + 1 + f(2-f) = 3 + 3f - f^2$$
$$H'_{f(t-2)} = (H_{f(t-2)} + H_{m(t-2)}f)/W_{f(t-2)}$$
$$W_{f(t-2)} = H_{f(t-2)} + H_{m(t-2)}f + P_{t-2}$$
$$H'_{f(t-3)} = [H_{f(t-3)} + H_{m(t-3)}f(2-f)]/W_{f(t-3)}$$
$$W_{f(t-3)} = H_{f(t-3)} + H_{m(t-3)}f(2-f) + P_{t-3}$$

As we discussed earlier, there are 4 different mating or family types for this model. For each of these family types, we can predict the proportion of female progeny within the family types for different aged progeny. As above, assume that f of the *FM* progeny are initially female and remain female throughout their life. The remainder of the *FM* progeny that are male may change each year to females with a probability f. In other words, the expected proportion of females in the progeny for the 4 mating types in Table 3 at age t are

$$\begin{aligned} X_{t(1)} &= f\sum_{i=0}^{t-1}(1-f)^i \\ X_{t(2)} &= \tfrac{1}{2}[1 + f\sum_{i=0}^{t-1}(1-f)^i] \\ X_{t(3)} &= \tfrac{1}{2}f\sum_{i=0}^{t-1}(1-f)^i \\ X_{t(4)} &= \tfrac{1}{4}[1 + 2f\sum_{i=0}^{t-1}(1-f)^i] \end{aligned} \tag{6}$$

Results

Two-Genotype Model of Guo et al. (1998)

Single Age-Class Model

As we discussed above, Guo et al. (1998) proposed a model in which *FM* individuals are always males and *FF* individuals are females and males in proportions f and $1-f$, respectively. First, let us examine Equations 1a to 1c. By substitution of W_f into Equation 1c, $P'_f = 1$, as expected because there is only one genotype that is female. Substitution of $P_f = 1$ and W_m into Equation 1a, and simplifying, gives

$$H' = \frac{\frac{1}{2}H}{1 - f(1 - \frac{1}{2}H)} \tag{7a}$$

Now, if we assume at equilibrium $H' = H = H_e$, then this equation becomes

$$H_e = \frac{2(f - \frac{1}{2})}{f} \tag{7b}$$

and $P_e = 1 - H_e$. Notice that f must be greater than 0.5 to result in a nonzero equilibrium frequency of heterozygotes.

Figure 1 gives the expected frequency of the 2 male genotypes for different values of f (solid lines). Notice that when $f = 1$, the model becomes a classic XY/XX sex determination, that is, one in which all *FM* individuals are males and all *FF* individuals are females. At the other extreme, when $f = 0.5$, there is no sex locus polymorphism and there are equal numbers of *FF* males and *FF* females. As f increases, the frequency of *FM* males increases and more *FF* individuals are females. When $f = 2/3$, there are equal numbers of *FM* and *FF* males. Because there are only 2 mating types that depend on the type of male, the frequency of the 2 mating types given in Table 2 are equal to the frequency of the 2 males as given in Figure 1.

When there is a polymorphism ($0.5 < f > 1.0$), it is reached fairly quickly. To illustrate, Figure 2 shows the increase over 10 generations of the frequency of *FM* males, assuming that there is initially only 1% of the *FM* genotype, for 3 levels of f. For example, for $f = 0.95$, after only 6 generations, the frequency of *FM* males has changed completely from the initial value of 0.01 to the equilibrium frequency of 0.947

Multiple Age-Class Model

The equilibrium values for the 2 male genotypes for the multiple age-class model (3 age classes here as discussed

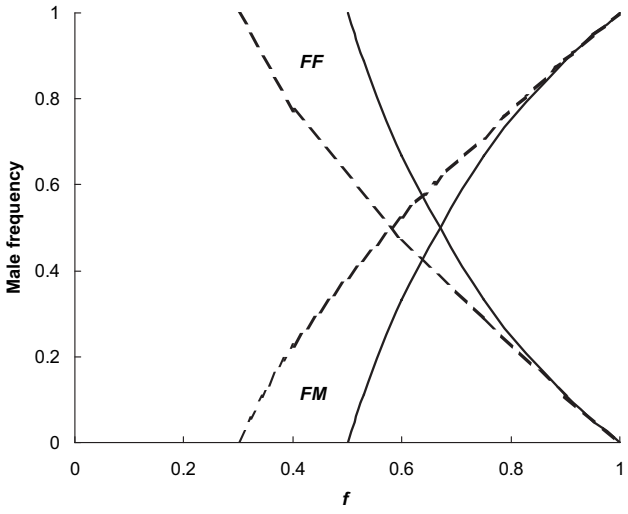

Figure 1. For the 2-genotype model of Guo et al. (1998), the effect of different levels of *f* on equilibrium frequencies of males of genotypes *FF* and *FM* for the single age-class model (solid lines) and the multiple age-class model (broken lines).

above) are also given in Figure 1 (broken lines). In this case, the values of *f* that result in a stable polymorphism are somewhat broader ($0.3 < f > 1.0$) than for the single age-class model. Intuitively, this is because the older age classes have more *FF* females, and therefore less *FF* males and more *FM* males, than for the single age-class model.

Now let us look at the expected proportion of females in the progeny at age *t* for each of the 2 mating types for different values of *f*. As examples, Table 4 gives the expected proportions of females in age classes 1, 2, and 3

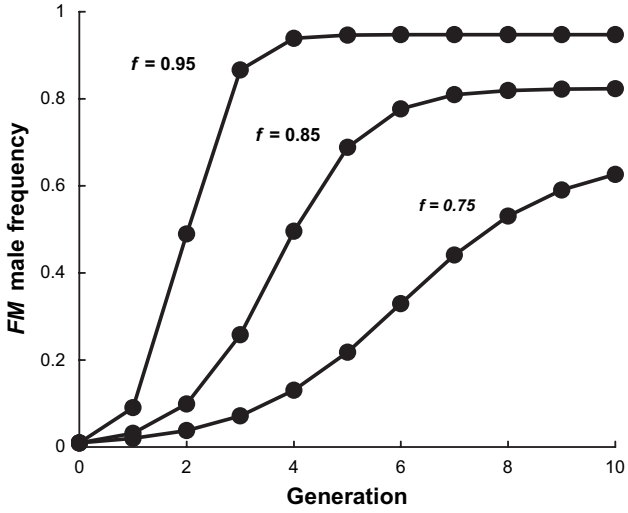

Figure 2. For the 2-genotype model of Guo et al. (1998) with a single age class, the increase over 10 generations of the frequency of *FM* males, assuming initially there is only 1% *FM* for 3 levels of *f*.

Table 4 For the 2-genotype model of Guo et al. (1998), the expected proportion of females at different progeny ages for the 2 different mating or family types

				Progeny age			
f	Mating	Male	Female	1	2	3	∞
0.3	1	*FM*	*FF*	0.15	0.255	0.328	0.5
	2	*FF*	*FF*	0.3	0.51	0.657	1.0
0.4	1	*FM*	*FF*	0.2	0.32	0.392	0.5
	2	*FF*	*FF*	0.4	0.64	0.784	1.0
0.5	1	*FM*	*FF*	0.25	0.375	0.438	0.5
	2	*FF*	*FF*	0.5	0.75	0.875	1.0
0.6	1	*FM*	*FF*	0.3	0.42	0.468	0.5
	2	*FF*	*FF*	0.6	0.84	0.936	1.0

from the 2 different mating types for 3 different values of *f*. For all the mating types, the proportion of females increases with age, with the biggest change occurring for mating type (2), *FF* × *FF*. For mating type (2), as age increases, the proportion of females approaches 1. For mating type (1), the proportion of females approaches 0.5 with increasing age. As a comparison with the observed data, if $f = 0.35$ for mating type 2, the expected values for the 3 age classes are 0.35, 0.578, and 0.725, consistent with the average values of 0.371, 0.549, and 0.750 given in Table 1 of Guo et al. (1998).

Three-Genotype Model

Single Age-Class Model

The 3-genotype model with a single age class also results in a balanced polymorphism. This can be intuitively understood when it is realized that for a normal autosomal locus with 3 genotypes, there would be 9 different mating types as shown in Table 5. Notice that 2 of the mating types that are missing in this model are the ones that produce only homozygotes, that is, mating types *FF* × *FF* and *MM* × *MM*, giving a basis for the balanced polymorphism.

Furthermore, this model results in an excess of heterozygotes at equilibrium. For example, if we assume that $f = 1 - f = 0.5$, that is, half of the *FM* progeny are females and half are males, and if we iterate the Equations 4 above, genotype proportions go quickly to a stable equilibrium, at which the frequencies of *FF*, *FM*, and *MM* are 0.207, 0.586, and 0.207, respectively.

A way to understand the basis for the excess in heterozygotes and this polymorphism is to realize that there are large differences in allele frequencies between the 2 sexes. If we again assume that $f = 0.5$, the equilibrium frequencies of

Table 5 The 9 possible mating types for an autosomal locus with the 4 permissible mating types in the 3-genotype model indicated as Yes in boldface

		Females		
		FF	*FM*	*MM*
Males	*FF*	No	No	No
	FM	**Yes**	**Yes**	No
	MM	**Yes**	**Yes**	No

FF and *FM* females are $P = 0.414$ and $H_f = 0.586$ and the equilibrium frequencies of *FM* and *MM* males are $H_m = 0.586$ and $Q = 0.414$. As a result, the frequencies of *F* in females and males are 0.707 and 0.293, respectively. The frequency of *FF* genotypes in the progeny is then the product of the frequency of *F* in females and *F* in males, or $p_f p_m = (0.707)(0.293) = 0.207$. Likewise, the frequency of *MM* progeny genotypes is $q_f q_m = (0.293)(0.707) = 0.207$, and the frequency of *FM* progeny genotypes is $p_f q_m + p_m q_f = 0.586$.

This model results in balanced polymorphism for all levels of *f* between 0 and 1. Figure 3 gives the equilibrium frequencies of the 2 types of females, *FF* and *FM* (broken lines), and the 2 types of males, *FM* and *MM* (solid lines), for different levels of *f*. When *f* is low, most females are *FF* because not many *FM* female progeny are produced, and most males are *FM* because mostly *FM* male progeny are produced.

Figure 4 shows the impact of different levels of *f* on the frequency of the 4 possible mating types for this model. When *f* is low, most *FM* individuals are males and nearly all the matings are between *FM* males and *FF* females, mating type 2 in Table 3. For example, when $f = 0.2$, 68.5% of the matings are of this type. On the other hand, if *f* is high, most *FM* individuals are female, and most of the matings are between *MM* males and *FM* females, mating type (3).

The overall strength of selection in this model is very high and as a result the equilibrium proportions are reached very quickly. As an example, Figure 5 gives the change in the frequency of the 4 genotypes over time from the equilibrium for $f = 0.01$ to that for $f = 0.5$. Even though the equilibrium genotypic frequencies for these 2 levels of *f* are quite different, within 10 generations the frequencies have changed from the $f = 0.01$ equilibrium so that they are all less than 1% different from those for the $f = 0.5$ equilibrium.

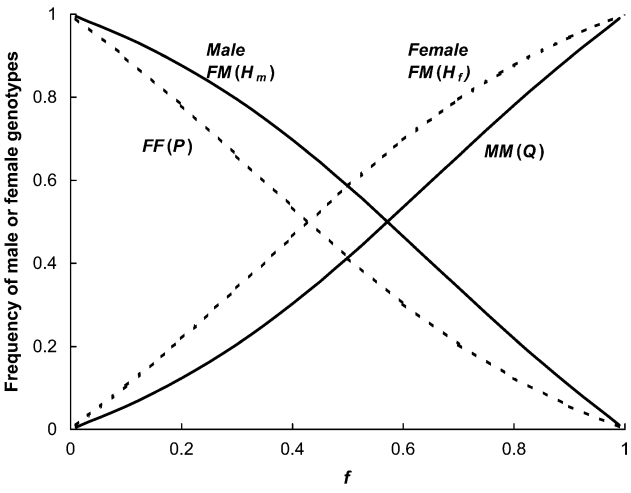

Figure 3. For the 3-genotype model and a single age class, the effect of different levels of *f* on the equilibrium frequencies of the 2 types of females, *FF* and *FM* (broken lines) and the 2 types of males, *FM* and *MM* (solid lines).

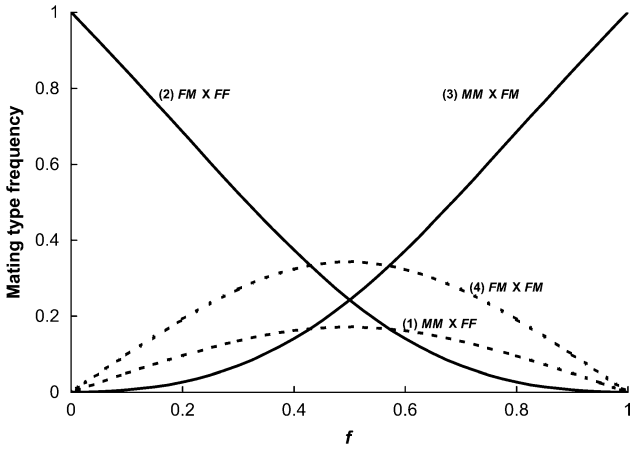

Figure 4. For the 3-genotype model and a single age class, the effect of different levels of *f* on the frequency of the 4 possible mating types.

Multiple Age-Class Model

Let us now examine how additional *FM* females from multiple age classes influence the equilibrium frequencies of the 2 types of males and the 2 types of females. Figure 6 gives these frequencies for different levels of *f*, as did Figure 3 for only one age class, and again there is a balanced polymorphism for all levels of *f*. If we compare these 2 figures, it can be seen that the effect for a given *f* level is, as expected, to increase the frequency of *FM* females (reduce the frequency of *FF* females) and to decrease the frequency of *FM* males (increase the frequency of *MM* males). For example, if $f = 0.4$ and there are 3 age classes, then the frequency of *FM* females is $H_f = 0.588$, compared with $H_f = 0.465$ when there is only one age class.

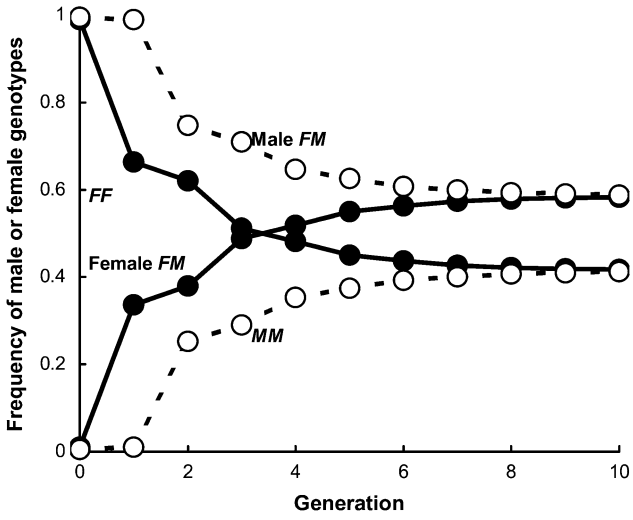

Figure 5. For the 3-genotype model and a single age class, the change in the frequency of the 2 types of females, *FF* and *FM* (solid lines) and the 2 types of males, *FM* and *MM* (broken lines) over time from the equilibrium for $f = 0.01$ to that for $f = 0.5$.

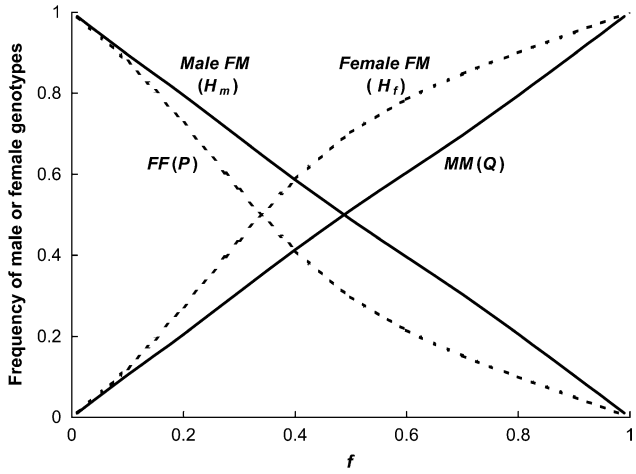

Figure 6. For the 3-genotype model and multiple age classes, the equilibrium frequencies of the 2 types of females, *FF* and *FM* (broken lines) and the 2 types of males, *FM* and *MM* (solid lines) for different levels of *f*.

Figure 7 shows the impact of different levels of *f* on the frequency of the 4 mating types, given 3 age classes. Again, the effect of a given increase in *f* is stronger than when there is only one age class. For example, the frequency of mating type (3), *MM* × *FM*, is higher than that for mating type (2), *FM* × *FF*, for all values of *f* greater than 0.4, rather than the 0.5, when there is only one age class (Figure 4).

Now let us look at the expected proportion of females in the progeny at age *t* for each of the 4 mating types for different values of *f*. As examples, Table 6 gives the expected proportion of females in age classes 1, 2, and 3 from the 4 different mating types for 3 different values of *f*. For all the mating types, the proportion of females

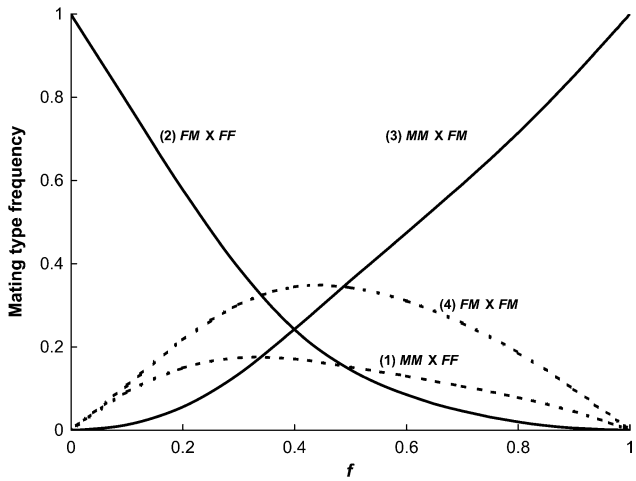

Figure 7. For the 3-genotype model and multiple age classes, the effect of different levels of *f* on the frequency of the 4 possible mating types.

Table 6 For the 3-genotype model, the expected proportion of females at different progeny ages for the 4 different mating or family types

				Progeny age			
f	Mating	Male	Female	1	2	3	∞
0.3	1	*MM*	*FF*	0.3	0.51	0.657	1.0
	2	*FM*	*FF*	0.65	0.755	0.828	1.0
	3	*MM*	*FM*	0.15	0.255	0.328	0.5
	4	*FM*	*FM*	0.4	0.505	0.578	0.75
0.4	1	*MM*	*FF*	0.4	0.64	0.784	1.0
	2	*FM*	*FF*	0.7	0.82	0.892	1.0
	3	*MM*	*FM*	0.2	0.32	0.392	0.5
	4	*FM*	*FM*	0.45	0.57	0.642	0.75
0.5	1	*MM*	*FF*	0.5	0.75	0.875	1.0
	2	*FM*	*FF*	0.75	0.875	0.938	1.0
	3	*MM*	*FM*	0.25	0.375	0.438	0.5
	4	*FM*	*FM*	0.5	0.625	0.688	0.75

increases with age, with the biggest change occurring for mating type (1), *MM* × *FF*. For mating types (1) and (2), as age increases, the proportion of females approaches 1. For mating types (3) and (4), the proportion of females approaches 0.5 and 0.75, respectively, with increasing age.

How close do these predicted changes of sex proportions fit the proportions of females in different age classes observed by Guo et al. (1998) (averages of 0.371, 0.549, and 0.75 for age classes 1, 2, and 3, respectively)? If we look at each mating type individually, then mating type (1), *MM* × *FF*, most closely fits the proportions observed. Examining the range of *f* values for this mating type, when $f = 0.35$, then the expected proportions of females, 0.35, 0.578, and 0.725, in age classes 1, 2, and 3, respectively, most closely fit the observed proportions (note that these are the same expectations given above for mating type 2 for the model of Guo et al. (1998) when $f = 0.35$). This mating type constitutes 17.5% of the mating types at equilibrium for $f = 0.35$. It is possible that there are by chance different mating types represented in the parents of the different age classes, particularly for the oldest age class, which consisted of only 88 individuals total from only 10 families.

Female Progeny Numbers and the 2-Genotype and 3-Genotype Models

The proportion of female progeny observed in families is more consistent with the 3-genotype than the 2-genotype model in 2 ways. First, the data in Table 1 show extensive variation over groups of families sired by different males, ranging nearly 4-fold from 0.163 to 0.604 for W2E. The 2-genotype model predicts only a 2-fold difference at age 1 in female progeny proportions between the 2 mating types, that is, between $f/2$ and f. On the other hand, the proportion of females for 3-genotype model can vary from $f/2$ to $(1 + f)/2$ for progeny from mating types (2) and (3), over a 4-fold difference when $f = 0.3$. Therefore, assuming that *f* is a constant value, the high observed variation over families of different males is more consistent with the 3-genotype model.

Perhaps more importantly, Guo et al. (1998) also found some heterogeneity for families of different females sired by the same male. Because all females in the 2-genotype model are *FF*, the 2-genotype model is not consistent with genetic variation determining the proportion of female progeny for different females. On the other hand, given polymorphism for the 3-genotype model, genetic variation can occur among females and result in genetic differences causing variation in the proportion of female progeny.

To examine this more closely, let us begin by looking at paternal half-sib families from cross W2E of Guo et al. (1998), in which there was homogeneity in the proportion of female progeny over female parents. For male 8 (Table 1), there were 3 female half-sib families that had female progeny proportions of 16.7%, 12.5%, and 19.6% with an average of 16.3% overall. All of these families could be from mating type (1) (*FM* × *FF*) of the 2-genotype model when $f = 0.35$ because the model yields a proportion of females of 17.5% (Table 2). (Here, $f = 0.35$ is used because, when $f = 0.3$, there is no stable polymorphism for multiple age classes, see Figure 1.) Similarly, this group of female half-sib families could be from mating type (3) (*MM* × *FF*) of the 3-genotype model because when $f = 0.35$ this model also yields a proportion of females of 17.5% (Table 6).

There was significant heterogeneity over half-sib families for sires 2 and 6 for the family set W2E. First, for male 2, the 3 female half-sib families had female progeny percentages of 71.0%, 44.2%, and 65.9% (Table 7). For the 2-genotype model, we can assume that all the matings are *FF* × *FF* and that $f = 0.591$ (the overall proportion of female progeny from male 2). However, this results in a significant χ^2 value of 6.56. On the other hand, using the 3-genotype model, the first and last families could be mating type *FM* × *FF*, which gives a proportion of females of 65%, when $f = 0.3$. The second family could be from mating type *FM* × *FM* which gives a proportion of females of 40% when $f = 0.3$. In this case, the χ^2 value is not significant. Note that both of these matings for the 3-genotype model have the same type *FM* male, as expected, but different females, *FF* and *FM*, to account for genetic variation among females.

Second, for the 3 female families for male 6 in family set W2E, the percentages of female progeny were 41.9%, 28.0%, and 10.0% (Table 7). For the 2-genotype model, we can assume that all the matings are *FM* × *FF* and that $f = 0.578$ (because the overall proportion of female progeny from male 6 is 0.289 and the proportion of female progeny at age 1 from mating *FM* × *FF* is $f/2$). Again this results in a significant χ^2 value of 6.04. On the other hand, using the 3-genotype model, the first 2 families could be mating type *MM* × *FF*, which gives a proportion of females of 30%, when $f = 0.3$. The third family could be from mating type *MM* × *FM* which gives a proportion of females of 15% when $f = 0.3$. Again, the χ^2 value is not significant and both matings for the 3-genotype model have the same genotype *MM* male, as expected, but different females, *FF* and *FM*, to account for genetic variation among females.

In both these examples for the 3-genotype model, the sex ratio in the progeny is explained by an f value of 0.3, that is, 30% of *FM* individuals are initially females and 70% are males and that each succeeding year 30% of the remaining *FM* males become females. Although this may be coincidental because of the families examined in detail here, it suggests that $f = 0.3$ is generally consistent with the observed data and a good working value for this parameter. On the other hand, the f value used to explain the progeny arrays as best possible for the 2-genotype model varies from 0.35 for the families of male 8 to 0.591 for the families of male 2.

Mating Type Frequencies and the 2-Genotype and 3-Genotype Models

From the theoretical results of the 2-genotype model above, an *FF-FM* polymorphism is only expected when f is between 0.5 and 1 for the single age-class model and between 0.3 and 1 for the multiple age-class model. The expected proportions of the 2 mating types, *FM* × *FF* (1)

Table 7 The observed and expected numbers of female progeny at 1 year of age from 2 paternal half-sib families in the W2E experiment (Guo et al. 1998)

			Expected	
Male × Female	*N*	Observed (%)	Two genotype (mating)	Three genotype (mating)
			$f = 0.591$	$f = 0.3$
2 × 4	31	22 (71.0)	18.3 (*FF* × *FF*)	20.2 (*FM* × *FF*)
2 × 5	43	19 (44.2)	25.4 (*FF* × *FF*)	17.2 (*FM* × *FM*)
2 × 6	41	27 (65.0)	24.2 (*FF* × *FF*)	26.6 (*FM* × *FF*)
			$\chi^2 = 6.56$ ($P < 0.05$)	$\chi^2 = 0.82$ (ns)
			$f = 0.578$	$f = 0.3$
6 × 16	31	13 (41.9)	9.0 (*FM* × *FF*)	9.3 (*MM* × *FF*)
6 × 17	25	7 (28.0)	7.2 (*FM* × *FF*)	7.5 (*MM* × *FF*)
6 × 18	20	2 (10.0)	5.8 (*FM* × *FF*)	3.0 (*MM* × *FM*)
			$\chi^2 = 6.04$ ($P < 0.05$)	$\chi^2 = 2.53$ (ns)

The expected female progeny numbers for the 2-genotype model of Guo et al. (1998) are for $f = 0.591$ (male 2) and $f = 0.578$ (male 6) and the given mating types. The expected female progeny numbers for the 3-genotype model assume that $f = 0.3$ and the given mating types (notice that the male genotype is the same for all 3 half-sib families within each male parent). The results of chi square goodness-of-fit tests with 2 degrees of freedom are shown for each case.

and *FF* × *FF* (2), are assumed to be equal to the expected proportions of the 2 types of males (Figure 1). Let us now compare these expectations to the observed proportions of different mating types for W2E, the family group examined at age 1 by Guo et al. (1998).

We can obtain the expected frequency of the 2 mating types for the 2-genotype model with multiple age classes by iterating Equation 2. If we assume that $f = 0.35$, as suggested above to best explain the proportion of female progeny from male 8 in W2E, then the frequency of mating type 1 (*FM* males) is only 12%. Of the 8 half-sib families in W2E, only one more, that from male 1, appears to be consistent with mating type 1. Given $f = 0.35$, the 3 families from males 5 (31.3% female progeny), 6 (26.6%), and 7 (34.2%) (Table 1) appear consistent with the more common mating type 2 (*FF* males), which predicts 35% female progeny. However, the other 3 half-sib families with male parents 2, 3, and 4 have 60.4%, 56.4%, and 50.8% female progeny, much too high to be explained by chance if $f = 0.35$.

On the other hand, from the theoretical results given above for the 3-genotype model, a *FF-FM-MM* polymorphism is expected for the complete range of f between 0 and 1. The expected genotype frequencies for the 3-genotype model with multiple age classes can be obtained by iterating Equation 5, and the expected frequencies of the 4 mating types can be calculated from these values. Let us assume that $f = 0.3$, as suggested above to best explain the proportion of female progeny from male 8 in W2E. We can then go through each of the 24 families in W2E and assign the most appropriate mating type. Table 8 gives both these observed proportions of the 4 mating types and the expected proportions generated from Equation 5. In general with $f = 0.3$, the observed proportions of mating types are generally consistent with but somewhat different than the theoretically expected mating types. When $f = 0.4$, there are somewhat closer predictions but still the observed proportions are also somewhat different from those expected.

Discussion

Genetic mechanisms of sex determination have long been of interest to evolutionary biologists, yet the exact mechanism for sex determination has not been determined in many animals. Sex is determined partially environmentally in cupped oysters, but controlled crosses suggest that genetic factors are important in sex determination. Guo et al. (1998) examined sex ratios in both full- and half-sib families of the Pacific oyster and found paternal control of sex ratio variation in families suggestive of 2 types of males. They proposed that a single major gene with 2 genotypes controls sex in the Pacific oyster with *FM* oysters being males and *FF* oysters maturing as males or females. Here, we show that such a model can, indeed, produce a stable polymorphism, for either single or multiple age-class populations, although under limited ranges of f, the probability that an *FF* individual matures as a female. However, the 2-genotype model of Guo et al. (1998) cannot explain heterogeneity of sex ratios observed in half-sib families with a single male parent and different female parents.

Thus, we propose here an alternative 3-genotype model that also produces a stable polymorphism, for either single or multiple age-class populations, but over all values of f between zero and one (here, f is the probability that an *FM* individual matures as a female). This model can account for sex ratio heterogeneity among male half-sib families because it features 2 types of females, *FM* and *FF*. Furthermore, the 2-genotype model cannot account for the proportion of females from the 24 families and the expected mating types in W2E. On the other hand, the 3-genotype model accounts for the frequencies of mating types inferred from the observed sex ratios of families although the expected proportions of mating types differ somewhat from those observed. Nevertheless, we have shown that simple genetic mechanisms may account for the broad features of sexual maturation and sex ratios in oyster families and populations.

Because Guo et al. (1998) found significant paternal effects on progeny sex ratios but less strong significant maternal effects, they inferred 2 kinds of males, *FM* and *FF*. The 3-genotype model proposed here has 2 kinds of females, explaining heterogeneity in female sex ratios. Guo et al. (1998) acknowledged that the design of the experimental crosses (i.e., females nested within males and factorial crosses with more males than females) diminished the power to detect maternal compared with paternal effects. In addition, the rate of maturation or growth affects the proportion of females in families (Coe 1932), so the time of sampling could have biased against observation of maternal effects. The 3-genotype model accounts for the greater bimodality of high female progeny in 2-year-old compared with one-year-old oysters (see Figure 1A,B in Guo et al. (1998) because proportions of females in progeny from *FM* versus *FF* females become more disparate with age.

The relationship between sex and growth is intriguing. Females are heavier than males, 10–12% in the same random bred crosses analyzed here (Hedgecock et al. 1993) and 41% in an F_2 family (Hedgecock D, Perry GML, Voigt M-L, in preparation). Thus, size or factors affecting growth (e.g., day degrees) may be a more important covariate of sex ratio than age itself. The observations of Coe (1936) that the proportion of females increased with environmental factors increasing growth (latitude, local temperature, and time in season) raises the possibility, for example, that f might vary with genetically determined family growth or

Table 8 The proportions of the 4 mating types observed in the 24 families of W2E for both $f = 0.3$ and 0.4 and that expected for the 3-genotype, multiple age-class model for both $f = 0.3$ or 0.4

		Mating type			
		1	2	3	4
$f = 0.3$	Observed	0.375	0.208	0.250	0.167
	Expected	0.174	0.389	0.135	0.302
$f = 0.4$	Observed	0.208	0.167	0.417	0.208
	Expected	0.171	0.242	0.243	0.344

with environment. This suggests experimental approaches to manipulating sex ratio, which might discriminate among the mating types.

There is a possibility that neither of these genetic models is completely correct, that is, sex is determined by more than a single major gene. A QTL mapping study shows one linkage group acting like a sex chromosome, with QTL at 6 other positions on 5 other linkage groups (Hedgecock D, in preparation). Detailed examination of experimental crosses using a large number of marker loci should help resolve the genetic basis of sex determination in oysters.

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