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Individual males in many animal species exhibit discrete modes of behaviour[1–3], but the genetic mechanisms underlying these differences are poorly understood. Here we investigate the genetics of the isopod crustacean *Paracerceis sculpta*, in which three different types of males coexist, each distinguishable from the others by their behavioural and morphological phenotypes[4,5]. Within families, alleles of the gene encoding the enzyme phosphoglucomutase (*Pgm* gene) are associated with particular male phenotypes, although no significant association between these characters exists population-wide. This suggests that *Pgm* is closely linked to a single genetic locus which controls male phenotype. We call this the *alternative mating strategy* (*Ams*) locus. We present evidence that two other factors—an autosomal gene, *transformer* (*Tfr*), and an extrachromosomal factor—interact with primary sex determination loci and with alleles at *Ams*, causing certain individuals to change sex, thereby biasing family sex ratios. A model based on our genetic analysis suggests that: first, polymorphism in male behaviour is controlled by the mendelian segregation of three alleles at the *Ams* locus; second, that family sex ratio is influenced by alternative alleles at the *Tfr* locus whose expression is influenced by the extrachromosomal factor; and third, that *Tfr* and *Ams* interact epistatically to determine the sex of the individual and, if male, its behaviour and external morphology.

Females are monomorphic in *P. sculpta*. Males, however, exhibit three distinct morphs that differ in reproductive behaviour: α-males are largest and defend harems within sponges using elongated posterior appendages; β-males invade harems by mimicking female behaviour and morphology; and γ-males invade harems by being small and secretive[5,6]. A genetic model has been proposed[7] to explain the persistence of the three male morphs at stable frequencies, in which three alleles at a single autosomal locus (*Ams*) show directional dominance and mendelian inheritance.

We tested this model[7] for male morphology using controlled laboratory crosses. We first examined mendelian inheritance at the *Pgm* locus[8] in 25/31 F_1 families in which one heterozygous and one homozygous parent were crossed. As three alleles were detectable at *Pgm*, we summarized alleles possessed by heterozygous parents as allele 1 or allele 2. The total numbers of progeny possessing these allele classes were 405 and 424, respectively, and individual crosses were homogeneous (*G*-test (ref. 9), $G_H = 31.01$) (d.f. = 24, $P > 0.10$). We considered *Pgm* inheritance to be mendelian.

The genetic model[7] suggested that field-collected β- and γ-males are heterozygous at the *Ams* locus, and thus should produce 50 : 50 ratios of α- and β-, or α- and γ-male sons, respectively, when crossed with field-collected females (see Methods). As most β- and γ-males were also heterozygous at the *Pgm* locus (11/13 and 7/10, respectively), we examined the association between *Pgm* genotype and male phenotype among F_1 progeny. If these loci were unlinked, we expected the progeny of males heterozygous at both loci to segregate four combinations of the two male morphs and two *Pgm* genotypes in equal frequency. The more severe the deviation from this expectation, the closer the linkage, thus, parental and recombinant classes were each pooled, then compared using a *G*-test (d.f. = 1)[9].

Of the 351 male progeny reared from 18 double-heterozygote *Pgm–Ams* crosses, 96.3% appeared in the parental class ($G = 368.9$, $P < 0.001$), a result indicating that *Ams* is closely linked to *Pgm* (separated by four map units), and one consistent with the hypothesis that a major gene causes phenotypic differences among males[7]. We confirmed, moreover, that male differences are not simply due to allelic differences at *Pgm*. Electrophoretic analysis over a two-year period (1987–88) showed no evidence of linkage disequilibrium[9] between *Pgm* allelomorphs and the three male morphs in nature ($D^2 = 0.004$, $P > 0.10$, $N = 292$). Thus *Pgm* and *Ams*, although linked, represent distinct genetic loci.

To examine mendelian inheritance at *Ams*, we unambiguously

Table 1 *Paracerceis sculpta* F_1 crosses

Cross-class	No. of families	No. of progeny	Weighted survivorship	Progeny phenotypes α	β	γ	F	N	Expected male frequency α : β : γ	G_{Ams}	Proportion of males†	$G_{sex\ ratio}$
$Ams^{\alpha}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	8	247	0.43	53	0	0	55	108	1.0 : 0.00 : 0.00	0.00	0.49	0.37
$Ams^{\beta}ms^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	12	1,308	0.49	59	267	0	317	643	0.50 : 0.50 : 0.00	143.60**	0.51	0.13
$Ams^{\beta}Ams^{\alpha} \times Ams^{\beta}Ams^{\alpha}$	1	107	0.39	0	28	0	14	42	0.25 : 0.75 : 0.00	<0.001‡	0.67	4.75*
$Ams^{\gamma}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	10	921	0.38	75	0	105	167	347	0.50 : 0.00 : 0.50	5.02*	0.52	0.49
	31	2,583	0.44	187	295	105	553	1,140				

F, number of females; *N*, total progeny; $G_{Ams} = G_{adj}$ (d.f. = 1) comparison of observed and expected male frequencies; $G_{sex\ ratio} = G_{adj}$ (d.f. = 1) comparison of observed and 1 : 1 sex ratio.
*$P < 0.05$; **$P < 0.001$.
† Proportion of males weighted by family size.
‡ Exact χ^2 test.

Table 2 Distribution of *Pgm* alleles among F_1 males and females in cross-classes 2 and 4

Cross-class	P_1Q_1	P_1Q_2	P_2Q_1	P_2Q_2	N	G_P	G_Q	G_{PQ}	Inference
$Ams^{\alpha}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	27	4	0	12	43	8.69*	2.85	33.00*	Sex change (1×)
	33	12	0	29	74	3.49	0.87	36.99*	Sex change (2×)
	10	1	2	13	26	0.62	0.15	17.45*	Sex change (2×)
	18	7	0	17	42	1.53	0.86	20.38*	Sex change (2×)
	19	11	0	3	33	25.64*	0.76	3.18	Sex change (1×)†
	14	0	10	11	35	1.41	4.95*	6.44*	Sex change (1×)†
	10	1	9	18	38	6.95*	0.00	8.88*	Sex change (1×)
	16	0	14	26	56	10.63*	0.29	14.65*	Sex change (1×)
	27	1	0	19	47	1.73	1.05	55.48*	Sex change (2×)
	17	1	0	10	28	2.32	1.30	30.19*	Sex change (2×)
$Ams^{\gamma}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	4	7	0	0	11	**	0.83	0.83	Sex change (1×)†
	5	5	6	9	25	1.01	0.36	0.36	Independent assort
	4	6	13	15	38	8.88*	0.42	0.00	Sex change (1×)†
	1	5	7	6	19	2.64	0.48	1.33	Independent assort
	10	10	8	9	37	0.24	0.03	0.03	Independent assort
	14	9	1	5	29	4.33*	0.47	5.94*	Sex change (1×)
	3	7	12	7	29	2.84	0.03	2.84	Independent assort

P_i, sex; Q_i, *Pgm* genotype: thus, P_1Q_1 represents β-males with *Pgm* allele class 1; P_1Q_2 are α-males with *Pgm* allele class 2; P_2Q_1 are females bearing a β-allele as indicated by *Pgm* allele class 1; P_2Q_2 are females bearing an α-allele as indicated by *Pgm* allele class 2. Inferences are explained in Methods. *$P < 0.05$; **$P < 0.001$.
† Crosses that appear to have undergone 1× or 2× sex change, but whose pattern of G_P G_Q and G_{PQ} tests were not consistent with confirmed sex-changed crosses because *Pgm* data were unavailable for all progeny; however, the identity of these crosses were confirmed with exact χ^2 tests in Table 3a.

Table 3 Inheritance of *Ams*, *Tfr* and ECF in progeny of *P. sculpta*

Ams cross-type	*Tfr* cross-type	ECF state	No. of families	No. of progeny	Weighted survivorship	Observed α-	β-	γ-	F	Expected α-	β-	γ-	F	N	Exact χ^2 probability
(a) Among F_1 progeny															
$Ams^{\alpha}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^1 \times Tfr^1Tfr^1$	M(–); F(–)	1	85	0.34	15	0	0	14	14.5	0	0	14.5	29	1.00
$Ams^{\beta}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(+); F(–)	2	194	0.72	0	37	0	87	0	45.88	0	78.12	124	0.11
	$Tfr^1Tfr^2 \times Tfr^1Tfr^2$	M(+); F(–)	1	95	0.49	5	13	0	29	2.82	20.68	0	23.5	47	0.06
			1	115	0.41	3	9	0	35	2.82	20.68	0	23.5	47	0.00*
	$Tfr^1Tfr^1 \times Tfr^2Tfr^2$	M(+); F(–)	2	227	0.40	0	51	0	41	0	46	0	46	92	0.35
	$Tfr^1Tfr^1 \times Tfr^1Tfr^2$	M(+); F(–)	5	589	0.49	41	134	0	118	38.09	146.5	0	108.41	293	0.34
	$Tfr^1Tfr^1 \times Tfr^1Tfr^1$	M(+); F(–)	1	85	0.47	10	23	0	7	10	20	0	10	40	0.55
$Ams^{\beta}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^1 \times Tfr^2Tfr^2$	M(+); F(–)	1	107	0.39	0	28	0	14	0	31.5	0	10.5	42	0.28
$Ams^{\gamma}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(–)	1	93	0.16	1	0	4	10	1.8	0	3.75	9.45	15	0.87
	$Tfr^1Tfr^1 \times Tfr^2Tfr^2$	M(–); F(–)	6	579	0.39	47	0	47	132	56.5	0	56.5	113	226	0.05
			1	94	0.36	5	0	11	18	8.5	0	8.5	17	34	0.02*
	$Tfr^1Tfr^1 \times Tfr^1Tfr^1$	M(–); F(–)	2	155	0.46	22	0	43	7	18	0	36	18	72	0.55
Totals			24	2,418	0.43	149	295	105	512	153.03	331.24	104.75	471.98	1,061	
(b) Among F_2 progeny															
$Ams^{\alpha}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(+)	2	52	0.58	0	0	0	30	0	0	0	30	30	1.00
	$Tfr^1Tfr^2 \times Tfr^1Tfr^2$	M(–); F(+)	1	29	0.31	3	0	0	6	1.17	0	0	7.83	20	0.10
$Ams^{\alpha}Ams^{\alpha} \times Ams^{\beta}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(+)	1	60	0.45	0	14	0	13	0	9.99	0	17.01	27	0.17
	$Tfr^1Tfr^1 \times Tfr^2Tfr^2$	M(–); F(+)	2	78	0.26	0	11	0	9	0	10	0	10	20	0.82
$Ams^{\beta}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(+); F(+)	4	148	0.28	0	16	0	25	0	15.17	0	25.83	41	0.87
	$Tfr^1Tfr^2 \times Tfr^1Tfr^2$	M(+); F(+)	3	109	0.40	4	16	0	24	2.64	19.36	0	22	44	0.46
	$Tfr^1Tfr^1 \times Tfr^2Tfr^2$	M(+); F(+)	1	37	0.62	0	12	0	11	0	11.5	0	11.5	23	1.00
	$Tfr^1Tfr^1 \times Tfr^1Tfr^2$	M(+); F(+)	4	158	0.34	8	23	0	23	7.02	27	0	19.98	54	0.57
$Ams^{\beta}Ams^{\alpha} \times Ams^{\beta}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(+); F(+)	1	46	0.57	0	15	0	11	0	14.62	0	11.38	26	1.00
$Ams^{\beta}Ams^{\alpha} \times Ams^{\gamma}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(+); F(–)	2	63	0.35	0	8	8	6	0	8.14	4.18	9.68	22	0.08
$Ams^{\gamma}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(+)	1	36	0.39	0	0	6	8	1.82	0	3.5	8.68	14	0.15
	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(+)	1	44	0.43	1	0	7	11	4.75	0	4.75	9.5	19	0.13
$Ams^{\gamma}Ams^{\alpha} \times Ams^{\beta}Ams^{\alpha}$	$Tfr^1Tfr^2 \times Tfr^2Tfr^2$	M(–); F(+)	1	40	0.65	0	15	0	11	0	9.62	3.38	13	26	0.04*
	$Tfr^1Tfr^1 \times Tfr^1Tfr^2$	M(–); F(+)	1	35	0.40	0	6	0	8	0	7	1.82	5.18	14	0.23
Totals			25	935	0.40	16	136	21	196	25.98	132.4	17.63	192.99	369	

Ams, alternative mating strategy locus; *Tfr*, transformer locus; ECF, extrachromosomal factor; M, male; F, female; *F*, number of females; *N*, total number of progeny; +, positive for ECF; –, negative for ECF. *$P < 0.05$.

assigned parental males and females to four cross-classes using *Pgm* genotypes in phase with α-, β- and γ-male phenotypes (Table 1). We pooled the total progeny within cross-classes to obtain the observed frequencies of females and males of different phenotypes. We then compared these frequencies with those predicted by the genetic model[7]. The predicted phenotypes appeared in the four cross-classes (Table 1), a result consistent with the presumed dominance relationships among the *Ams* alleles ($Ams^{\beta} > Ams^{\gamma} > Ams^{\alpha}$)[7]. However, according to strict mendelian expectations, crosses involving β- and γ-sires showed an excess of β- and γ-sons, respectively (Table 1). Moreover, pooled sex ratios deviated from unity only in cross-class 3 (Table 1), whereas family sex ratios within cross-classes 2 and 4 were heterogeneous ($G_H = 62.88$ (d.f. = 9, $P < 0.001$) and 19.45 (d.f. = 5, $P < 0.005$), respectively). Given evidence that *Pgm* and *Ams* are closely linked, apparent mendelian inheritance at *Pgm*, but not at *Ams*, as well as heterogeneous sex ratios within cross-classes, indicated that additional factors must influence male phenotype.

Both genetic and extrachromosomal factors affect family sex ratios in peracarid crustaceans[10–17]. Certain autosomal genes cause genetic females to mature as males, whereas extrachromosomal factors (from either bacteria or virions) appear to produce the opposite effect[10–17]. As the *Pgm/Ams* complex in *P. sculpta* is autosomal[7], we reasoned that if sex-ratio biasing factors exist, they should cause deviations in the observed frequencies of *Pgm* alleles between males and females, in the same families that showed sex-ratio biases and excesses of β- and γ-males. Differently put, if sex-ratio biasing factors caused males to mature as females and females to mature as males, we predicted an apparent interaction between *Pgm* and sex, even though these traits were unlinked (Fig. 1).

To test this hypothesis, we examined the distribution of *Pgm* alleles among male and female progeny in F_1 cross-classes 2 ($Ams^{\beta}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$) and 4 ($Ams^{\gamma}Ams^{\alpha} \times Ams^{\alpha}Ams^{\alpha}$) (Tables 1, 2). Independent assortment occurred in 4/17 F_1 crosses, all involving γ-sires. The remaining 13/17 F_1 crosses showed evidence of one-way or two-way sex change (Table 2), indicating that sex-ratio biasing factors exist in *P. sculpta*. Lethal sex-limited or autosomal alleles are unlikely to have produced these deviations because sex-ratio biases occurred in both directions (G_P in Table 2), and because *Pgm* showed mendelian inheritance in 16/17 crosses (G_Q in Table 2). Variation in male-morph and sex-ratio frequencies, within cross-classes presumed to be homogeneous with respect to their *Ams*- and sex-determination genotypes (for example, cross-classes 2 and 4, in Table 2), indicated that expression of sex-ratio biasing factors is contingent on individuals' allelic states at *Pgm/Ams* and at primary sex-determination loci. Moreover, evidence of two-way sex change suggested that sex-ratio biasing factors in *P. sculpta* were both genetic and extrachromosomal[10,12,13,15–17].

We devised a model to explain the observed variation in F_1 phenotype frequencies in which one autosomal (*Tfr*, for *transformer*) and one extrachromosomal factor (ECF) exist (factor effects are explained in Methods; Fig. 2). Like previous hypotheses regarding sex-ratio biasing factors[10,12,13,15–17], we assumed that primary sex determination involved female heterogamety[7]. Unlike previous models, however, we assumed that two-way sex change is caused by alternative alleles at a single autosomal locus (*Tfr*), and that the effects of ECF are contingent on allelic states at *Ams*, *Tfr* and primary sex-determination loci (Fig. 2). Our model thus provided a testable explanation for male- and female-biased sex ratios, for one- and two-way sex change, for the inheritance of the *Pgm/Ams*

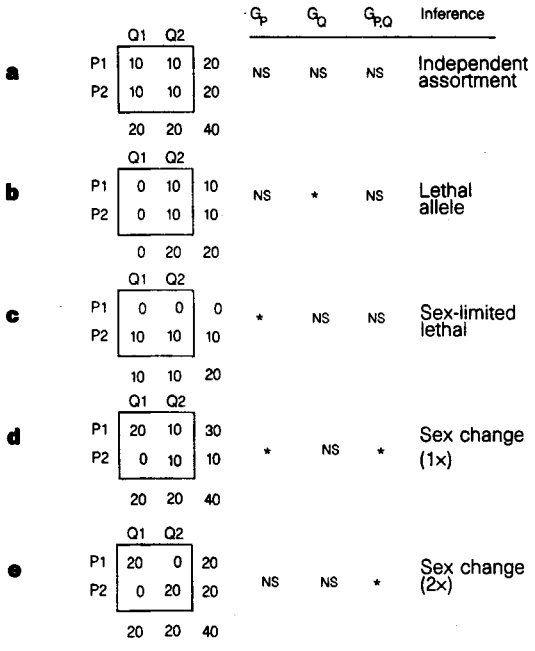

Figure 1 Detection of sex-ratio biasing factors in *P. sculpta*. 2 × 2 tables show hypothetical allelic combinations among 40 progeny produced by a P_1P_1, Q_1Q_2 male crossed with a P_1P_2, Q_1Q_1 female; P represents sex (P_1, males; P_2, females); Q = *Pgm* (Q_1, allele class 1; Q_2, allele class 2); *G* tests (G_P, G_Q, $G_{P,Q}$) show significant (asterisk) and non-significant (NS) deviations in sex ratio and *Pgm* allele frequency, as well as interactions between sex and *Pgm*; **a**, independent assortment; **b**, lethal allele indicates mortality regardless of sex; **c**, sex-limited lethal indicates mortality regardless of Q_i genotype; **d**, P_2Q_1 interaction causes females to mature as males; **e**, P_2Q_1 and P_1Q_2 interactions cause females to mature as males and males to mature as females.

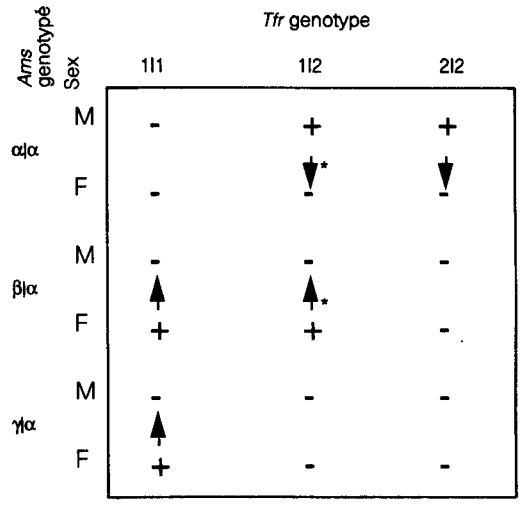

Figure 2 Effects of *Tfr* and ECF on *Ams* and primary sex determination loci: α, β, γ are alleles at *Ams*; M, male; F, female; Tfr^i represent alleles at *Tfr*; minus sign, no effect of *Tfr* on *Ams*-sex combination; plus sign, sex change, with an arrow indicating the direction of change; asterisk, effect produced by interaction of ECF with *Tfr*, *Ams* and primary sex-determination genotypes. The apparent effect of ECF is to enhance or suppress the expression of the Tfr^2 allele depending on allelic states an individual's *Ams* and primary sex-determination loci.

complex, and for the surplus of β- and γ-males in F_1 families (Table 1; Fig. 3).

We tested our model by assigning *Ams* and *Tfr* genotypes, as well as ECF states (Table 3a) to all parental individuals using unambiguous *Pgm* genotypes and apparent *Tfr* genotypes (Table 2). We then compared observed and expected frequencies of male and female F_1 progeny using exact χ^2 tests[18]. We found no significant deviation of observed from expected frequencies in 22/24 F_1 families (for N progeny, $N = 1{,}061$; Table 3a). Moreover, the presumed *Tfr*-genotypes of parental individuals conformed to Hardy–Weinberg expectations (exact $\chi^2 = 0.21$).

We further tested our model by combining (1) unambiguous *Ams* genotypes (determined from the *Pgm* genotypes of F_1 parents and F_2 families; Tables 1, 2), with (2) predicted *Tfr* genotypes for F_2 progeny (determined from the apparent *Tfr* genotypes of their F_1 parents; Table 2), with (3) the predicted ECF state of F_2 progeny (determined from the apparent ECF state of their F_1 parents; Table 3a), to generate expected male-morph frequencies and sex ratios for all F_2 progeny (Table 3b). Using the same methods for comparing observed and expected progeny frequencies as those described for F_1 families, we found no significant deviation in male morph or sex ratios in 24/25 F_2 families (Table 3b). Although ECF was initially detectable only in parental β-males, this factor was evidently transmitted to individuals of both sexes, because this factor's interaction with a range of *Ams* and *Tfr* genotypes predictably biased F_1 and F_2 family sex ratios (Table 3b).

The relative frequencies of the three male morphs, as well as local sex ratios, are known to influence male and female fitness in *P. sculpta*[7,19,20]. Biases in male morph and sex ratios, moreover, arise and vanish without pattern within patchily distributed spongocoels[6,20]. Conditional strategies are unlikely to evolve in such unpredictable environments[21–24]. Thus, our model of interaction between *Ams*, *Tfr*, ECF and primary sex-determination loci is consistent with known aspects of this species' biology and with established theory. Our results demonstrate the mendelian inheritance of male mating behaviour and sex factor loci, whose alleles interact with each other, as well as with an apparent extrachromosomal sex-ratio biasing factor. Interactions among these factors could rapidly shift population sex ratios in response to the dynamics of this species' mating system. Allele frequencies at *Ams* and *Tfr* conform to Hardy–Weinberg expectations[7] (Table 3a), perhaps because fitness interactions between alleles at both loci cycle rapidly[3,25]. Thus our results also demonstrate that genetic polymorphisms and epistasis affecting fitness can arise and persist in nature[26,27]. □

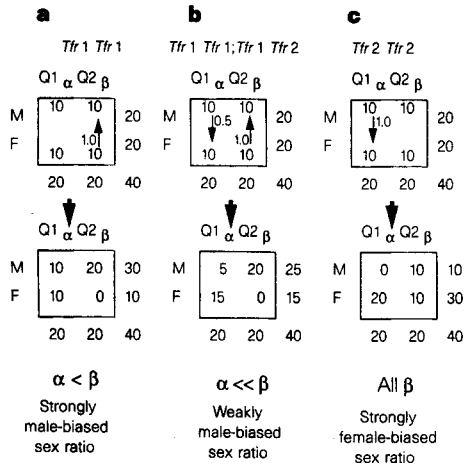

Figure 3 Effects of *Tfr* and ECF on mendelian inheritance at the *Ams* locus: upper 2 × 2 tables show hypothetical allelic combinations among 40 progeny from $Q_{i\alpha}Q_{1\alpha}$ female × $Q_{1\alpha}Q_{2\beta}$ male crosses (Q_{ij}, *Pgm*/*Ams* complex, where *i* is the *Pgm* allele and *j*, the *Ams* allele; M, males; F, females); small arrows indicate sex transformations resulting from epistatic and extrachromosomal interactions; *Tfr* genotypes are shown above each upper table; the numbers near the arrows indicate the proportions of transforming individuals; large arrows point to lower 2 × 2 tables, which show the results of sex transformations on α : β male morph- and sex ratios; **a**, Tfr^1Tfr^1, $Q_{1\alpha}Q_{2\beta}$ females mature as males; **b**, Tfr^1Tfr^2, $Q_{1\alpha}Q_{1\alpha}$ males mature as females owing to ECF; all $Q_{1\alpha}Q_{2\beta}$ females mature as β males owing to *Tfr* and *Ams* interaction; **c**, Tfr^2Tfr^2, $Q_{1\alpha}Q_{1\alpha}$ males mature as females.

Methods

Genetic crosses and electrophoresis. Male isopods and virgin females[28] were collected from sponges[6,29] and maintained as pairs until females became gravid[6]. The F_1 generation included eight α-males, six β-males and five γ-males, each crossed to haphazardly selected females, to yield eight α-families, 13 β-families and 10 γ-families. We reared F_1 animals to maturity[7] and produced an F_2 generation from six F_1 α-males, 13 F_1 β-males and four F_1 γ-males each crossed with F_1 daughters of β-males, as well as two F_1 β-males each crossed to F_1 daughters of γ-males. F_2 animals were reared under the same conditions as F_1 animals. Tissue samples from all adults of each generation were electrophoresed and stained for *Pgm* activity[8].

Estimation of expected *Ams* frequencies for the F_1. Previously described methods[7] estimate that over 99% of field-collected individuals possess $Ams^{\alpha}Ams^{\alpha}$ (0.86), $Ams^{\beta}Ams^{\alpha}$ (0.02) or $Ams^{\gamma}Ams^{\alpha}$ (0.11) genotypes[7], limiting the possible allelic combinations for F_1 progeny. Females are evidently heterogametic in this and in related sphaeromatid species (S.M.S. and C.S., unpublished electrophoretic data)[10,17,30]. Thus, we presumed that *P. sculpta* females carry alleles at the *Ams* locus at the same frequencies as described for males[7], and we expected primary sex ratios to equal unity.

Detection of genetic interactions. The genetic model[7] indicated that the *Pgm*/*Ams* complex and primary sex determination loci in *P. sculpta* are unlinked. Thus, a cross between a homogametic male, heterozygous at *Pgm*/*Ams*, and a heterogametic female, homozygous at *Pgm*/*Ams*, would yield four combinations of two sexes and two *Pgm* genotypes in equal frequency. Using P_1 to represent males and P_2 for females, Q_1 as *Pgm* allele class 1 and Q_2 as *Pgm* allele class 2, we plotted the four progeny genotypes in a 2 × 2 table (Fig. 1a) and examined deviations in the table using *G*-tests. We identified sex-ratio biases by comparing $\Sigma(P_1Q_i)$ with $\Sigma(P_2Q_i) = G_P$, deviations from mendelian expectations at *Pgm* by comparing $\Sigma(P_iQ_1)$ with $\Sigma(P_iQ_2) = G_Q$, and interactions between sex ratio and *Pgm* frequency by comparing $(P_1Q_1 + P_2Q_2)$ with $(P_1Q_2 + P_2Q_1) = G_{P,Q}$. We predicted five possible patterns of G_P, G_Q and $G_{P,Q}$ deviations among the F_1 families (shown in Fig. 1): a, independent assortment between primary sex factors and the *Pgm*/*Ams* complex would yield four combinations of two sexes and two *Pgm* genotypes in equal frequency, no significant G_P, G_Q and $G_{P,Q}$ deviations, and indicate no effect of sex-ratio biasing factors; b, lethal factors causing mortality unrelated to sex would cause deficiencies in the frequencies of *Pgm* alleles, significant G_Q deviations, but no deviations in sex ratio (G_P), and no interaction between *Pgm* and sex ($G_{P,Q}$); c, factors causing sex-limited mortality would cause consistent deficiencies in the frequency of one or the other sex, consistent G_P deviations, but no deviations in *Pgm* frequency (G_Q), and no interaction between *Pgm* and sex ($G_{P,Q}$); d, factors causing one-way sex change (genetic males maturing as females, for example) would cause no deviation in *Pgm* frequencies (G_Q), but would generate consistent sex-ratio (G_P) deviations, as well as significant *Pgm*–sex interactions ($G_{P,Q}$); e, factors causing two-way sex change would only show significant $G_{P,Q}$ interactions.

Effects of *Tfr* and ECF. We let *Tfr* be a diallelic, autosomal locus whose alleles (Tfr^1, Tfr^2) interact with, but assort independently of, alleles at *Ams* and at primary sex-determination loci (Fig. 2). Tfr^1Tfr^1 was assumed to have no effect on males of any *Ams* genotype, and no effect on $Ams^{\alpha}Ams^{\alpha}$ females. However, females bearing Tfr^1Tfr^1, as well as β- or γ-alleles at *Ams*, were assumed to mature as males, with phenotypes determined by their *Ams* allelic state. Tfr^2Tfr^2 was assumed to have no effect on females of any *Ams* genotype, and no effect on β- or γ-males. However, $Ams^{\alpha}Ams^{\alpha}$ males bearing Tfr^2Tfr^2 were assumed to mature as females. Tfr^1Tfr^2 heterozygotes were assumed to affect

only individuals descended from field-collected β-males, and then affect only two *Ams* genotypes: Tfr^1Tfr^2 females bearing β-alleles were assumed to mature as β-males, and Tfr^1Tfr^2, $Ams^{\alpha}Ams^{\alpha}$ males were assumed to mature as females. This latter effect assumed that the Tfr^2 allele interacts with ECF, which initially occurred only in parental β-males, but which was transmitted to $F_{1\text{-}2}$ individuals of both sexes and a range of *Ams* genotypes (Table 3b).

Testing the model. In Tables 3a,b, exact probabilities were Bonferroni-adjusted ($0.05/k$, where k is the number of tests) when multiple crosses with identical *Ams* and *Tfr* genotypes, as well as ECF states were tested; similar crosses with nonsignificant exact probabilities were pooled and the exact χ^2 probability for the pooled frequencies reported; primary sex-determination genotypes were unambiguously determined from *Pgm* genotype frequencies within families; the apparent *Tfr* genotypes among 36 parents (24 crosses) were 15 Tfr^1Tfr^1, 12 Tfr^1Tfr^2 and 9 Tfr^2Tfr^2; expected genotypes calculated from inferred allele frequencies conform to Hardy–Weinberg expectations, exact χ^2 probability 0.21.

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Typographical Errors in Table 2:

The first cross class (#2 from Table 1) should be $Ams^{\beta}Ams^{\alpha}$ x $Ams^{\alpha}Ams^{\alpha}$

Typographical Errors in Table 3:

Using "No. of progeny" as the identifier of crosses, two corrections are necessary: (1) the *Ams* genotype of Cross 107 should be $Ams^{\beta}Ams^{\alpha}$ x $Ams^{\beta}Ams^{\alpha}$; (2) the *Tfr* genotype of Cross 44 should be $Tfr^{1}Tfr^{2}$ x $Tfr^{1}Tfr^{2}$.