Sex-Linked Inheritance of a Cuticular Pigmentation Marker in the Marine Isopod, *Paracerceis sculpta* Holmes (Crustacea: Isopoda: Sphaeromatidae)

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Cuticular pigmentation is highly variable in *Paracerceis sculpta*, a Gulf of California isopod. Individuals bearing the distinctive pattern we call *Str* (*Stripe*) exhibit a longitudinal band of dark pigmentation on the proximal portion of each dorsal body segment and appear "striped" when viewed from above. In field samples collected over a 10 year period, over 90% of all individuals scored as *Str* ($N = 62$) were females ($G = 21.3$, $P < .001$, $N = 9598$). Three generations of laboratory-reared *Str* females, when crossed to unmarked males, yielded 1:1 sex ratios, 98% *Str* daughters (46/47) and no *Str* sons ($N = 56$). Sons from these families never produced *Str* daughters. The sex-limited expression of this cuticular marker in three consecutive generations indicates that sex determination in *P. sculpta* involves female heterogamety (ZW = females, ZZ = males) and that *Str* is W-linked. Our results

are consistent with studies documenting female heterogamety in flabelliferan and oniscoidean isopods, and suggest that chromosomal sex determination may be common within the Isopoda.

Crustacean sex determination mechanisms are varied and diverse (Bull 1983; Hurst 1993). Combinations of allelic and chromosomal sex factors are widespread (review in Ginsburger-Vogel and Charniaux-Cotton 1982), and genetic as well as extrachromosomal factors are known to affect family sex ratios in peracarids (Bull 1983; Heath and Ratford 1990; Hurst 1993; Juchault and Rigaud 1995; Juchault et al. 1992; Legrand et al. 1987; Rigaud and Juchault 1993; Rousset et al. 1992; Shuster and Sassaman 1997). Sex factors appear to be dispersed throughout the genome in certain marine and terrestrial isopods, and may be sensitive to epistatic or environmental variation (Heath and Ratford 1990; Juchault et al. 1992; Sassaman 1978). The sex-limited expression of cuticular pigmentation patterns has been documented in two genera of flabelliferan isopods to date (Legrand et al. 1987).

Cuticular pigmentation markers in isopods appear to be controlled by dominant Mendelian alleles at autosomal loci, which exist at low frequency in natural populations (Heath 1979; Legrand-Hamelin 1976; Shuster 1989). This observation leads to three predictions: most individuals bearing cuticular markers in nature are expected to be heterozygous at the marker locus; both males and females are expected to bear such markers in equal frequency; and marked individuals are expected to produce 1:1 ratios of marked : unmarked progeny when crossed to unmarked individuals.

When cuticular marker loci are located on heterochromosomes influencing sex determination, only members of the heterogametic sex are expected to express the marker. Most studies demonstrating chromosomal sex determination in isopods suggest that females are the heterogametic sex (ZW = females, ZZ = males; Ginsberger-Vogel and Charniaux-Cotton 1982; Juchault and Rigaud 1995; Legrand et al. 1987). In particular, females are heterogametic in *Dynamene bidentata,* a sphaeromatid isopod inhabiting European and north African coasts (Legrand-Hamelin 1976).

Paracerceis sculpta is a sphaeromatid isopod crustacean inhabiting the northern Gulf of California (Figure 1). Inheritance of sex in this species is consistent with female heterogamety (Shuster and Sassaman 1997). However, chromosomal sex determination has not been confirmed due to a lack of known sex-linked markers. In this article we document a sex bias in the expression of a cuticular pigmentation marker (*Str* = striped; Figure 1) in population samples of *P. sculpta* collected over a 10 year period, and we document the inheritance of this marker in three generations of laboratory-reared isopods. Our results indicate that sex determination in *P. sculpta* involves female heterogamety (ZW = females, ZZ = males) and that *Str* is W linked.

Materials and Methods

Field Collections

Isopods were collected from the spongocoels of the intertidal sponge *Leucetta losangelensis* in the northern Gulf of California between 1984 and 1994. All individuals were sexed, measured to the nearest 0.125 mm, and identified by cuticular pigmentation pattern (Shuster 1989). We tabulated these observations by sex and month, summed all observations, and using a *G* test (Sokal and Rohlf 1995), compared the number of individuals of each sex bearing *Str* (striped) with the numbers of individuals that were unmarked or which bore some marker other than *Str* (indicated "+").

Laboratory Experiments

A field-collected, sexually mature female (Shuster 1991) bearing *Str* was crossed with an unmarked α male (Figure 1) from a laboratory lineage (α-1-1) that consistently produces families with 1:1 sex ratios. Individuals from the α-1-1 lineage are homozygous for the Ams^{α} allele at *Ams* (*Alternative mating strategy*), an autosomal locus that controls male maturation rate, male external morphology, and male mating behavior (Shuster and Sassaman 1997). α-1-1 individuals are also homozygous for the Tfr^{1} allele at *Tfr* (*Transformer*), another autosomal locus that causes sex reversal, depending on an individual's genotype at *Ams* and at primary sex determination loci (Shuster and Sassaman 1997). Since Ams^{α} and Tfr^{1} alleles do not interact, the use of $Ams^{\alpha}Ams^{\alpha},Tfr^{1}Tfr^{1}$ sires in this and in subsequent crosses (see below) minimized the possibility of sex-ratio distortion within families. Progeny were separated from the female at parturition, placed into individual, sterilized glass petri dishes, and reared to maturity at 24°C on coralline algae (*Amphiroa* sp.) and brine shrimp flakes, with seawater changes every 4 days, as described in Shuster and Sassaman (1997).

Five of the F_1 females were crossed to unmarked α-1-1 sires, and the F_2 generation was reared to maturity as described above. Five F_2 females were crossed to unmarked α-1-1 sires, three F_2 males were crossed to unmarked α-1-1 females, and the F_3 generation was reared to maturity as well. For each generation, all *Str* individuals were recorded at birth as well as at maturity, when all surviving individuals were measured and sexed.

We investigated Mendelian inheritance of *Str* by comparing the frequency of marked and unmarked individuals at birth and at maturity, within and among families. In all comparisons we expected 1:1 ratios of marked : unmarked individuals. We also investigated the inheritance of family sex ratio by comparing the number of male and female individuals within and among families. Under chromosomal sex determination, we expected 1:1 sex ratios in all families. All comparisons were performed using heterogeneity *G* tests (Sokal and Rohlf 1995).

We investigated the association of *Str* and sex by pooling all laboratory-reared adult males and females into *Str* and unmarked (+) classes. If *Str* and sex were unlinked, we expected equal frequencies of *Str* and unmarked individuals in both sexes. We tested the deviation from this expectation using an exact chi-square test (Toquenaga Y, personal communication). Lastly, we investigated the possible transmission of *Str* through male lineages by examining the number of *Str* individuals produced when sons of *Str* females were crossed to unmarked α-1-1 females.

Results and Discussion

Field Collections

In monthly samples collected over a 10 year period, the frequency of *Str* in the northern Gulf of California *P. sculpta* population never exceeded 8% within 1 month, and was less than 0.02 overall (mean ± 95% CI = 0.015 ± 0.006, N = 9598). These results are consistent with cuticular pigmentation markers reported in other isopod species (Heath 1979). Over 90% of all *Str* individuals collected were female (0.903; N = 62; G = 21.3, $P <$ .001, N = 9598), indicating a significant sex bias in the expression of *Str* in nature. Similar sex biases in cuticular marker expression are reported in *Idotea* and *Dynamene* (Legrand et al. 1987).

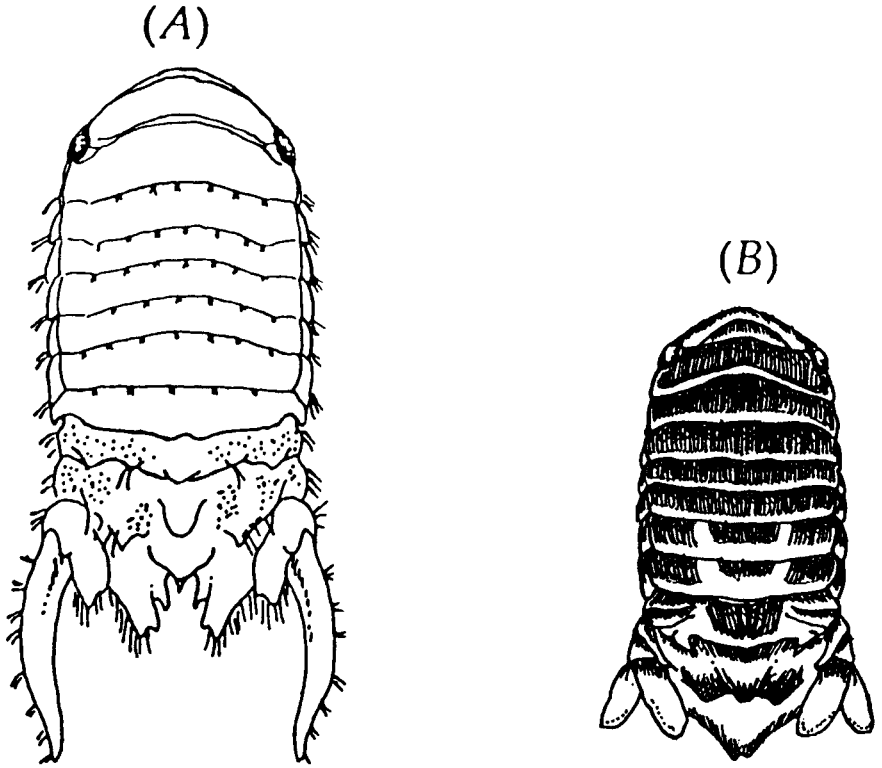

Figure 1. **(A)** Male and **(B)** female *Paracerceis sculpta*; the pigmentation pattern on the female represents *Str* (striped); redrawn after Shuster 1991).

Laboratory Experiments

In the three generations in which *Str* females were crossed to unmarked males, *Str* showed Mendelian inheritance at birth, within and among all families ($\Sigma G_{i[df=11]} = 8.93$, $P > .50$; $G_{Pooled[df=1]} = 1.26$, $P > .20$; $G_{Heterogeneity[df=10]} = 7.67$, $P > .50$; Table 1). Among adults, both *Str* and family sex ratio showed Mendelian inheritance within and among all families ($\Sigma G_{i[df=11]} = 7.75$, $P > .70$; $G_{Pooled[df=1]} = 0.79$, $P > .50$; $G_{Heterogeneity[df=10]} = 6.96$ and 6.57, $P > .70$, respectively; Table 1). In all but one case, in the F_3 generation, all females expressed *Str*, whereas all males were unmarked, indicating close linkage between *Str* and sex (Exact chi-square probability = 2.55×10^{-19}, $N = 103$). Unmarked F_2 males ($N = 3$) crossed to unmarked females produced no *Str* progeny of either sex ($N = 44$), indicating that *Str* is not transmitted through male lineages. These results are consistent with Mendelian inheritance of *Str* and with chromosomal sex determination involving female heterogamety in this species.

The decreased fecundity of F_{2-3} families compared to the F_1 family is explained by the negative relationship between body size and fecundity in *P. sculpta* (Shuster 1991). Isopods maintained in incubators at 24°C (F_{2-3}) were smaller in size and less fecund than the field-collected parental *Str* female, who had matured at a cooler temperature (17°C–20°C in March; Shuster and Guthrie, in press). An episode of overfeeding caused higher mortality among F_3 individuals compared to F_{1-2} individuals. Despite these differences in fecundity among generations, no differential mortality was detectable between *Str* and non-*Str* individuals, or between males and females (Table 1). Although genetic factors are known to cause reversal of sexual phenotype in *P. sculpta* and in other isopods (Juchault and Rigaud 1995; Legrand et al. 1987; Shuster and Sassaman 1997), our use of α sires from families with unbiased sex ratios (lineage α-1-1) and the lack of biased sex ratios within and among our F_{1-3} families indicate that factors responsible for sex reversal were not present in these crosses.

The single non-*Str* female observed in the F_3 generation may represent a female whose maternal *Str*-bearing W chromosome had undergone recombination with a non-*Str*-bearing segment of its corresponding Z chromosome. This hypothesis cannot be confirmed because no *Str* male was found in the F_3 family in which the unmarked female appeared (Table 1). However, six (0.0006; $N = 9598$) *Str* males were observed in field collections. The relative rarity of these males is consistent with their identity as recombinant individuals. Alternatively, these individuals could be the result of misclassification of cuticular markers in the field. One particular pattern, "scale," observed primarily in α males, resembles *Str* but involves a somewhat different distribution of cuticular pigmentation (i.e., a longitudinal band of dark pigment on the *distal* portion of each dorsal body segment, with regular, anterior-directed projections; this pattern gives its bearers a "scaled" as well as "striped" appearance; Johnson K, unpublished data). The inheritance of scale, as well as the frequency of recombination of *Str* in labora-

Table 1. Heterogeneity *G* tests for Mendelian inheritance of *Str* and sex ratio in *Paracerceis sculpta* (F_1–F_3)

Generation	At birth[a]				At maturity					
					Str		+			Sex ratio[b]/*Str*[c]
	Str	+	*N*	*Gi*	F	M	F	M	*N*	*Gi*
F_1	34	38	72	0.22	14	0	0	15	29	0.03
F_2	13	9	22	0.73	8	0	0	5	13	0.70
	6	8	14	0.29	1	0	0	3	4	1.50
	9	20	29	4.28[d]	2	0	0	5	7	1.33
	17	21	38	0.42	1	0	0	5	6	2.91
	4	8	12	1.36	2	0	0	4	6	0.68
F_3	18	24	42	0.86	8	0	1	8	17	0.06
	27	21	48	0.75	2	0	0	2	4	0.00
	18	18	36	0.00	2	0	0	3	5	0.20
	14	14	28	0.00	4	0	0	3	7	0.14
	21	22	43	0.02	2	0	0	3	5	0.20
Total	181	203	384	8.93	46	0	1	56	103	7.75

[a] $G_P = 1.26$, $P > .10$, $G_{H[df=10]} = 7.67$, $P > .50$.
[b] $G_P = 0.79$, $P > .10$; $G_{H[df=10]} = 6.96$, $P > .10$.
[c] $G_P = 1.18$, $P > 0.10$, $G_{H[df=10]} = 6.57$, $P > .50$.
[d] $P < .05$.