

# Evidence for female heterogamety in two terrestrial crustaceans and the problem of sex chromosome evolution in isopods

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Female heterogamety (WZ type) has been demonstrated in the terrestrial isopods *Oniscus asellus* (Oniscidae) and *Eluma purpurascens* (Armadillidiidae), by making crosses between two genetic females (one of them experimentally reversed into a functional neo-male). The WW individuals generated by such crosses were viable and fertile females. These data, plus the frequent monomorphism of sex chromosomes and the coexistence of two heterogamety types (XX/XY and WZ/ZZ), indicate that sex chromosome differentiation in isopods is at a primitive stage. The evolution of sex chromosomes in this group of crustaceans is discussed, and it is suggested that this evolution has been disturbed by parasitic sex factors.

**Keywords:** *Eluma purpurascens*, heterogamety evolution, Oniscidea, *Oniscus asellus*, sex determination, sex-ratio distorters.

## Introduction

Our poor knowledge of sex determining mechanisms in crustacea results from, in considerable measure, to the difficulties in establishing the heterochromosomal sex in this group. Such knowledge is essential for assessing the evolution of sex determination, particularly in terrestrial isopods where sex determination is often disturbed by parasitic elements (Juchault *et al.*, 1993, 1994). Heterogametic mechanisms have been demonstrated in gonochoric crustaceans by three methods: cytogenetics, sex-linkage of markers and crosses between females and neo-males (experimentally reversed females) (review in Ginsburger-Vogel & Charniaux-Cotton, 1982).

Karyotypic analysis is often difficult because of the large number of small chromosomes in many crustacean species. This is the case in terrestrial isopods (Oniscidea) in which the diploid number varies from 16 to 94 often being as great as 50, and chromosomes rarely exceed 2  $\mu\text{m}$  in length (Artault, 1977). Nevertheless, sex chromosomes have been identified morphologically in a few species of isopods (see Table 3).

Although few species have been extensively studied, sex-linked genes appear to be rare in Crus-

tacea and are only known in three species: the branchiopod *Artemia salina* (Bowen, 1963) and two marine isopods, *Idotea balthica* and *Dynamene bidentata* (Table 3). Female heterogamety (WZ) has been demonstrated in these three species by crossing individuals exhibiting genetic polychromatism.

Reversing genetic females into males (creating neo-males) by the early implantation of the androgenic gland is an accurate method by which heterogametic sex can be determined. Such crosses have demonstrated female homogamety (XX) in the amphipods *Orchestia cavimana* and *O. gammarellus* (Ginsburger-Vogel, 1972, 1973) and in three terrestrial isopods: *Porcellio dilatatus dilatatus*, *Armadillidium nasatum* and *Helleria brevicornis* (Table 3). Female heterogamety has been shown in the Oniscidea *Armadillidium vulgare*, *Porcellio dilatatus petiti*, and in the Valvifera *Idotea balthica*. Cytogenetic studies failed to show any morphological differentiation of heterochromosomes in some of these species (Artault, 1977; Table 3). Only one case of a cross between a female and a neo-male has been reported for Crustacea other than the Peracarida. This was in the prawn *Macrobrachium rosenbergii*, in which the females are heterogametic (WZ) (Malecha *et al.*, 1992). Successful matings of two andrectomized males (= neo-females) with normal males of this species have provided further evidence of male

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homogamety (ZZ) (Sagi & Cohen, 1990). Nevertheless, the finding of unexpected sex ratios among crosses implies that 'the genetic determination of sex in prawns must be more complicated than a simple female-heterogametic mechanism' (Malecha *et al.*, 1992).

This paper describes the results for two terrestrial isopods showing female heterogamety. The results have been integrated into previously obtained results to provide a clearer picture of sex chromosome evolution in this group of crustaceans.

**Material and methods**

Two Oniscidea species were investigated: *Oniscus asellus* (Oniscidae) and *Eluma purpurascens* (Armadillidiidae). *Oniscus asellus* were collected in a garden at Celles-sur-Belle (Deux-Sèvres, France) and *E. purpurascens* in woodlands near Sevpret (Deux-Sèvres, France). Progeny obtained from wild gravid females (F<sub>0</sub> progeny) showed a 1:1 sex ratio (results not shown, but see control crosses in Tables 1 and 2). Feminizing *Wolbachia* infection similar to that observed in many isopod species (Martin *et al.*, 1973; Juchault *et al.*, 1994) was not detected, by either electron microscopy or PCR (Juchault *et al.*, 1994). These preliminary results indicate that sex in these populations of *O. asellus* and *E. purpurascens* is probably not under the control of non-Mendelian genetic elements (Legrand *et al.*, 1987).

The heterogametic sex was diagnosed by breeding animals whose sex has been experimentally reversed by hormone treatment, using the technique of Juchault & Legrand (1964). Full-sib very young females (4 mm in *O. asellus*; 3 mm in *E. purpurascens*) were implanted with one androgenic gland from an adult male. The androgenic glands (organ responsible for male hormone synthesis) survived in their new hosts, and continued to produce male hormone (Juchault & Legrand, 1964). The implanted females became neo-males 350 days after the implantation. These neo-males are thus chromosomal females with a male phenotype, male behaviour and male physiology. They were crossed with their intact (normal) sisters, and produced F<sub>1</sub> progeny. From these F<sub>1</sub>, full-sib males and females were crossed to obtain the F<sub>2</sub> generation.

**Results**

***Oniscus asellus***

Seven normal males and seven neo-males from the same F<sub>0</sub> progeny were crossed with a full-sib female.

The sex ratio did not deviate from 1:1 in control crosses ( $\chi^2$  test nonsignificant in all cases). The sex ratio was always female-biased in crosses involving neo-males ('experimental' crosses), and never significantly different from 1♂/3♀ ( $\chi^2$ -test). The 17 F<sub>2</sub> from three different 'experimental' F<sub>1</sub> produced either broods with a balanced sex ratio (10 crosses, producing 130♂/119♀), or all-female broods (seven crosses, producing 0♂/147♀) (Table 1).

***Eluma purpurascens***

Eight males and four neo-males from the same F<sub>0</sub> progeny were crossed with their sister. The results were similar to those for *O. asellus*. The sex ratios of the broods were balanced in control crosses, and female-biased (1♂/3♀) in 'experimental' crosses. The eleven F<sub>2</sub> from one 'experimental' F<sub>1</sub> produced either amphogenous broods (eight families;

**Table 1** Sex ratio in offspring of *Oniscus asellus*

F <sub>1</sub>			F <sub>2</sub>		
Family no.	♂	♀	Family no.	♂	♀
1	11	13	Aa	8	6
2	5	9	Ab	16	6
3	24	17	Ac	23	16
4	11	12	Ad	14	9
5	10	8	Ae	9	10
6	11	17	Af	0	27
7	10	10	Ag	25	26
			Ah	10	5
			Ai	0	24
			Aj	0	21
A	5	18			
B	3	15	Ba	0	13
C	6	20	Bb	15	20
D	5	22	Bc	5	11
E	2	13	Bd	0	18
F	5	19			
G	7	21	Ca	0	11
			Cb	0	33
			Cc	5	10

In the first generation (F<sub>1</sub>), the family numbers 1–7 denote crosses between a normal male and one of its sisters (control crosses), while families A–G denote crosses between a neo-male (see text) and one of its sisters ('experimental' crosses). In the second generation (F<sub>2</sub>), families beginning with the same capital letter indicate crosses between full-sib individuals from the same F<sub>1</sub> progeny. Vertical bars indicate crosses in which one male was mated with two or three females.

**Table 2** Sex ratio in offspring of *Eluma purpurascens*

F <sub>1</sub>			F <sub>2</sub>		
Family no.	♂	♀	Family no.	♂	♀
1	9	12	Aa	0	41
2	11	21	Ab	31	30
3	16	25	Ac	21	29
4	22	37	Ad	16	18
5	17	23	Ae	17	21
6	24	21	Af	0	38
7	35	33	Ag	16	15
8	30	23	Ah	24	19
A	7	25	Ai	0	8
B	2	10	Aj	5	5
C	7	18	Ak	13	21
D	11	37			

This table follows similar conventions to those in Table 1.

143♂/158♀) or all-female broods (three families; 0♂/87♀) (Table 2).

### Interpretation

As the results for both species were similar, crosses in these two isopods can be interpreted in the same way. Crosses involving neo-males can be seen as crosses between two heterogametic (WZ) individuals:

$$\text{neo-}\delta\text{WZ} \times \text{♀WZ} \rightarrow 1/4\delta\text{ZZ} + 1/2\text{♀WZ} + 1/4\text{♀WW}.$$

This is based on the assumption that WW individuals are viable. The F<sub>2</sub> crosses confirm this interpretation, and also indicate that WW females are fertile: the WZ females produced broods with a 1:1 sex ratio, and the WW females produced all-female broods. F<sub>2</sub> crosses also indicate that there was one WW female for two WZ females in the F<sub>1</sub> offspring.

### Discussion

*Oniscus asellus* and *E. purpurascens* showed female heterogamety. These results are in addition to those previously obtained in isopods (Table 3). Both heterogamety types are represented in the Oniscidea, with a slight majority of species having female heterogamety. But both types of heterogamety can occur within a family (Porcellionidae, Armadillididae), within a genus (*Armadillidium*), and even within a single species (*Porcellio dilatatus dilatatus* and *P. d. petiti*), but most notably in individuals of the same population of a subspecies (*P. d. dilatatus*, [Legrand *et al.*, 1980]). Male and female hetero-

gamety in a species is rare, but has been reported for *Chironomus tentans* (Diptera) (Thompson & Bowen, 1972) and the platyfish *Xiphophorus* (Gordon, 1954; Kallman, 1965). Our data indicate that the heterogametic system is not yet fully established in the Isopoda.

*Oniscus asellus* and *E. purpurascens* WW females are viable and fertile. The viability of such an unusual genetic combination has also been reported for the Oniscidea *Armadillidium vulgare* and *Porcellio dilatatus*, and for the amphipod *Orchestia gammarellus* (Ginsburger-Vogel & Magniette-Mergault, 1981). The sex chromosomes in these species must have large homologous segment, for there to be such viability. This contrasts with the situation in mammals and insects, where the sex chromosomes are often so differentiated that YY individuals are inviable or sterile (Bull, 1983). It is generally accepted that the morphological similarity of the sex chromosomes indicates a relatively early stage of specialization of a pair of ancestral chromosomes carrying sex determinants (Bull, 1983; Charlesworth, 1991). The isopod group may well fall in this category. There are only tiny morphological differences between the sex chromosomes in *Porcellio rathkei* and *P. laevis* (Mittal & Pahwa, 1980, 1981), and incipient sex chromosome differentiation has been proposed to account for the slight morphological difference in the male-specific chromosome in *Asellus aquaticus* (Rocchi *et al.*, 1984). The rare extreme sex chromosome differentiation observed in isopods (Table 3) results more from major chromosomal rearrangements than from real chromosomal differentiation. For example, the male chromosome is lacking in *Tecticeps japonicus* and the W chromosome differentiation in *Jaera marina* results from translocation.

The members of the isopod suborder Oniscidea have very little sex chromosome differentiation. However, this suborder is considered to be the most recently evolved group in the Isopoda. Sex chromosome monomorphism and the coexistence of the two types of heterogamety is thus surprising, and the cause for this lack of sex chromosome differentiation must be found. Sex determination in this group is highly distorted by nonchromosomal sex factors: *Wolbachia* bacteria reverse chromosomal males into neo-females in several species of Oniscidea, and induce large distortions of the sex ratio by inhibiting 'male gene' expression (Martin *et al.*, 1973; Juchault *et al.*, 1974, 1994, unpublished results). These feminizing *Wolbachia* have major consequences for the evolution of host sex determination for three reasons.

**Table 3** Heterogametic types in isopod crustaceans

Suborder	Heterogamety type		
	♂ X0/♀ XX	♂ XY/♀ XX	♂ ZZ/♀ WZ
Asellota		<i>Asellus aquaticus</i> ** (2)	<i>Jaera marina</i> (5 ssp.)* (5)
Flabellifera	<i>Tecticeps japonicus</i> * (1)		<i>Dynamene bidentata</i> †(6)
Valvifera			<i>Idotea balthica</i> (7)
Oniscidea		<i>Porcellio dilatatus dilatatus</i> † (3)	<i>Porcellio dilatatus petiti</i> † (8)
			<i>Porcellio rathkei</i> ** (9)
		<i>Armadillidium nasatum</i> (4)	<i>Porcellio laevis</i> ** (10)
		<i>Helleria brevicornis</i> (3)	<i>Armadillidium vulgare</i> † (11)
			<i>Eluma purpurascens</i> (12)
			<i>Oniscus asellus</i> (12)

Symbols indicate species with extreme (\*), slight (\*\*), and no (†) heteromorphism of sex chromosomes. Absence of symbols: no data about heteromorphism.

References: (1) Niiyama (1956); (2) Rocchi *et al.* (1984); (3) Juchault & Legrand (1964); (4) Juchault & Legrand (1979); (5) Staiger & Bocquet (1954, 1956); (6) Legrand-Hamelin (1976); (7) Tinturier-Hamelin (1963); Legrand-Hamelin (1977); (8) Legrand *et al.* (1974); (9) Mittal & Pahwa (1980); (10) Mittal & Pahwa (1981); (11) Juchault & Legrand (1972); (12) this study.

First, because infected mothers generally produce more daughters than do uninfected ones, *Wolbachia* can cause the disappearance of the female chromosome from infected populations (Taylor, 1990; Juchault *et al.*, 1993). Secondly, *Wolbachia* can give rise to a new parasitic feminizing element (the f factor, which also reverses chromosomal males to neo-females) in *A. vulgare*. This element behaves like a mobile DNA element (Legrand & Juchault, 1984). Although f factor inheritance is mainly non-Mendelian, this element can also evolve by stable insertion into the host nuclear genome, creating a new female sex chromosome (Juchault & Mocquard, 1993). As *Wolbachia* and the f factor change ZZ males to females, and conversely, as WZ females (and even WW females [Hasegawa & Katakura, 1983]) can be changed to males, it has been assumed that males and females have all the genes needed for complete differentiation in both sexes (Legrand *et al.*, 1987). Lastly, nuclear genes for resistance to feminizing elements have been selected in populations where *Wolbachia* and the f factor are present (Juchault *et al.*, 1992; Rigaud & Juchault, 1993). A dominant autosomal gene (*M*) can restore the male sex in the presence of the f factor (i.e. *mm* ZZ + f individuals are females and *Mm* ZZ + f individuals are males). This *M* gene is believed to inhibit the gene inhibiting the 'male gene' expression (*M* also represses the expression of the W chromosome, thus *Mm*WZ individuals have a male phenotype). The *M* gene may be selected in a population infected by the f factor, and the final state of this selec-

tion would be essentially male heterogamety at the *M* locus, a situation similar to a dominant Y system of sex determination (Rigaud & Juchault, 1993). Thus, here, male heterogamety can be seen as a by-product of an intragenomic conflict in a species with an ancestral female heterogamety.

We therefore propose that epigenetic sex factors can prevent sex chromosome differentiation in Oniscidea by repeatedly changing the locations of sex-determining genes on their host's chromosomes. However this assumes that the association between sex ratio distorters and Oniscidea occurred at an early stage of sex chromosome evolution. Further experimental and theoretical studies are needed to test this hypothesis.

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