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# Roles of resource and partner availability in sex determination in a parasitic copepod

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Because sexuality plays an essential role in gene transmission and consequently in the evolution of species, investment into male or female function constitutes a key factor in the reproductive success of individuals. Environmental sex determination permits adaptive sex choice under unpredictable environmental conditions, where the environment affects sex-specific fitness, and where offspring can predict their likely adult status by monitoring an appropriate environmental cue. The parasitic copepod *Pachyppygus gibber* displays three sexual phenotypes (i.e. one female and two kinds of male) which are environmentally determined (i.e. after conception and in response to environmental cues). Here, we report an experimental analysis on the combined action, during larval development, of availability of food resources and sexual partners in the sex determination of this species.

**Keywords:** evolution; parasite; sex determination; food resources; mating; copepod

## 1. INTRODUCTION

The theory of sex allocation predicts that the sexual determinism of an individual and of its offspring should be sensitive to, and so respond to, the potential gains of possessing a male or female function (Charnov & Bull 1977; Charnov 1982), providing there is genetic variability for sex-determination mechanisms. In this context, two main factors should be responsible for optimal sex determination: (i) the cost of producing a male or a female within the physical and nutritional characteristics of the environment, and (ii) the probability of encountering a suitable sexual partner. When the environment is unpredictable for parent and offspring, selection should favour sex determination after conception, based on the environmental conditions experienced by the different individuals (i.e. environmental sex determination or ESD) (Charnov & Bull 1977, 1989; Charnov 1982). ESD has been reported in different taxa of vertebrates and invertebrates (Bull 1983; Adams *et al.* 1987; Korpelainen 1990; McCabe & Dunn 1997). Charnov & Bull's model for the evolution of ESD predicts that the environment experienced during development is variable and has differential effects on the fitness of males and females (Bull 1983; Bull & Charnov 1988; Charnov & Bull 1989). For instance, when the energetic cost of being male differs from that of being female, it has been suggested that the available resources are likely to influence sex determination (Petersen 1972). However, in unpredictable and highly patchy environments, natural selection should also presumably favour individuals that can adjust their sex according to the sex of the partner already present in their patch.

*Pachyppygus gibber* (Copepoda: Notodelphyidae) is a parasitic copepod of the sea squirt *Ciona intestinalis*, in which it feeds on plankton filtered by the host. After hatching, the life cycle of this copepod involves seven, free-swimming, non-feeding stages (nauplius 1–6 and copepodid 1, respectively). Copepodid 2 is the infective stage, and then another three copepodid stages (copepodid 3–5), feeding within the host, precede the adult stage. Three sexual phenotypes have been described in this species (Hipeau-Jacquotte 1988): two males and one female (figure 1). The first male phenotype, named 'atypical', is very small and actively leaves its host to visit other hosts in order to search for females. Conversely, the 'typical' male is larger and unable to swim outside its host. Females are larger than the two males and are also unable to swim. It has been proposed that sex is under environmental determinism in this species. Indeed, atypical males develop in young hosts, and typical males and females develop in older ones, suggesting that resource availability is responsible for ESD (Hipeau-Jacquotte 1988). Knowing that *P. gibber* can be reared outside the host in monospecific culture fed by the unicellular algae *Pavlova lutheri* (Hipeau-Jacquotte 1988), the first aim of this study was to demonstrate experimentally the key role of food availability on sex determination in this parasitic copepod species. Subsequently, we investigated in controlled conditions the combined action of availability of food resources and sexual partners in the sex-determination process.

## 2. MATERIAL AND METHODS

A sample ( $n=100$ ) of female *P. gibber* bearing eggs in their marsupial pouch were collected from sea squirts *Ciona intestinalis* randomly sampled in the Thau lagoon (Southern France,

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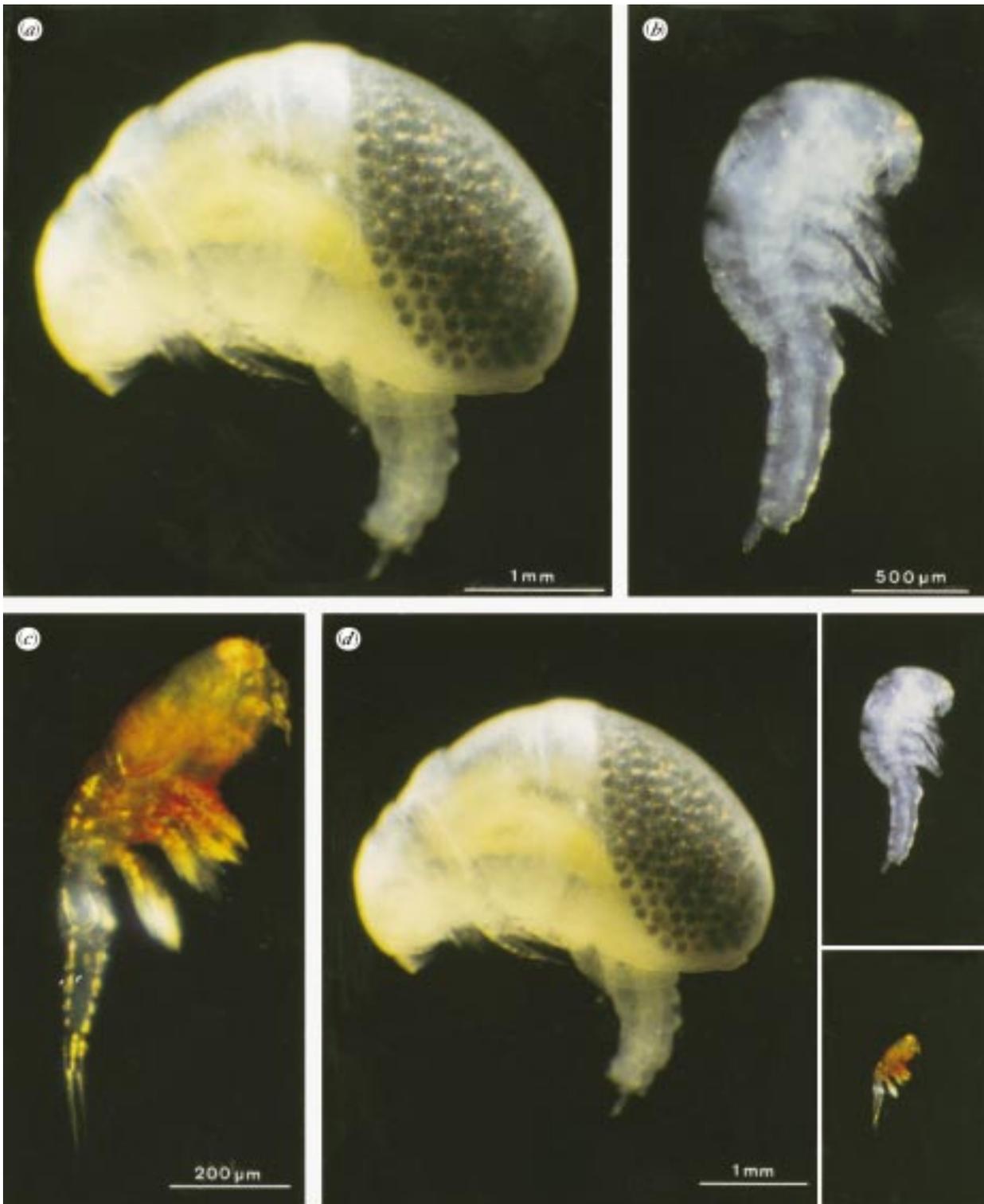


Figure 1. Sexual phenotypes observed in *Pachypygus gibber*. (a) Female displaying eggs in her marsupial pouch; (b) typical male; (c) atypical male; (d) the three phenotypes at the same scale.

43°25' N, 3°35' E) during March 1995. Copepod females were maintained alive in filtered lagoon water in order to obtain nauplii (i.e. first larval stage) release. All nauplii were then pooled together and grown under these laboratory conditions (i.e. filtered lagoon water) until the development of the copepodid 2, which is the infective stage. Indeed, the different nauplii stages and the copepodid 1 stage do not feed. We reared

copepodid 2 of *P. gibber* outside the host in monospecific culture fed by the unicellular algae *Pavlova lutheri* provided by the 'Station de Lagunage' of Mèze (France) and reared at the 'Station Méditerranéenne d'Ecologie du Littoral' of Sète (France).

To test the influence of resource availability on sex determination and mortality in copepodid development, we conducted experiments where copepodids were reared in four

Table 1. *Experimental conditions in which copepodids were reared*

experimental conditions	
A	unfiltered seawater
B	filtered seawater with $5 \times 10^5$ cells ml <sup>-1</sup> of <i>Pavlova lutheri</i> (final concentration <sup>a</sup> )
C	filtered seawater with $2 \times 10^6$ cells ml <sup>-1</sup> of <i>Pavlova lutheri</i> (final concentration)
D	filtered seawater with $4 \times 10^6$ cells ml <sup>-1</sup> of <i>Pavlova lutheri</i> (final concentration)
E	condition D where one virgin female was added at the beginning of the experiment
F	condition C where one typical male was added at the beginning of the experiment

<sup>a</sup>Estimates of final concentration of *P. lutheri* (number of cells ml<sup>-1</sup>) were checked under a 'Cellule de Malassez' (Fiala & Billy 1979).

Table 2. *Influence of resource on sex determination and mortality of copepodids*

(Experimental conditions A, B, C and D are described in table 1.)

experiments	A	B	C	D
atypical male	8	11	3	0
typical male	0	0	15	2
female	0	0	0	20
mortality	52	49	42	38
total	60	60	60	60

environmental conditions: A, B, C and D (table 1). Copepodids were reared in two other environmental conditions, E and F (table 1), to test the influence of sexual partner on sex determination and mortality in copepodid development.

In each experiment, one copepodid 2 was individually reared in a Petri dish in one of the described environmental conditions (i.e. A–F). To avoid genetic bias, copepodids 2 were chosen randomly in the pool of copepodids obtained from the protocol previously described. The Petri dish environment of each experiment was renewed each day to avoid bacterial and fungal development. For each experiment, (i) each sexual phenotype was determined at the copepodid 5 stage according to Hipeau-Jacquotte (1978, 1980), and (ii) mortalities and differential developmental times were noted.

### 3. RESULTS AND DISCUSSION

Development and mortality of copepodids (i.e. stages 2–5) under the four (A–D) feeding regimes are presented in table 2. The results show the strong influence of food resources on sex determination and larval mortality rates. Indeed, the proportion of the different sex phenotypes observed differed significantly among experiments (Fisher's exact probability test,  $p < 0.0001$  in each paired comparison), except for the A–B comparison ( $p = 0.62$ ). Concerning mortality, Fisher's exact probability tests revealed significant differences only for the A–C, A–D and B–D paired comparisons ( $p < 0.044$  in each case). We also observed significant differences between the development time of atypical males (12–15 d; mean = 14.2,

Table 3. *Influence of sexual partner on sex determination and mortality of copepodids*

(Experimental conditions E and F are described in table 1.)

experiments	E	F
atypical male	0	5
typical male	13	2
female	0	9
mortality	17	14
total	30	30

s.d. = 0.88), typical males (19–21 d; mean = 20.0, s.d. = 0.84) and females (23–25 d; mean = 24.1, s.d. = 0.79) (Kruskal–Wallis ANOVA test,  $\chi^2 = 48.23$ ,  $p < 0.0001$ ; each pair of comparisons is significantly different).

Thus, only atypical males were obtained in poor feeding conditions A and B, whereas females were obtained only in the richest environment, D, suggesting a greater cost in energy and time to make a female than a male.

Our results also demonstrated that, in addition to food availability, an existing sexual partner had a strong influence on sex determination (table 3). Indeed, in the richest environment, D, where female development is expected (table 2), a significant proportion of copepodids differentiated into typical males when a virgin female was initially present (table 3, Fisher's exact probability test, comparison between environments D and E,  $p < 0.0001$ ). In fact, no females were produced under condition E. Similarly, whereas in environment C copepodids mainly evolved into typical males (table 2), a significant proportion of copepodids developed into a different phenotype (i.e. atypical male or female) when a typical male was initially added (table 3, Fisher's exact probability test, comparison between environments C and F,  $p = 0.00001$ ). Concerning mortality, Fisher's exact probability tests revealed no difference between experiments D and E ( $p = 0.64$ ), but a significant difference between experiments C and F ( $p = 0.04$ ). Indeed, mortality was lower in experiment F (47%) than in experiment C (70%). This might be explained by the higher proportion of atypical males observed in experiment F (31%) compared with experiment C (17%) (Fisher's exact probability test,  $p = 0.01$ ).

For animals where there is a differential cost in making a male or a female, all demonstrations of resource-dependent ESD are indirect, inferring food resource availability from host size or host weight (Petersen 1973, 1977; Harlos *et al.* 1980). For *P. gibber*, in which development time and body size support the hypothesis of a differential cost in the production of males (i.e. atypical or typical) or females, we have demonstrated directly the influence of food resource availability on ESD. ESD in *P. gibber* may be favoured by the unpredictable patchiness (space and time) of resource distribution (i.e. host individuals).

Among the natural populations of *C. intestinalis*, the mean abundance of *P. gibber* is generally low, with a patchy distribution of possible mates (Becheikh *et al.* 1996). Our results show that selection may have favoured an ESD that also takes into account whether a sexual partner is already present in the host. Interestingly, in an inappropriate environment for female development (resource constraint; experiment C, table 2), the presence

of a male also induced an increase in the proportion of atypical males produced. These atypical males are the only adult stages able to change host patch in order to seek a female. This could illustrate the combined role of resource availability and local mate competition in *P. gibber* sex determination. Our results thus support the prediction of Charnov & Bull (1977, 1989) that ESD should be selected when the environment into which an individual develops has a differential effect on male and female fitness.

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