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Parental control of sex ratio in *Gammarus duebeni*, an organism with environmental sex determination

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Abstract

Parental sex ratio control was investigated in *Gammarus duebeni*, an amphipod with an environmentally mediated sex determining system. The effect on the F2 generation sex ratio of the photoperiodic conditions experienced by a) the P generation during and after copulation, b) the F1 generation before and after sex determination, and c) the F2 generation themselves during the period of sex determination, was tested. The photoperiodic conditions the F2 generation experienced during the period of sex determination had a significant effect on their sex ratio (more males were produced under long-day than under short-day conditions), but the photoperiodic conditions experienced by the F1 generation males and females or the P generation on the F1 male's side had no effect on the F2 sex ratio. However, the photoperiodic conditions the P generation on the F1 female's side experienced significantly affected the F2 sex ratio. When these animals experienced long-day conditions the F2 generation became female biased and when they experienced short-day conditions, male biased. It is proposed that the mechanism of control operates through the F1 generation mothers whilst in an embryonic stage of development in the P generation mother's brood pouch. The photoperiodically mediated effects of the embryonic F1 generation mother and the F2 generation on sex determination are additive. A mechanism by which both F1 generation maternal and F2 generation sex ratio control could operate in the field is proposed.

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Introduction

There may be circumstances when parents can increase their fitness by manipulating the sex ratio of their offspring. Trivers and Willard (1973) proposed that selection favoured sex ratio adjustments in response to environment, so that parents controlled their offspring's sex ratio according to their ability to invest. Their model was directed mainly at polygynous vertebrates in which the breeding success of males is more variable than females and so more likely to be influenced by parental investment. Field trials have tested and are in agreement with their predictions (Austad and Sunquist, 1986; Zuleta and Bilenca, 1992): mothers in a good condition invested in male offspring and mothers in a poor condition, female offspring.

In partially bivoltine animals in which generations overlap and males or females of each generation could compete for mates, parents may be able to avoid inter-generation competition by manipulating the sex ratio of their offspring. This was proposed in Werren and Charnov's (1978) and Seger's (1983) models of sex ratio selection. Patterns of alternating sex ratios are often seen in partially bivoltine insects (e.g. Brockmann and Grafen, 1992), and Werren and Charnov (1978) and Seger (1983) have found sex ratio data amongst some of these insects consistent with their predictions.

In organisms with environmental sex determination (ESD), in which the sex of the embryo remains labile until some time after conception and is then fixed in response to an environmental cue, parental control of offspring sex ratio is thought to be an inferior strategy in cases when the offspring have better information about the period in which they must develop (Charnov and Bull, 1977). However, there may still be circumstances when parental sex ratio control is advantageous. For example, it could be a strategy to ensure the differential production of the sexes or to prevent excessive sex ratio biases if environmental cues became unreliable, or if harsh environmental conditions delayed breeding.

I investigated here the possibility of parental sex ratio control in one organism with ESD: the amphipod curstacean *Gammarus duebeni*. In the laboratory young *G. duebeni* became predominantly male under long-day conditions and predominantly female under short-day conditions (Bulnheim, 1967, 1978; Naylor et al., 1988a; Watt and Adams, 1994). However, in the field there may be a complex interaction of environmental cues that determine sex (Watt and Adams, 1993). The dynamics of the population under investigation are relatively complex, having a long breeding season for its latitude (Watt, 1989), probably due to the effects of the North Atlantic Drift which increases winter temperatures. In addition, the environmental cue determining sex may have a temperature as well as a photoperiod component (Watt and Adams, 1993).

Materials and methods

Gammarus duebeni were collected from Gansey beach on the Isle of Man, U.K. (National Grid Reference SC 216686). Eleven pre-copula pairs were taken into the laboratory (P generation; Fig. 1). Each was kept in a perspex pot containing 250 mls

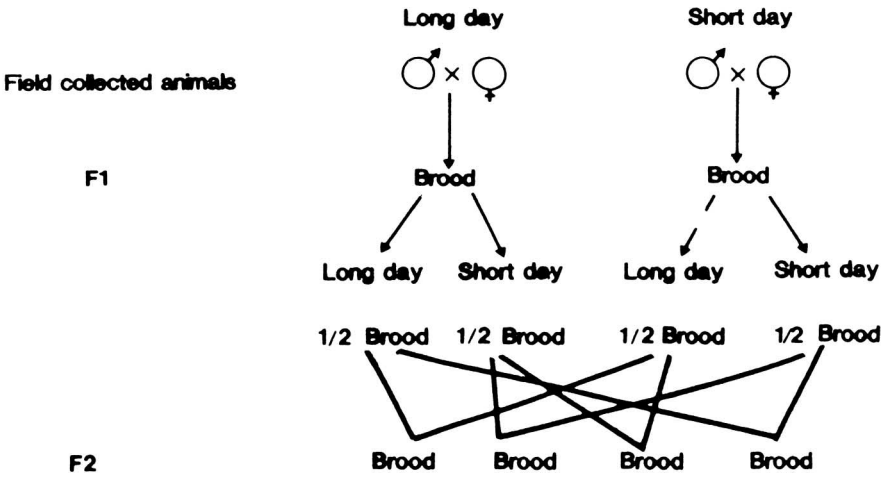

Fig. 1. The crossing design. Field collected pairs (P generation) of *Gammarus duebeni* were allocated to long-day or short-day environmental cabinets. Once they had mated their broods (F1) were divided in half and each half was reared under the parental or complementary photoperiod. On reaching sexual maturity, several months later, half broods from different families were crossed at random. Broods (F2) from these crosses were halved, as before, reared under long- or short-day conditions and sexed 3–4 months later.

of brackish water (specific gravity 1005°). Pairs were allocated to environmental cabinets illuminated with fluorescent lights with an intensity level of approximately 2200 lux and spectral range of 300–800 nm, and kept under one of two controlled light regimes: long day (16 hours light/8 hours dark) and short day (8 hours light/16 hours dark) (Fig. 1). These light regimes lie on either side of the threshold photoperiod for male production (estimated as 13 hours light) in this and other *G. duebeni* populations. Four pairs were maintained under long-day conditions and seven pairs under short-day conditions. Cabinets were kept at 15° C and *Enteromorpha* was provided as food.

Once a pair had copulated the male was removed. This means that both the male and the female of a pair experienced the same daylength and so the effects of photoperiod experienced by the P generation males and females could not be separated. Brood incubation took 20 days at 15° C (Kinne, 1959). Immediately after the brood was released from her marsupium, the female was removed to prevent her from eating the young. These F1 generation broods, which varied in size from 10–30 young, were divided in half: one half was raised under the maternal photoperiod and the other half under the other light regime (Fig. 1) to control for genetic factors. ESD takes effect 2–3 weeks after the brood has been released from the maternal marsupium (Naylor et al., 1988a).

The young were cultured for a further 3–4 months until they reached 8 mm in length and could be sexed. Distinctive secondary sexual characters appear when animals reach 8 mm. Females possess five pairs of oostegites arising from the second to sixth peraeopods and males, which lack oostegites, possess a pair of

penial papillae ventrally between the fifth pair of walking legs. Once sexed, males and females were cultured for a further 4 months until they reached sexual maturity. Once sexually mature, at about eight months, females develop fringed setae on the oostegites. In males sexual maturity is less obvious, but after eight months most males are able to carry a female in pre-copula and mate.

F1 males and females which had been reared under long- and short-day conditions were randomly allocated to pots for mating (Fig. 1). A t-test confirmed that mating was indeed random: short day female biased broods were not more likely to be crossed with long day male biased broods (t tests between F1 generation mother and F1 generation father LD and SD sex ratio data, $P > 0.05$ in both cases). The broods from these crosses (F2 generation) were split, raised under long-day and short-day light regimes as before, and sexed after three months.

Thus, there were three generations of animals: (1) the P generation originally collected from the field; (2) the offspring of the P generation – the F1 generation; and (3) their offspring – the F2 generation. Animals from each generation were subjected to long-day and short-day conditions; for the field-caught P generation these treatments began after sex determination, at the coupling stage, and continued during brooding for the female (the male was removed after copulation). For the F1 generation and the F2 generation the photoperiod treatment included the period of sex determination.

I tested the effects of a) P generation photoperiod, b) F1 generation photoperiod, and c) their own photoperiod, on the sex ratio of the F2 generation. There were 52 broods in the F2 generation in total. Data analysis used maximum likelihood methods with the GLIM (Generalized Linear Interactive Modelling) statistical package, a process of statistical modelling which allows an ANOVA like approach to data which is not normally distributed and which has varying sample sizes for different treatments (Aitkin et al., 1990). Since the results were all ratios (number of males/total) binomial errors were specified which results in the mean proportions ($\pm$SEs) corresponding to treatment levels estimated on a logit scale ($\log_e(P/1-P)$). Standard errors were estimated on a logit scale. Sex ratio was taken as a property of the brood and not of an individual within a brood.

Results

Significantly more males were produced in the F2 generation when reared under long-day conditions than under short-day conditions (analysis of deviance, $P < 0.025$, Table 1. N.B. since the residuals from the model fits are chi square (G) values rather than sum of squares, the process is called analysis of deviance rather than ANOVA): this confirmed that sex determination has a photoperiodic component in this population. The photoperiodic conditions, which either the males or the females of the F1 generation experienced during rearing, had no effect on the F2 offspring sex ratio, nor the photoperiod experienced by the P generation on the F1 male's side (Tab. 1). However, the photoperiodic conditions the P generation on the F1 female's side experienced had a significant effect on the F2 sex ratio (analysis of deviance, $P < 0.005$). More males were produced in the F2 generation when these

Table 1. The mean proportion of male (sex ratio = SR) *Gammarus duebeni* in the 52 F2 generation broods produced when each generation (the F2 generation, the male and female F1 generation and the *P* generation on the F1 male and female side), shown in the left hand side column (G), experienced long-day and short-day light regimes. Mean SR calculated in each case by taking the average SR of the 52 broods. Upper and lower limits of standard errors are shown. Probabilities (*P*) are those derived from the analysis of deviance and test for differences between the proportion of males in long-day and the proportion of males in short-day conditions.

G	Long-day			Short-day			*P*
	Mean SR	SE upper	SE lower	Mean SR	SE upper	SE lower	
P female side	0.31	0.35	0.26	0.52	0.56	0.49	<0.005
P male side	0.41	0.45	0.36	0.46	0.50	0.43	ns
F1 female	0.425	0.46	0.39	0.47	0.51	0.42	ns
F1 male	0.44	0.47	0.40	0.46	0.51	0.41	ns
F2	0.55	0.59	0.51	0.34	0.38	0.30	<0.025

P generation animals experienced short-day photoperiods than when they experienced long-day photoperiods (Tab. 1). Thus, the F2 generation sex ratio is affected both by the photoperiod experienced by the P generation animals on the F1 generation female's side and by the classical ESD mechanism. The P generation (on the F1 female's side) × F2 generation interaction was not statistically significant, which is consistent with the effect of these two mechanisms being additive (Tab. 2). Since the P generation females would have been brooding the F1 generation females in their brood pouches when they experienced the photoperiod, it is likely that the F2 generation is not affected indirectly by the P generation but directly by the embryonic F1 females.

In order to determine whether F1 males or females differed in their ability to produce males, the effects of F1 male and female germ-lines on the F2 sex ratio were investigated. Since any effects on the F1 germ-line were confounded with the photoperiodic conditions experienced by the P generation, it was necessary to use a

Table 2. Mean proportion of males (sex ratio) in F2 generation broods showing the additive effect of the photoperiod experienced by the embryonic F1 female and the F2 generation (LD = long day, SD = short day). Upper and lower limits of standard errors are shown. When F1 females experience LD conditions there is a 25% reduction in the proportion of males in the F2, whereas when the F2 generation experience LDs there is a 23% increase. *N* = total number of F2 broods in each case.

F1 female photoperiod	F2 photoperiod	F2 sex ratio	SE upper	SE lower	*N*
SD	LD	0.67	0.72	0.61	14
SD	SD	0.41	0.45	0.36	14
LD	LD	0.39	0.45	0.33	12
LD	SD	0.19	0.26	0.14	12

Table 3. The mean proportion of male *Gammarus duebeni*($\pm$SE) produced in F2 generation broods by F1 females with specific germ-lines. There is a significant difference between F1 females in their ability to produce males ($F = 5.8$, $P < 0.025$). N = the number of F2 generation broods in each case.

F1 female germ-line	Proportion of males	N
SD1	0.54 ± 0.05	14
SD2	0.00 ± 0.00	2
SD3	0.25 ± 0.25	2
SD4	0.72 ± 0.05	2
SD5	0.55 ± 0.10	8
LD1	0.00 ± 0.00	10
LD2	0.30 ± 0.14	6
LD3	0.00 ± 0.00	2
LD4	0.66 ± 0.09	6

nested model and test for F1 generation germ-line variation within P generation photoperiod blocks. F1 females differed in their ability to produce males (analysis of deviance, $P < 0.001$; Tab. 3), F1 males did not ($P > 0.05$).

Discussion

In *Gammarus duebeni* from Gansey the sex ratio of the F2 generation was significantly affected both by the photoperiod the P generation on the F1 female's side experienced and by that which the F2 generation themselves experienced. During the period of exposure to photoperiod the P generation females would have been carrying the potential F1 generation mothers amongst the eggs in their brood pouch, and so the photoperiod could be acting directly on these in the form of an early maternal effect, rather than on the P generation animals themselves. It is proposed that this is the mode of action by which this sex ratio control takes place; the F1 generation mothers monitor the environmental conditions early in development and based on these predetermine the sex ratio of future broods so that the sex which benefits most from the imminent conditions dominates. It was only the F1 generation females that were affected by the photoperiodic conditions they experienced in the embryonic state, while the conditions that the F1 generation males experienced during incubation had no effect on the F2 sex ratio.

The direct effect of the photoperiodic conditions experienced by young *G. duebeni* on their sex ratio is well documented (Bulnheim, 1967, 1978; Naylor et al., 1988a; Watt and Adams, 1994), but that of the embryonic mother has not been recorded before for this, or any other, organism with ESD. There is, however, some indication that female leopard geckoes, with temperature dependent sex determination, are able to choose nest sites on the basis of temperature in the laboratory (Bull et al., 1988) which may imply some maternal control of sex ratio. In *Gammarus duebeni* the effect of the maternal photoperiod and that experienced by her brood

on the sex ratio of the brood itself is almost equally strong, but they are in opposition. Exposure of a given brood to long-day conditions increases male production by 25%, while exposure of the brood's embryonic mother to long-day conditions reduces male production by 25%. The two effects are additive – an early maternal long-day treatment exactly negates a brood long-day treatment, returning the population of males to the 40% value found when both treatments are short day.

How the maternal control is exerted is unknown. In the broad bean aphid *Aphis fabae*, winged gynoparae development is regulated by pre- and post-natal photoperiod. Hardie and Lees (1983) found that the induction of gynoparae was maternally-controlled during the early larval instars of the parent generation, but that during later stages of embryonic development the presumptive gynoparae responded directly to photoperiod through the mother's abdominal wall. The presumptive gynoparae remained sensitive to photoperiodic conditions during early postnatal development.

Gammarus duebeni females with differing germ-lines varied in their ability to produce males suggesting an additive genetic control mechanism operating independently of photoperiodic cues. However, the effects of microsporidian parasites, which have been found to distort brood sex ratios in other populations of *G. duebeni*, cannot be ruled out (A. Dunn, pers. comm.).

Under field conditions, the differential production of males and females has been recorded for a number of *G. duebeni* populations (Naylor et al., 1988b; Watt and Adams, 1993): males are produced early in the season and females later. Males benefit more from large size, and therefore a long growth period, than do females (Adams et al., 1989) and so it is selectively advantageous for them to be born first. However, in all cases studied there is a mismatch between the photoperiodic conditions under which males and females are determined in the field and those that bring about sex determination in the laboratory (Watt, 1989; Watt and Adams, 1993). In the laboratory more males are produced under long-day than under short-day conditions whereas in the field, because animals start reproducing in the winter months (Hynes, 1954; Naylor, 1986; Watt and Adams, 1993), the first young of the season, the males, are born into short-day conditions.

Using monthly temperature data collected at Gansey (Hynes, 1954) to estimate brood incubation time, the period from brood release to sexual maturity (6–11 months, sex of young determined in first 3-weeks – see methods) and the length of time females are in pre-copula before mating, it is possible to estimate the length of time for an embryonic mother to produce her own offspring (Tab. 4). Based on these criteria this period is on average 11.3 months, but may vary from 8–14 months if calculations consider rates in good (highest possible temperature) and bad (lowest possible temperature) years.

This means that the F2 generation offspring are produced about one year after their F1 generation mothers initially experienced the photoperiodic conditions. Thus, embryonic mothers exposed to short days early in the season, would give rise to male biased broods at the same time the following year, and embryonic mothers exposed to long days female biased broods later in the season. This is exactly the

Table 4. The time period for an embryonic mother to produce her own sex determined offspring based on a mean annual temperature at Gansey of 11.2 ± 1.1° C (from Hynes, 1954). The sex of the offspring is assumed to be determined 3 weeks after release from the maternal brood pouch – the period when young are sensitive to photoperiod in the laboratory (Naylor et al., 1988a).

Stage of development	Period length (days)	Time since maternal egg (days)
maternal brood incubation	30.79 ± 3.6	30.79 ± 3.6
maternal sexual maturity	224	254.79
maternal pre-copula	9.5	264.29
offspring brood incubation	30.79 ± 3.6	295.08
offspring sex determination	21	316.08

order of sex-differential production found in the field and which could not previously be explained by the laboratory data.

However, imposed on this is the direct response of the female's offspring to photoperiod. If the embryonic mothers experience a short-day photoperiod and her offspring about a year later a long-day photoperiod then, because the two effects are additive, the broods will be male biased. However, if both the mother and her offspring experience short-day conditions then the two treatments exactly negate each other and genetic mechanisms of sex ratio control come into operation so that the sex ratio approximates 0.5. Similarly, a mother experiencing a long-day and her offspring a short-day photoperiod gives rise to female biased offspring and a mother experiencing a long-day and her offspring a long-day photoperiod gives a 0.5 sex ratio.

Based on the minimum and maximum time taken for an embryonic mother to develop and produce her offspring and assuming females are capable of reproducing most of the year, it is possible to determine when the effects of the mother's and the offspring's mechanisms of sex ratio control would give rise to male biased, female biased and non-biased broods in the field at Gansey (Fig. 2). The threshold daylength for male production is taken as 13 hours light (see methods) so that from October to March days are short and from April to September days are long. Thus, embryonic mothers experiencing photoperiods from October to March will predetermine broods to be male biased (offspring – March to September) and from April to September broods will be predetermined as female biased (offspring – October to March). Through the operation of the two mechanisms of control this would mean that male biased broods would be produced in a good year between June and September and in a bad year a proportion the following year in April; the remainder of the time genetic mechanisms of sex ratio control would maintain the

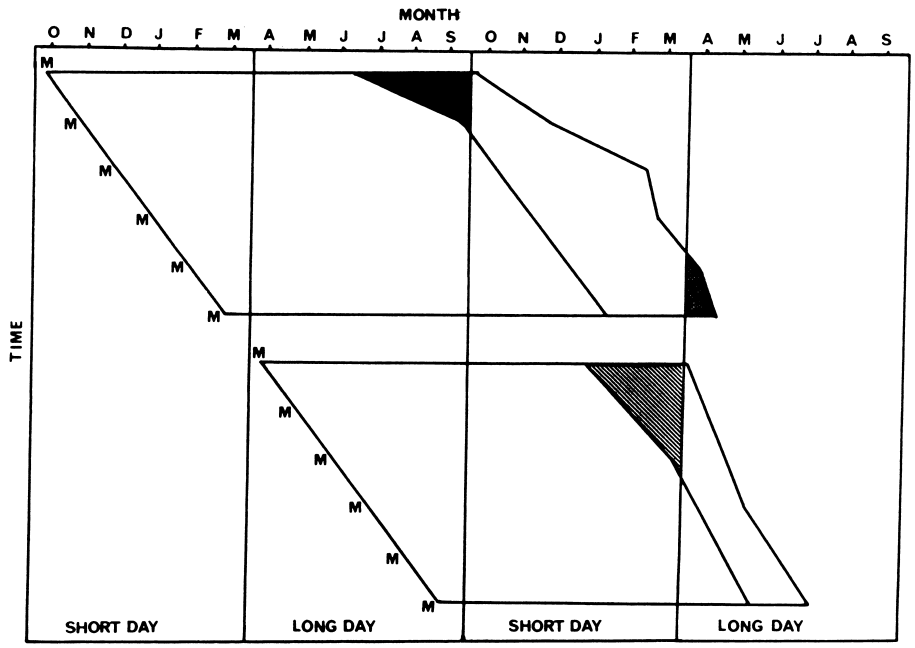

Fig. 2. The proposed model of male and female production at Gansey based on the operation of maternal and brood mechanisms of sex ratio control. Embryonic mothers (M) experience long-day or short-day photoperiods whilst in their own mothers brood pouch and predetermine their offspring broods to be male or female biased. These broods are produced 8–11 months later depending on whether it is a good (highest possible temperatures) or a bad (lowest possible temperatures) year. The predetermined offspring are sensitive to photoperiod themselves. However, the brood and maternal mechanisms are in opposition; short-day maternal photoperiods and long-day brood photoperiods give rise to male biased broods (dark stippled blocks) and long-day maternal and short-day brood photoperiods to female biased broods (lined block). Either long-day maternal and long-day brood or short-day maternal and short-day brood effects completely negate each other and sex ratio control is genetic (plane blocks). Thus, the sex ratio of the broods depends on the photoperiod experienced by both the mother in an embryonic state and that which they themselves experienced when photoperiodically sensitive (at 3 weeks, Naylor et al., 1988a). Temperature data based on that for Gansey (Hynes, 1954).

sex ratio around 0.5. Female biased broods would be produced from January to March in good years only. The rest of the time genetic mechanisms of sex ratio control would be in operation.

The operation of two mechanisms of sex ratio control would ensure both male and female production in good and in bad years and a population sex ratio which did not become overly biased in one direction or the other but stayed around 0.5. According to this model, sex-differential production would only take place in good years, but in bad years a small proportion of broods would be male biased and could perhaps delay reproduction until the following season so that they would be larger than any of the males produced then.

It remains to be tested as to whether this is the pattern of male and female production in the field. However, it does provide a means by which both maternal and brood mechanisms of sex ratio control, both observed in animals from Gansey, could operate in the field. Further studies are required in order to determine the extent to which sex ratio control is under maternal control in other populations of *Gammarus duebeni* and to identify the source of this control.

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