

# An analysis of inherited factors affecting the sex ratio in the rice weevil, *Sitophilus oryzae* L.

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Observations of a significant female bias in one geographical strain of the rice weevil, *Sitophilus oryzae* L., stimulated a detailed investigation of sex ratio in three geographical strains. Sex ratios from intra-strain crosses were not usually biased, but certain inter-strain crosses were consistently associated with female biased sex ratios. Foodtype did not affect the sex ratio. Sex ratio bias was continued through generations and reciprocal crosses produced dissimilar sex ratios. A model of the genetic basis of sex ratio determination in *S. oryzae* was developed involving the interaction of a non-chromosomal, maternally inherited factor and a Y-linked factor. Evidence supporting Y chromosome involvement in the model and the similarity of certain features of the model to hybrid dysgenesis in *Drosophila* were discussed.

## INTRODUCTION

The sex ratio is an important fitness character of any sexually reproducing organism. Fisher (1930) first pointed out that the sex ratio would be determined by the relative amounts of effort devoted to sons and daughters by the parents. If sons and daughters require the same expenditure of time and activity by the parents before the offspring become independent, the optimum sex ratio would be 1:1 in an idealised random mating population. However, in some species, although sons and daughters require the same amount of energy expenditure by the parents, the primary sex ratio is biased in favour of females. Hamilton (1967) noted that such bias often occurs when males compete with siblings more than with unrelated males for the same group of females; Hamilton called the phenomenon local mate competition and showed that the optimum sex ratio is female biased when males compete for mates with relatives. Extreme examples of sex ratio bias occur in some groups of arrhenotokous arthropods and such examples are usually interpreted as resulting from local mate competition (Hamilton, 1967; Charnov, 1982).

The sex ratio of the rice weevil, *Sitophilus oryzae* L., has been reported as not deviating significantly

from 1:1 (e.g., Richards, 1947; Evans, 1977), but Shazali (1982) noted that the sex ratio in a population of *S. oryzae* from Hyderabad, India, was significantly biased in favour of females. The work described here analyses the sex ratio in the Indian strain (I) used by Shazali (1982) and in two geographical strains of *S. oryzae* collected in St. Augustine, Trinidad (T) and Tanzania, Africa (A). The aim of the experiments was to determine whether different geographical strains produced different sex ratios and what the genetic basis of such differences was.

## EXPERIMENTAL DETAILS

Experimental grain was disinfested at a temperature of 80°C for 4 hours (Coombs *et al.*, 1977). Grain moisture content was then allowed to equilibrate in a constant temperature and humidity (CTH) room at 30°C and 70 per cent r.h.. The grain was placed in large plant propagators to a depth of 2–3 cms and left in the CTH room for a period of two months prior to the experiments to equilibrate (Pixton and Warburton, 1968).

All female insects used were virgin. The collection of virgin *S. oryzae* is easy since females will not mate until 48 hours have elapsed from adult

emergence from grain (Singh and Soderstrom, 1963). Sexing was performed under  $\times 12$  magnification using sexually dimorphic rostrum characters (Halstead, 1963).

(i) *Experiment 1: Sex ratio variation on wheat*

The three strains were paired up in all nine possible strain  $\times$  strain combinations. A male and female were placed in a 50 mm high  $\times$  25 mm diameter glass tube with approximately 3 g of wheat and capped with a perforated plastic top. Each strain  $\times$  strain combination was replicated 12 times. The insects were allowed to oviposit for 20 days and then removed from the tubes. The tubes were inspected daily after the 28th day from the beginning of the experiment. Emerging offspring were removed from the tubes and sexed. For each strain  $\times$  strain combination, six of the 12 replicates were selected at random; three sib:sib pairings were set up for each chosen replicate and allowed to oviposit for 20 days in order to produce F2 offspring.

(ii) *Experiment 2: Sex ratio in relation to food*

The three strains were paired up in all nine possible strain  $\times$  strain combinations. A male and a female were placed in a 50 mm  $\times$  25 mm glass tube with 3 g of either wheat, sorghum or yellow split-pea. For each food, six replicates of each strain  $\times$  strain combination were set up. The insects were allowed to oviposit for one week before being transferred to another tube containing 3 g of fresh food and the procedure was continued until the insects died of old age. At no time were the insects transferred to a different foodtype. The reason why insects were transferred to fresh food weekly was that this experiment was part of a broader investigation of life history characters in relation to age (Holloway, 1984). In order to make the data comparable with experiment 1, offspring from the first 21 days of oviposition were combined. Newly emerging offspring were sexed but not retained.

## RESULTS

(i) *Sex ratio data*

More than half of the crosses showed significant differences between replicate heterogeneity for sex ratio and therefore the usual binomial distribution variance could not be used to calculate confidence intervals for the strain  $\times$  strain combination sex

ratios. Therefore, the following expression was used which does not assume that the data between replicates is binomially distributed (J. H. Roger, pers comm):

$$\text{Var. } \hat{R} = \frac{N}{\left(\sum_{i=1}^N y_i\right)^2} (Sxx - 2\hat{R}Sxy + \hat{R}^2Syy) \quad (1)$$

where

- $x_i$  = number of males in  $i$ th replicate,
- $y_i$  = total number in  $i$ th replicate,
- $N$  = number of successful replicates,
- $\hat{R}$  = sample sex ratio (proportion male)
- $Sxx$  = corrected sum of squares of  $x_i$ ,
- $Syy$  = corrected sum of squares of  $y_i$ ,
- $Sxy$  = corrected sum of cross products of  $x$  and  $y$ .

Using expression (1), the null hypothesis  $H_0: \hat{R} = 0.5$  was tested against each strain  $\times$  strain combination sex ratio. The sex ratios from the 1st and 2nd generations (experiment 1) are shown in table 1.

**Table 1** Sex ratios (proportion male) in the F1 and F2 (Experiment 1). The stars represent significance levels of deviations from  $H_0: \hat{R} = 0.5$  (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ )

a) Sex ratios in F1

Male parent	Female parent		
	T	I	A
T	0.471	0.463*	0.410*
I	0.343***	0.466	0.532
A	0.311***	0.412**	0.493

b) Sex ratios in F2

Male parent (P1)	Female parent (P1)		
	T	I	A
T	0.442*	0.483	0.446
I	0.327***	0.319***	0.480
A	0.381***	0.359***	0.470

Experiment 1 was carried out entirely on wheat. To exclude the possibility that foodtype was affecting sex ratio, data were extracted from experiment 2 which involved wheat, sorghum and yellow split-pea. Experiment 2 was not designed specifically to collect sex ratio data; therefore, in order to make results from the two experiments comparable, only data from the first three weeks of oviposition in experiment 2 were used in the analysis. Sex ratios

**Table 2** Sex ratios (proportion male) on wheat, sorghum and yellow split-pea (Experiment 2). The stars represent significance levels of deviations from  $H_0: R = 0.5$ . (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ )

a) Sex ratios from wheat

Male parent	Female parent		
	T	I	A
T	0.319*	0.466	0.494
I	0.273*	0.358**	0.508
A	0.248*	0.386*	0.467*

b) Sex ratios from sorghum

Male parent	Female parent		
	T	I	A
T	0.452	0.513	0.426**
I	0.264**	0.343	0.511
A	0.242**	0.388	0.484

c) Sex ratios from yellow split-pea

Male parent	Female parent		
	T	I†	A
T	0.388	—	0.555
I†	—	—	—
A	0.286**	—	0.527

† The Indian strain was unable to survive on yellow split-pea.

from wheat, sorghum and yellow split-pea are shown in table 2.

Tables 1 and 2 indicate that on many occasions the sex ratio deviated significantly from 0.5 and that sex ratio bias occurred independently of food-type. On the whole, sex ratios were biased in favour of females and of the 11 intra-strain sex ratios in tables 1 and 2, 10 were female biased. *S. oryzae* is a weak flier (Kiritani, 1965) and its powers of dispersal are limited. Therefore, like so many pests of stored products, populations are often isolated in grain stores for several generations (Smith and Lessells, 1985). Thus it is possible that populations of *S. oryzae* experience a level of local mate competition (Hamilton, 1967), such that a bias in favour of females is evolutionarily stable (Maynard Smith and Price, 1973); hence the slight sex ratio bias in all strains may be an adaptive trait. The proportion male of all intra-strain cross data pooled was 0.444.

Inspection of the sex ratios in tables 1 and 2 shows that certain categories of inter-strain cross tended to produce significantly biased ratios and

that reciprocal crosses differed. Thus the deviations from the mean sex ratio are non-random.

(ii) *The model*

Three proximate mechanisms were considered which might account for the observed sex ratio deviations:

- a. differential mortality
- b. sex linked drive
- c. maternally inherited factors.

If males are less likely to survive to adult than females (*i.e.*, differential mortality), then one would expect brood size and proportion male in that brood to be positively correlated, since the more males that die the greater the distortion in the sex ratio and the smaller the brood size. No such correlation was found ( $r = -0.41$   $p > 0.1$ ) (Holloway, 1984).

The sex linked drive model assumed that a male factor and a female factor interacted additively to affect the brood sex ratio. The strength of the male factor (X-linked drive) was fixed for each strain, but varied between strains. The magnitude of the effect of the antagonistic female factor varied between strains in relation to the size of the male effect in the same strain. The direction and degree of bias of the brood sex ratios of inter-strain crosses depended on the relative strengths of the male and female factors carried by the parents. The sex linked model may be interpreted as a test of whether the two sexes are locked in an "arms race", since the male and female factors are assumed to act antagonistically. In other words, the optimum brood sex ratio for the female is not necessarily the sex ratio preferred by the male. The sex linked drive model was examined (Holloway, 1984), but was unable to account for the observed sex ratio data.

In *S. oryzae* the male is the heterogametic sex (Smith, 1952). A model (MI model) was developed involving a maternally inherited, non-chromosomal factor and an independent male factor. The nature of the extrachromosomal agent is not known, but the maternal factor could act either directly on the fertilised eggs (selectively removing those containing a Y chromosome) or by altering the probabilities that X and Y-bearing sperm will fertilise eggs (Unterberger and Kirsch, 1932). The data indicate that factors in Trinidad females favoured X chromosomes the most and African females had least effect on the probability of fertilisation by Y-bearing sperm.

Intra-strain crosses did not produce the most biased sex ratios and therefore the magnitude of

effect of the male factor of a particular strain seems to vary in relation to the magnitude of effect of the female factor in the same strain. In a sense, the male effect conferred "resistance" to the Y chromosome against the effect of the female factor. For example, a strong Trinidad male factor would confer resistance to the Y chromosome against the female factors from all three strains and the sex ratio would reflect the true proportions of X and Y-bearing sperm. If the male factor is not strong enough to confer full resistance to the Y chromosome, Y-bearing sperm suffer a reduced probability of achieving fertilisation compared with a resistant Y-bearing sperm. A full prediction of resistance and susceptibility of Y-bearing sperm to the maternal effects in all nine strain × strain combinations is given in table 3.

**Table 3** Prediction of interactive effect of male and female factors on the transmission of Y chromosomes to the next generation

a) Relative magnitudes of male and female effects in the three strains.

Strain	Male effect	Female effect
T	Strong	Strong
I	Medium	Medium
A	Weak	Weak

b) Predicted response of Y carrying sperm or zygote to female effect encountered.

Male parent	Female parent		
	T	I	A
T	Resistant	Resistant	Resistant
I	Susceptible	Resistant	Resistant
A	Susceptible	Susceptible	Resistant

*(iii) The analysis*

The data used to test the MI model were those presented in tables 1, 2a and 2b. The data were fitted by maximum likelihood to the MI model using the GLIM computer package (table 4). The fit of the MI model was compared with a full model (M + F + [M \* F]) (table 5) which assumed that the probability that an offspring was male was different in all nine cells of the matrix (table 4a).

Three of the four F values in table 5 are not significant, that is the MI model did not give a significantly worse fit than the full model; thus the MI model provided a reasonable fit to the data. The data producing a significant F value were those from the F2 (table 1b). It can be seen from table

**Table 4** MI model fitted by GLIM computer package

$$\log \frac{\text{Probability male}}{\text{Probability female}} = \mu + mf_{ij} + e_{ij}$$

where

- $\mu$  = total mean of all data sets
- $m_i$  = size of male effect of *i*th strain
- $f_j$  = size of female effect of *j*th strain
- $e_{ij}$  = binomial error term

and

$$mf_{ij} = 0 \text{ for } i \neq j$$

a) Sex ratio matrix predicted by MI model

Male parent	Female parent		
	T	I	A
T	a	a	a
I	b	a	a
A	c	d	a

where a, b, c, d represent the expected sex ratios from the various strain × strain crosses

**Table 5** Comparison of MI model with full model (M + F + [M \* F])

a) Data from F1 (Table 1a)

Source	DF	Deviance	MS	F
MI - (M + F + [M * F])	5	11.59	2.32	1.45 0.1
M + F + [M * F]	96	153.38	1.60	< p < 0.25
MI	101	164.97		

b) Data from F2 (table 1b)

Source	DF	Deviance	MS	F
MI - (M + F + [M * F])	5	32.25	6.45	3.19 0.005
M + F + [M * F]	148	299.09	2.02	< p < 0.01
MI	153	331.34		

c) Data from wheat (table 2a)

Source	DF	Deviance	MS	F
MI - (M + F + [M * F])	5	18.41	3.68	1.78 0.1
M + F + [M * F]	42	86.76	2.07	< p < 0.25
MI	47	105.17		

d) Data from sorghum (table 2b)

Source	DF	Deviance	MS	F
MI - (M + F + [M * F])	5	17.56	3.51	1.84 0.1
M + F + [M * F]	41	78.21	1.91	< p < 0.25
MI	46	95.77		

1b that the sex ratio not fitting the MI model is that associated with I × I cross. To test whether the I × I sex ratio was the only one not fitting the MI model, the model was modified so that Indian

Y-bearing sperm were susceptible to the Indian maternal factor. In other words, the matrix in table 4a now reads with a letter e corresponding to the I × I combination. The data from the F2 (table 1b) were fitted to the modified maternal inheritance model (MIM model) (table 6).

**Table 6** Comparison of MIM model with full model (M + F + [M \* F]) for F2 data (table 1b)

Source	DF	Deviance	MS	F
MIM - (M + F + [M * F])	4	3.79	0.95	0.47
M + F + [M * F]	148	299.09	2.02	
MIM	152	302.88		

The MIM model did not give a significantly worse fit than the full model. Thus the I × I sex ratio in the F2 (table 1b) was the only sex ratio not fitting the MI model. No reason could be given why the I × I cross produced a significantly biased sex ratio in the F2.

DISCUSSION

The good fit of the maternal inheritance (MI) model to the data provides evidence of independent male and female factors which interact to affect the sex ratio in *S. oryzae*. The female effect seems to be inherited as a maternal, non-chromosomal factor, but the data do not allow further speculation as to the nature of the maternal factor.

Since an independent male factor is intrinsic to the MI model, the involvement of the Y chromosome is necessary. In many organisms, the Y-linked genes appear to be non-functional, possibly reflecting the operation of Müller's ratchet (Müller, 1964; Felsenstein, 1974). Theoretical models have been developed concerning the evolution of inert Y chromosomes (Nei, 1970; Charlesworth, 1978) and Hamilton (1967) suggested that Y chromosomes are genetically inert as a defence against the dangers of Y-linked drive.

The sex determination mechanism in *S. oryzae* is a neo-XY system (Smith, 1952). The neo-XY system in *S. oryzae* is thought to have developed from an Xy system, having a minute y chromosome, as is still found in the closely related species *S. granarius*. Neo-XY sex determination mechanisms usually develop from XO (or Xy) systems thought the centric fusion of an acrocentric X chromosome and an acrocentric autosome (White, 1973). If the fusion spreads through the popula-

tion, the unfused autosome is then limited to the male and is termed the neo-Y chromosome. Presumably, since crossing over between the neo-X and neo-Y chromosomes in *S. oryzae* is very restricted (Smith, 1952), Müller's ratchet is operating and slowly increasing the genetic load associated with the neo-Y chromosome (Haldane, 1957; Lewontin, 1974). In time, the neo-Y chromosome in *S. oryzae* may evolve to become dysfunctional (Maynard-Smith, 1978). However, much of the neo-Y chromosome in *S. oryzae* stains euchromatically (Smith, 1952), indicating the presence of functional genes which may include a chromosomally inherited male factor on the neo-Y chromosome affecting sex ratio.

Several aspects of the MI model resemble the phenomenon of hybrid dysgenesis in *Drosophila* (Kidwell *et al.*, 1977; Engels, 1979; Kidwell, 1979). Hybrid dysgenesis is a term used to describe a syndrome of aberrant traits observed when certain strains of *Drosophila* spp. are crossed. The underlying nature of hybrid dysgenesis is not well understood, but hypotheses have been formulated in terms of transmissible, but probably not Mendelian, factors (Kidwell and Kidwell, 1976; Picard, 1976). One factor is passed on by the male and another by the female; their interaction leads to dysgenesis (Kidwell and Kidwell, 1976). Since separate male and female factors are involved, reciprocal crosses do not always yield similar results, as found in the present study. One of the traits encompassed in the syndrome is the distortion of transmission ratios, which may manifest itself as a biased sex ratio.

There is evidence of maternally inherited factors affecting sex ratio in other organisms. Goldschmidt (1931) found that the sex ratio of the gypsy moth, *Lymantria dispar* L., was affected by a maternally inherited factor, and certain types of sex ratio bias in *Drosophila* spp. are known to be caused by agents (often spirochaetes) passed on through the öoplasm (Malagolowkin *et al.*, 1959; Poulson and Sakaguchi, 1961). It is known that symbiotic relationships exist between micro-organisms and insects with a restricted diet deficient in certain essential nutrients (Chapman, 1971, chapter 5), including Coleoptera surviving on dry cereal (*e.g.*, *S. oryzae*). In some beetles, micro-organisms are inherited by the offspring from the mother *via* the öoplasm (Brooks, 1963). It is possible that the sex ratio is affected in *S. oryzae* by micro-organisms transmitted maternally giving a maladaptive sex ratio bias and that the male effect (in the MI model) has evolved to correct the maternally determined sex ratio bias.

*Acknowledgements* I would like to thank Dr R. H. Smith for his constructive criticisms and careful reading of the manuscript, Dr J. H. Roger for his advice concerning the statistical analysis and Professor K. Simkiss for providing research facilities. The Indian and African strains of *S. oryzae* were provided by Dr P. Dobie of the Tropical Development Research Institute (Slough) and the Trinidad strain was collected by Dr R. H. Smith on a visit to Trinidad funded by the British Council. The work was part of a project funded by a NERC studentship.

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