

# The importance of cytoplasmic male killing elements in natural populations of the two spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae)

GREGORY D. D. HURST\*, MICHAEL E. N. MAJERUS AND LINDA E. WALKER

*Department of Genetics, Downing Street, Cambridge, CB2 3EH*

*Received 27 November 1991, accepted for publication 11 March 1992*

---

*Adalia bipunctata*, the two spot ladybird, is polymorphic for a cytoplasmically inherited element which produces female-biased sex ratios by effecting the death of male offspring during embryogenesis. The levels of this element were assessed in Cambridge populations. Six out of 82 females tested showed both a female-biased sex ratio and low egg hatch rates consistent with the presence of this element. Population sex ratios were assessed by collecting pupae from the Cambridge area. The population sex ratio was found to be 1.15:1, female biased, significantly different from 1:1, and consistent with predictions based on a model incorporating the observed level of the sex ratio element in the population.

ADDITIONAL KEY WORDS:—Natural occurrence – population sex ratio.

## CONTENTS

Introduction . . . . .	195
Methods . . . . .	197
Assessment of the level of the sex ratio distorting element in the Cambridge population . . . . .	197
Assessment of population sex ratio . . . . .	197
Results . . . . .	197
The level of the sex ratio distorting element in the Cambridge population . . . . .	197
Population sex ratio in the Cambridge population . . . . .	198
Prediction of the population sex ratio from the level of the sex ratio distorting element in the Cambridge population . . . . .	198
Discussion . . . . .	200
Acknowledgements . . . . .	201
References . . . . .	201

## INTRODUCTION

Genes which affect the sex ratio that are inherited either on sex chromosomes or through the cytoplasm face different selection pressures with respect to the sex ratio from those on autosomes (Cosmides & Tooby, 1981). Consider a novel mutation of a gene on the X chromosome which prevents the formation of

\*Author to whom correspondence should be addressed.

gametes bearing a Y chromosome in the heterogametic sex. If the fertility of such individuals with half their gametes defective is greater than one half that of individuals bearing the wild type chromosome, then the mutant gene will increase in the population in the short term. The individuals bearing such genes will produce female-biased sex ratios, and the population sex ratio will become female biased. This is the phenomenon of meiotic drive. In the longer term genes on the Y chromosome or autosomes which suppress such activity will be favoured, as a significant fitness loss accrues to individuals bearing so many defective gametes. A similar conflict of interest as to the sex of progeny exists between genes inherited through the cytoplasm and nuclear genes. Cytoplasmic genes only gain transmission through females (Birky, 1978). They are at an evolutionary dead end in males (Lewis, 1941; Hamilton, 1967). As a consequence, any cytoplasmic gene with the ability to bias sex allocation towards daughters will spread. One way in which this is achieved is to liberate, for use by daughters, resources which are either held in, or potentially used by, male offspring by effecting the death of male offspring during embryogenesis. This is the phenomenon of early male killing (Hurst, 1991). Recent work on the two spot ladybird, *Adalia bipunctata*, has shown cytoplasmic genes, probably bacterial in nature, to be responsible for the production of female-biased sex ratios in this species (Hurst, Majerus & Walker, 1992). The trait has two symptoms: a low hatch rate of eggs due to the death of male zygotes during embryogenesis coupled with a female-biased sex ratio as measured on emergence as adults (Hurst *et al.*, 1992). A high proportion of the daughters from such broods will show similar symptoms, the trait being maternally transmitted with some inefficiency in transmission between generations. The maintenance of this element in the population in the face of incomplete transmission may be explained by certain features of the ecology of the *A. bipunctata*. Firstly, an increase in the hatch rate of female offspring may be produced by lowering the rate of cannibalism on late hatching females by early hatching adults due firstly to the decrease in the number of early hatching individuals (many fewer early hatching males) and, secondly, an increase in the number of eggs that can be cannibalized by the early hatching larvae (eggs containing dead brothers are present). Secondly, the hatching females cannibalize the unhatched eggs bearing male zygotes. This provides an increase in the longevity (Banks, 1956) and speed (Walker, 1962) of the first instar larvae. This, when considered in the light of the high mortality of first instar larvae through starvation, caused by a combination of not finding and inefficiency in capturing aphids (Wratten, 1973), may provide a significant advantage to the surviving individuals from the death of some male offspring. Thirdly, competition for resources between siblings after dispersal from the eggs may be lowered through a decrease in the local density of larvae associated with the death of brothers. Fourthly, the cannibalism of siblings, a behaviour observed predominantly when resources are low, may be reduced for the same reason: male killing may lower the local density of larvae competing for resources. All of these factors may act to increase the probability of survival of individuals from clutches in which males have died. Most of the benefitting individuals will be females which bear the sex ratio distorting element, and thus account for continued existence of the element in the population in spite of some inefficiency in transmission.

We here assess the level of this element in the Cambridge population of *A. bipunctata*. Past work in Russian populations has shown that large deviations

in population sex ratio occur in regions where sex ratio distorting elements exist (Lus, 1947). However, Lus failed to record the level of the trait in these populations. We therefore measured the population sex ratio by sampling. We use a simple equation to estimate the expected population sex ratio from the level of the element in the population, the sex ratio produced by such individuals and the survival advantage gained by such individuals over those from broods where no male biased mortality occurs. This latter value is estimated from an adaptation of Hurst's (1991) model connecting the level of the element with the vertical transmission efficiency of the element and the advantage gained by the individuals of broods where males are killed. The consistency of the expected and observed population sex ratios suggests that the population sex ratio is affected by the sex ratio distorting elements.

#### METHODS

##### *Assessment of the level of the sex ratio distorting element in the Cambridge population*

Female *A. bipunctata*, either in copulating pairs, or found singly, were collected during May and early June, 1991 from five sites all within 1.5 km of the centre of Cambridge. If these females were not collected in cop, then a male was added. The pairs of adults were fed on nettle aphids and allowed to produce eggs over a period of time. The hatch rates of egg clutches were recorded, and the sex of progeny determined on emergence as adults using the sexing criteria of Randall, Majerus & Forge (1992). The sexing of ladybirds by this method, as tested by gonadal dissection, is over 98% accurate. If females produced both low egg hatch rates and a strongly female-biased sex ratio, then these were considered to bear the male killing element. An attempt was made to demonstrate the heritability of the trait through further crosses in each case where both the above criteria were met.

##### *Assessment of population sex ratio*

Ladybird pupae were collected throughout the month of July from lime trees (*Tilia europaea*) and nettle beds (*Urtica* spp.) within the Cambridge area. These pupae were allowed to eclose in the lab. The resulting ladybirds were scored as to species and sex. Sex was determined by dissection of the gonads.

#### RESULTS

##### *The level of the sex ratio distorting element in the Cambridge population*

Six of the 82 females tested showed both a low hatch rate and female-biased sex ratio and were therefore considered to carry the sex ratio trait (Fig. 1). Each of these broods proved to show a sex ratio significantly different from 1:1, and the heritability of the condition was successfully tested in five of these (dark squares in figure) (Table 1). Mortality of the progeny prevented the testing of heritability in the sixth line. The reason for mortality in this line is thought to be due to a lack of aphids and not inherent low fitness. The remaining 76 crosses produced, in total, 1254 male progeny and 1323 female progeny. This is not significantly different from 1:1 ( $\chi^2_{v=1} = 2.41$ , NS), suggesting that the boundary conditions for accepting crosses as showing the sex ratio trait are not unrealistic.

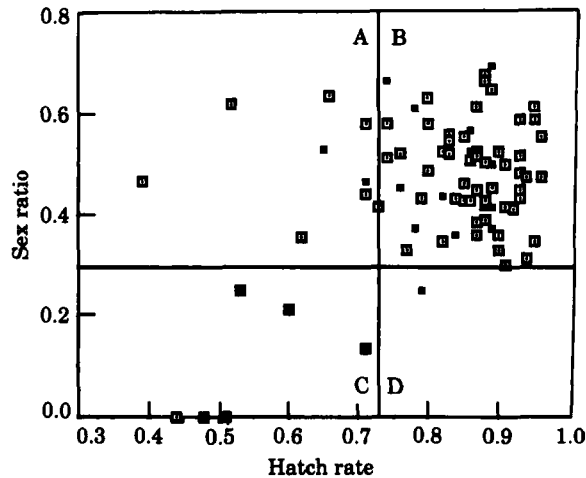


Figure 1. The sex ratio and egg hatch rates of 82 crosses of individuals collected from the Cambridge area. Dots represent crosses where less than 20 adult progeny were scored for sex. Squares represent crosses where greater than 20 progeny were scored for sex. Filled squares represent those crosses from which female progeny were shown to produce a biased sex ratio in their progeny. Lines represent the cut off point for determination as being of low sex ratio and low hatch rate. Those in region A have low hatch rate, but no evidence of a female-biased sex ratio. Those in region B have both high hatch rates and sex ratios close to 1 : 1. Those in region C have both low hatch rates and female-biased sex ratios. Those in region D have female-biased sex ratios but no evidence of low hatch rates. Only crosses in region C were diagnosed as being bearers of the male killing element.

#### *Population sex ratio in the Cambridge population*

The population sex ratio measured during July 1991 was 847 females: 737 males; 46.5% males. This is significantly different from a 1 : 1 ratio ( $\chi^2_{v=1} = 7.64$ ;  $P < 0.01$ ).

#### PREDICTION OF THE POPULATION SEX RATIO FROM THE LEVEL OF THE SEX RATIO DISTORTING ELEMENT IN THE CAMBRIDGE POPULATION

The sex ratio produced by a generation of adults depends on three factors:

- (a) the proportion of females,  $x$ , bearing the sex ratio distorting element.
- (b) the proportion of sons of females bearing the element which survive,  $s$ , as determined by the difference between the number of daughters and sons derived from such crosses.
- (c) the relative survival abilities,  $b$ , of the progeny of such individuals when compared with females which do not bear the element.

Such that,

$$\begin{aligned} \text{Expected sex ratio} &= \frac{\text{Number of males produced}}{\text{total progeny}} \\ &= \frac{(1-x) + xsb}{2(1-x) + xb(1+s)}. \end{aligned} \quad (1)$$

Estimation of variables:

$x$  is estimated from above data ( $x = 6/82 = 0.073$ );

$s$  may be estimated from the number of males produced by each of the sex ratio females. If we assume that the number of sons produced at fertilization was

TABLE 1. The sex ratios and hatch rates from crosses classified as being of the sex ratio type, and the results of crosses involving the daughters produced by such crosses

Cross	Parents				Cross	Progeny			
	Males	Females	Sex ratio	Egg hatch rate		Males	Females	Sex ratio	Egg hatch rate
B:	10	63	0.137	0.72	B : a	2	29	0.065	0.39
					B : b	3	70	0.041	0.44
					B : c	0	11	0	0.37
AP:	11	33	0.25	0.53	AP : a	1	19	0.050	0.36
					AP : c	3	26	0.103	0.53
					AP : d	12	37	0.245	0.64
FC25:	0	42	0	0.48	B25 : a	3	8	0.273	0.70
					B25 : b	0	13	0	0.38
					B25 : d	0	17	0	0.44
					B25 : e	8	44	0.182	0.55
FC26:	0	39	0	0.51	B26 : a	2	31	0.061	0.55
FC39:	8	30	0.210	0.60	B39 : b	1	13	0.071	0.55
					B39 : d	3	4	0.428	0.78
					B39 : e	3	4	0.428	0.81
FC44:	0	47	0	0.44	No progeny successfully crossed				

equal to the number of daughters produced, then the survival rate of sons from infected females is a simple proportion of male progeny/female progeny. Taking each female as an independent point,  $s = 0.13$  (mean of six crosses);

$b$  may be estimated from the level of the element in the population and measurements of transmission efficiency of the element between generations, if the above level is assumed to be at an equilibrium. If we consider the element at level  $p_1$  in the population at generation 1, then at generation 2 the level of the element,  $p_2$  will be:

$$p_2 = \frac{abp_1}{1 - p_1 + bp_1}$$

where  $a$  = transmission efficiency of the element, mother to adult daughter,  $b$  = advantage accruing to a member of a clutch where males die over members of a clutch where this does not occur.

The change between generations,  $p_1 - p_2$ , is:

$$\Delta p = \frac{p(ab - 1) + p^2(1 - b)}{1 - p + bp}$$

Now, at equilibrium,  $\Delta p = 0$ , i.e.

$$p = 0 \text{ (trivial)}$$

$$\text{or } p = \frac{1 - ab}{1 - b};$$

so, for real equilibria,  $b = \frac{p - 1}{p - a}$  (II)

The mean transmission efficiency,  $a$ , of the element from mother to adult daughter can be estimated from past breeding studies used in this and past studies (Hurst, Majerus & Walker, 1992). Over the last year, a total of 77

daughters from 23 crosses which showed the sex ratio trait have been crossed. Of these crosses, 65 showed the sex ratio trait. The other 12 showed no evidence of the sex ratio trait, and in no case was any evidence of the sex ratio trait found in future generations of these lines. The transmission efficiency, calculated as the mean transmission efficiency of the 23 crosses (which certainly appear to vary in transmission efficiency), is  $a = 0.87$ .

If we take the level of the element measured in this work to be an estimate of  $p$ , using our above estimate of transmission efficiency,  $a$ , this yields, from II,  $b = 1.163$ .

Now, if we assume that the advantage gained by offspring through being in a clutch where males eggs fail to hatch is purely in terms of increased survival of early larval instars, then, from I, the sex ratio at the pupal stage will be:

$$\text{expected sex ratio} = 0.481.$$

This figure is not significantly different from the sex ratio observed in the population ( $\chi^2_{v=1} = 1.56$ ;  $P > 0.1$ ; NS).

#### DISCUSSION

The above work demonstrates firstly that the sex ratio distorting element exists at significant levels in British populations of *A. bipunctata*. Furthermore, the consistency of the expected with actual population sex ratios, and the deviation of both from 0.5, suggests that these may have real effects in distorting population sex ratios. The above calculation does, of course, only provide an estimate of the population sex ratio expected due to the male killing element when certain assumptions are true. Principally, the estimate of the survival advantage accruing to individuals in clutches where male death occurs requires that the level observed is an equilibrium one. Past studies have shown that such elements can stay at equilibrium in populations over long periods of time (Clarke, Johnston & Johnston, 1983). It should also be noted that the level of the element used in this calculation is, in fact, an estimate, and will have an error on it. This said, the calculated value of  $b$  is of the correct order when we consider the nature of the advantages that accrue to members of clutches where some male zygotes perish. Analysis of wild samples of recently hatched clutches suggest that between 5 and 10% of all individuals fail to hatch due to cannibalism by early hatching larvae (Hurst, G., personal observations). This level would be significantly reduced due to the dilution effect. Secondly, the importance of unsuccessful foraging by first instar larvae in larval mortality is profound (Wratten, 1973). Eating an egg both decreases the amount of resources required for ecdysis to the second instar and the time to find the first aphid after hatching. The estimated survival advantage,  $b$ , is not therefore, an unreasonable one.

The main error in the estimation of population sex ratio will derive from an error in our observed level of the element in the population. This error derives from three sources. Firstly, a sampling error will exist, an inevitable limit imposed by the restrictions in breeding so many ladybirds. Secondly, an error will exist due to the spontaneous reversion of adults from sex ratio distorted individuals to normal individuals over time: the retrogressive sex ratio trait (Hurst *et al.*, 1992). The sex ratio we measured would have been produced through clutches laid in early June. The level of the element was estimated from

individuals collected in May and early June. A low level of reversion would be expected in this time, and hence an slight underestimate of the level of the element in May will exist. This will, however, only produce a small error, spontaneous reversion being a rare event, seen only once in sixty-five crosses which showed the sex ratio trait in the laboratory. Thirdly, considerable rigour was applied in classification of individuals as to the sex ratio trait, only individuals having both low egg hatch rates and producing a low sex ratio being included as sex ratio individuals. In some cases, insufficient data on sex ratios may have prevented the correct identification of the trait in these individuals. Our estimate is probably on the conservative side, and the level of the sex ratio element closer to 10% than 5%. If the element is at a level of 5% and 10% in the population, this gives us expected population sex ratios of 0.487 and 0.474 respectively. The conservatism of our estimate of the level of the element in the population renders the correlation between the level of the element and the population sex ratio more firm.

In conclusion, we believe the observed population sex ratio bias to be primarily attributable to a significant level of the cytoplasmic sex ratio distorting element in the Cambridge population. In order to corroborate this thesis, it will be necessary to develop a quick, accurate assay for the presence of the element using cytological or molecular biological techniques. With such an assay, large sample sizes may be used to obtain more accurate estimates of the level of the trait in the population. A more accurate estimate of transmission efficiency may also be obtained. In addition, the sampling of different populations over geographical space may allow further testing of the importance of sex ratio distorting elements in producing biases in population sex ratios.

#### ACKNOWLEDGEMENTS

The authors wish to thank Dr Laurence Hurst for discussion of this work. We are very grateful to Mr Dennis Parkinson, Mr Alan Feast and Mr Roger Ison for their invaluable assistance. We thank the Bursar and Fellows of Churchill College for permission to work in the grounds of Churchill College. The work was carried out in a laboratory funded by the Wolfson Foundation. Greg Hurst is the recipient of a grant from the S.E.R.C.

#### REFERENCES

- Banks CJ.** 1956. Observations on the behaviour and early mortality of coccinellid larvae before dispersal from egg shells. *Proceedings of the Royal Entomological Society of London A*, **31**: 56–61.
- Birky CW Jr,** 1978. Transmission genetics of mitochondria and chloroplasts. *Annual Review of Genetics* **12**: 472–512.
- Clarke C, Johnston G, Johnston B.** 1983. All female broods in *Hypolimnas bolina* (L.) A resurvey of West Fiji after 60 years. *Biological Journal of the Linnean Society* **19**: 221–235.
- Cosmides LM, Tooby J.** 1981. Cytoplasmic inheritance and intragenomic conflict. *Journal of Theoretical Biology* **89**: 83–129.
- Hamilton WD.** 1967. Extraordinary sex ratios. *Science* **156**: 477–488.
- Hurst LD.** 1991. The incidences and evolution of cytoplasmic male killers. *Proceedings of the Royal Society of London B* **244**: 91–99.
- Hurst GDD, Majerus MEN, Walker LE.** 1992. Cytoplasmic male killing elements in *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). *Heredity* **69**: 84–91.
- Lewis D.** 1941. Male sterility in natural populations of hermaphroditic plants: the equilibrium between females and hermaphrodites to be expected with different types of inheritance. *New Phytologist* **40**: 56–63.

- Lus YY. 1947.** Some rules of reproduction in populations of *Adalia bipunctata*: non-male strains in populations. *Doklady Akademii Nauk SSSR* **57**: 951–954.
- Randall K, Majerus MEN, Forge H. 1992.** Characteristics for sex determination in British ladybirds, Coleoptera: Coccinellidae. *Entomologist* **111**: 109–122.
- Walker MF. 1962.** *The comparative efficiency of some aphid predators*. Unpublished Thesis, University of Oxford.
- Wratten SD. 1973.** The effectiveness of the coccinellid beetle *Adalia bipunctata* (L.) as a predator of the lime aphid, *Eucalliptreus tiliae* (L.). *Journal of Animal Ecology* **42**: 785–802.