SHORT COMMUNICATION

Sex ratio in ladybirds (Coccinellidae)

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Introduction

The sex ratio is an important fitness character of any sexually reproducing organism. In a random mating, diploid population, Fisher (1930) argued that the sex ratio will evolve towards unity providing that the two sexes require the same investment of energy. If one sex requires more effort on the part of the parents to rear to maturity, then the sex ratio should be biased towards the cheaper sex. However, in some cases the sex ratio is clearly biased even though the two sexes are equally 'expensive'. During earlier studies on the 7-spot ladybird, *Coccinella septempunctata* (Holloway *et al.*, 1991) and the 2-spot ladybird, *Adalia bipunctata* (de Jong *et al.*, 1991) we noticed a biased sex ratio. In both studies the ladybirds were collected from the field. Ladybirds overwinter as adults (Hodek, 1973) and, whilst it is possible that local mate competition (Hamilton, 1967) may influence sex ratios, it could also be that these biases occur as a result of differential winter mortality. This seems particularly feasible considering Brakefield's (1985) finding that lighter ladybirds have a lower chance of surviving the winter months than heavier individuals. Male ladybirds are indeed lighter than females. Another possibility has been proposed by Hurst *et al.* (1992) who found that cytoplasmically inherited agents, possibly bacteria, are responsible for male biased sex ratios in populations of 2-spot ladybirds derived from England. Hurst *et al.* found that the factor was passed on through females and resulted in up to 100% of the male eggs in some broods failing to hatch.

A large experiment was carried out in the laboratory to study the quantitative genetics of life history and the defence system in the 2-spot ladybird (Holloway *et al.*, 1993). However, since the design of the experiment required families to be reared it also yielded information about the sex ratio and perhaps also clues as to which

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factors are operating in these species of ladybirds that result in biased sex ratios.

Methods and Results

The precise design of the experiment is described elsewhere (Holloway *et al.*, 1993) and is only summarized here for convenience. Over 300 2-spot ladybirds (typica morph) were collected from the field (Breda, the Netherlands) in September 1990. These ladybirds were placed unsexed in groups of fifteen in 9 cm diameter petri dishes. They were fed daily with live pea aphids (*Acyrthosiphon pisum* (Harris)), which were also reared in the laboratory on bean plants. The animals were kept throughout the experiment under constant conditions ($20 \pm 1°C$ and 18:6 h light:dark). The petri dishes were checked daily for eggs and if batches were found the adults were transferred to a clean petri dish. After 20 days the number of egg batches being produced was low and these adults were discarded.

The newly hatched larvae were kept in groups of ten and fed aphids daily. As they moulted to second instar the groups were split into batches of five. This was done to minimize the risk of cannibalism which frequently occurs as large larvae will willingly eat smaller siblings. Third instars were split into groups of three per petri dish and fourth instars were placed in dishes as pairs. When one of the fourth instar larvae pupated the remaining larva was transferred to a clean petri dish. Following this procedure 313 virgin adult ladybirds were reared.

Before being paired up the ladybirds were sexed using abdominal characters (Majerus & Kearns, 1989; de Jong *et al.*, 1991). Sexing accuracy was checked by putting thirty-nine pairs together. Thirty-eight of the pairs had copulated within 5 min, indicating a degree of accuracy between 97% and 100%. The sexes were paired up at random and 134 pairs were set up. These F_1 adults were fed daily with aphids and checked for eggs. After about 3 weeks few egg batches were being produced and these adults were discarded. The F_2 insects were reared through

to adult as described above. The F_2 adults were finally frozen and sexed.

The sex ratio of the F_1 insects was significantly biased in favour of females (134:179, $\chi^2 = 6.47$, $P < 0.05$). Of the 134 pairs that were set up, ninety produced offspring surviving through to adults. 1302 F_2 insects were produced. There was among family heterogeneity in the sex ratio ($\chi^2_{89} = 125.4$, $P < 0.01$), so the usual binomial distribution variance could not be used to calculate confidence intervals for the overall F_2 sex ratio. Therefore, the procedure used by Holloway (1985) was adopted where the following expression was used to attach errors to the sex ratio:

$$\text{Var. } R = \frac{N}{\sum\limits_{i=1}^{N} y_i} (Sxx - 2RSxy + R^2Syy)$$

where x_i = number of males in the ith replicate, y_i = total number in the ith replicate, N = number of successful replicates, R = sample sex ratio (proportion male), Sxx = corrected sum of squares of x_i, Syy = corrected sum of squares of y_i, and Sxy = corrected sum of cross products of x and y.

This expression allows standard errors to be attached to the sex ratio without the assumption that the data are binomially distributed between families. The sex ratio in the F_2 was 0.409 ± 0.032 (standard error) which was significantly different from 0.5 ($t = 2.84$, $P < 0.01$). Table 1 summarizes the sex ratios found in the present study and those of Holloway *et al.* (1991) for the 7-spot ladybird and de Jong *et al.* (1991) for the 2-spot ladybird. It is clear from Table 1 that the sex ratios do not differ significantly from each other.

Table 1. Sex ratios (proportion male) of ladybird populations from the field (F) and bred in the laboratory (L).

F/L	Species	Sex ratio ($\pm$SE)	Source
F	7-spot	0.387 ± 0.046	Holloway *et al.*, 1991
F	2-spot	0.338 ± 0.042	de Jong *et al.*, 1991
L	2-spot (F_1)	0.428 ± 0.028	Present study
L	2-spot (F_2)	0.409 ± 0.032	Present study

Fig. 1 shows the distribution of sex ratios among the families. Only families containing ten or more offspring are included. The mode is around 0.4 or just below. Two families showed a very high sex ratio (0.82 and 1.0), but these were families of only seventeen and eleven individuals, respectively.

Discussion

Lus (1947) and Shull (1948) both reported the occurrence of ladybirds that produce all female broods and more recently Hurst *et al.* (1992) noted a similar phenomenon in populations of ladybirds derived from England. Hurst
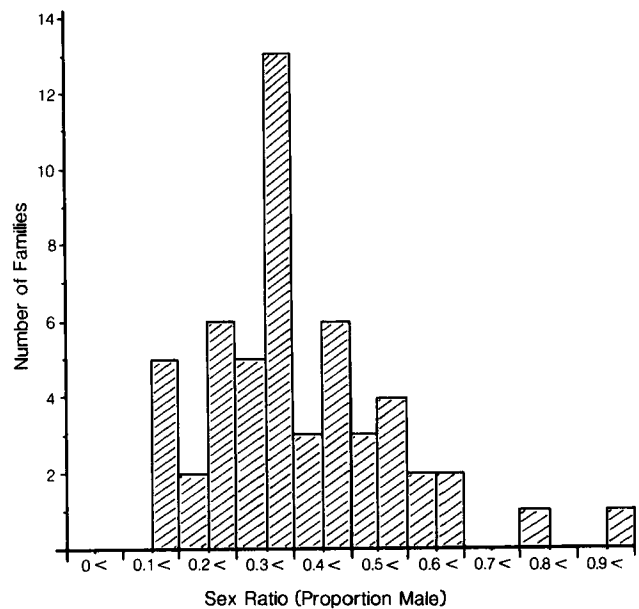

Fig. 1. Distribution of sex ratios (proportion male) of families containing at least ten individuals (fifty-three families).

et al.'s work clearly shows that cytoplasmically inherited factors are responsible for the skewed sex ratio in 2-spot ladybirds by selectively killing male eggs. They found from a sample of twenty-six pairs from the wild four families that produced a highly biased sex ratio, the remaining twenty-two families had sex ratios around 0.5. Similar male killing bacteria may be operating in Dutch populations of *A. bipunctata* but, if so, it must be more prevalent than in English populations with far more broods being affected. Hurst *et al.* (1992) found broods containing between 0% and 35% males. None of the broods shown in Fig. 1 contained less than 15% males, so although the prevalence could be higher the virulence may be lower. Geographic variation in the sex ratio in *A.bipunctata* has been reported by Lus (1947) which was apparently caused by variation in the male killing element.

The results indicate that differential overwintering mortality is an unlikely explanation for the observed sex ratios as they were found in populations from the field (that had overwintered) and also from the laboratory. A further possibility concerns the finding of Holloway *et al.* (1993) that female larvae have a faster growth rate than males, although a similar development period. Smaller larvae are prone to cannibalism by larger larvae even when aphids are abundant and at a given time males will be on average smaller than female larvae. A sex ratio bias could occur, therefore, through a higher incidence of cannibalism of male larvae and such a mortality factor could indeed produce the results that are reported here, both in the field and in the laboratory. It is possible that more than one factor is operating in unison to produce the observed sex ratio bias, but further work is needed to clarify the problem.

Acknowledgments

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