Heredity **81** (1998) 683–691

Received 28 April 1998, accepted 19 June 1998

Extreme variation in the prevalence of inherited male-killing microorganisms between three populations of *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Females from three populations of *Harmonia axyridis* (Pallas) were assayed for two phenotypical indicators of the presence of male-killing endosymbionts: low egg hatch-rates and strongly female-biased progenic sex ratios. Samples from Sapporo City, Japan, and the Altai Mountains, Mongolia, but not from Novosibirsk, Russia, were found to contain some females displaying both of these traits. Furthermore, there was a profound difference in the prevalence of infection between the Japanese and Mongolian populations. The proportion of females infected from the Japanese sample was approximately 0.49, whereas that from Altai was only 0.02. The trait was inherited by more than 99% of the progeny of infected females. The trait was inherited maternally with the same high efficiency over five generations. Treatment with antibiotics produced a small increase in the production of males from the male-killing lines, suggesting that the male-killer is bacterial in nature. Causes of variation in prevalence are discussed, and the consequences of high levels of infection with an efficiently transmitted male-killing microorganism on host population demography are considered.

Keywords: Coccinellidae, cytoplasmic microorganisms, *Harmonia axyridis*, male-killing, maternal inheritance, sex ratio.

Introduction

Maternally inherited female-biased sex ratio traits have been found in a number of species of coccinellid (*Adalia bipunctata* — Lus, 1947; Hurst *et al*., 1992; *Harmonia axyridis* — Matsuka *et al*., 1975; Hu, 1979; Gotoh & Niijima, 1986; *Menochilius sexmaculatus* — Niijima & Nakajima, 1981; *Hippodamia quinquesignata* — Shull, 1948; *Coleomegilla maculata* — Hurst *et al*., 1996; *Adonia variegata* — Hurst *et al*., 1998a), and are suspected in several others (Majerus, 1994; Majerus & Hurst, 1997).

Identification of the agents responsible for male-killing in several coccinellids has revealed male-killing to be caused by bacteria (in *A. bipunctata*, Rickettsia — Werren *et al*., 1994; Spiroplasma — Hurst *et al*., 1998b; Wolbachia — G. D. D. Hurst *et al*., unpubl. data; in *Coleomegilla maculata* and *Adonia variegata*, Blattabacterium — Hurst *et al*., 1997a, 1998a).

Majerus & Hurst (1997) have argued that coccinellids are a model system for the study of the evolution of male-killing endosymbionts and their consequences for their hosts. This argument is based on a knowledge of the advantages of male-killing in coccinellids: resource reallocation from male-killed embryos to sibling females (Hurst *et al*., 1992) and reduction in sibling cannibalism of infected female embryos (Majerus, 1994), and on the evolution of male-killing in several widely divergent bacterial symbionts of coccinellids.

Harmonia axyridis exhibits all the features that make it liable to invasion by male-killers (Majerus & Hurst, 1997). Eggs are laid in batches. Neonate larvae consume unhatched eggs (Kawai, 1978; Ng, 1986; Osawa, 1989). This consumption has been shown to confer a significant survival advantage on

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*Correspondence. E-mail: tmom@mole.bio.cam.ac.uk neonates, particularly at low aphid density (Osawa, 1992). Furthermore, some female specimens of *H. axyridis* are known to produce highly female-biased progenic sex ratios (Matsuka *et al.*, 1975), the female-bias trait is maternally inherited, although with varying levels of transmission efficiency (Matsuka *et al.*, 1975), and the trait is antibiotic sensitive (Gotoh & Niijima, 1986). It is probable that this coccinellid is infected with one or more male-killing bacteria (for review, see Hurst & Majerus, 1993).

This paper describes studies on the prevalence of male-killing in three Asian populations of *H. axyridis*. Substantial variation in the prevalence of male-killers in these populations is reported. The effect of antibiotics on the trait, using tetracycline and chloramphenicol, is reassessed, and the possible effects of male-killing on the demography of these populations are discussed.

Materials and methods

Initial detection of the male-killer trait

Collections of *H. axyridis* were made at overwintering sites in a suburb of Sapporo City, Japan (Sapp stock), in spring 1994, in the Altai Mountains, Mongolia, in autumn 1994 (Altai stock) and at Novosibirsk, Russia, in autumn 1994 (Novo stock). These stocks were transferred to Cambridge. The sample sex ratio was obtained. Each individual was anaesthetized with CO_2, and its sex was ascertained from the presence or absence of a male-specific pronounced notch in the ventral portion of the last segment of the abdomen. The ladybirds were kept as mixed-sex, single-population stocks in 9 cm Petri dishes and fed daily on pea aphids (*Acyrthosiphon pisum*). Mating pairs were removed to fresh Petri dishes when observed. Because the original Sapp and Altai stocks were female-biased, some males were returned to the stock dishes after mating. In this way, 94, 46 and 11 isofemale lines were created from the Sapp, Altai and Novo stocks, respectively. When clutches of eggs had been laid, the ladybirds were removed to fresh Petri dishes. The eggs were counted and, once they had hatched, the hatch-rates were recorded. Unhatched eggs were scored as either yellow, indicating no obvious sign of embryonic development, or grey, indicating that the embryos had developed but failed to emerge from the eggs.

All larvae from the Sapp and Novo crosses were reared to adulthood when aphid availability allowed. All larvae from Altai females in which the egg hatch-rate was lower than 75% were retained, fed on pea aphids daily and sexed on emergence as adults. However, because of a shortage of pea aphids, larvae from those females that produced a high egg hatch-rate ($>75\%$) were discarded except for one or two clutches that were reared to ensure that some males resulted.

The inheritance of the male-killing trait

To confirm our scoring of the male-killing trait, its inheritance was tested. Female progeny from 15 Sapp sex ratio lines (producing 26 crosses), 18 Sapp normal lines (22 crosses), the one Altai sex ratio line (three crosses) and eight normal Altai lines (10 crosses) were outcrossed to males from their own populations. These crosses were treated in the same way as the original crosses. As further crosses were produced from the progeny of these crosses and subsequent generations (up to F_5), egg hatch-rates and progenic sex ratios were recorded as a matter of course.

Sensitivity to antibiotics

Previous studies on heritable female-biased sex ratios in coccinellids have revealed the trait to be curable by treatment with antibiotics (Gotoh & Niijima, 1986; Hurst *et al.*, 1992) and associated with bacterial endosymbionts. To establish whether this was true of the male-killing trait in both Sapp and Altai samples of *H. axyridis*, infected and uninfected stocks from both populations were treated with tetracycline. In addition, infected and uninfected lines from Sapporo were tested with a second antibiotic, chloramphenicol.

Females from both infected and uninfected Sapp and Altai lines were mated, fed on pea aphids and allowed to oviposit. The egg hatch-rates and progenic sex ratios from these females were recorded. From each group (infected Sapp, uninfected Sapp, infected Altai, uninfected Altai), some females were then fed, for approximately 1 h per day, on a diet of pure golden syrup, over a period of 4 weeks. The rest were fed for the same period daily on a diet of golden syrup containing 10% w/v tetracycline. At other times, the females were fed on pea aphids. Four Sapp SR and four Sapp N lines fed for 4 weeks with golden syrup were subsequently fed on chloramphenicol in golden syrup for 4 weeks. The hatch-rates, the number of yellow eggs and grey eggs and the sex ratios from egg clutches laid subsequent to these treatments were recorded.

Results

Initial detection of the male-killer trait

The sex ratios (given throughout as the proportion of males) in the three samples were: Sapp 0.394 ($n = 287$); Altai 0.442 ($n = 197$); Novo 0.533 ($n = 30$). The female bias in the Sapporo population is highly significant ($\chi^2_1 = 12.97$, $P < 0.001$), but the sex ratios of the other samples are not significantly different from 1:1.

The egg hatch-rates and sex ratios produced by each of the Sapp and Altai isofemale lines are given in Tables 1 and 2, respectively. Summary data for the Novo lines are given in Table 3.

Sapp lines were divided into six classes on the basis of egg hatch-rates, sex ratio and the number of progeny produced as follows. Normal (N): >5 progeny in family and sex ratio consistent with 1:1; sex ratio (SR): >5 progeny, egg hatch-rate <0.7 and only female progeny; incomplete sex ratio (iSR): >5 progeny, egg hatch-rate <0.7, significantly female-biased sex ratio but with at least one male produced; high hatch-rate sex ratio (hhrSR): as for SR above, but egg hatch-rate >0.7; incompletely ascertained (?N/?SR): as for N/SR above, but <6 progeny produced (data not shown).

Using these criteria, 30 out of 94 Sapp lines were classed as SR, one as iSR (single male in 21 progeny), one as hhrSR (egg hatch-rate of 0.74, producing 11 female progeny) and 10 as probably bearing a male-killer (?SR). If the families producing fewer than six progeny (?N/?SR) are excluded from the analysis, on the basis of incomplete ascertainment, the frequency of the sex ratio trait is 32 from 65 or 0.49 (95% confidence interval for prevalence = 0.37 − 0.61).

Altai lines were split into three classes as follows. Normal (N): at least one male produced; probable normal (?N): no progeny resulting, but egg hatch-rate > 0.7; sex ratio (SR): egg hatch-rate <0.7 and only female progeny.

Only one of the 46 lines (2.2%) was classed as SR, producing 21 females ($P = 0.000\,000\,48$).

None of the 11 Novo lines produced either low egg hatch-rates or a significantly female-biased sex ratio.

Analysis of the egg hatch-rates from the Sapp SR, iSR, hhrSR and N lines gave means of 0.418 from the three sex ratio classes combined, and 0.755 for the N lines. The mean egg hatch-rate from the Altai normal lines was 0.794. The egg hatch-rate from the single Altai SR line was 0.49, this line being one of only three of the 46 Altai lines in which less than half the eggs hatched.

Conclusion A strong female-biased sex ratio, associated with a low egg hatch-rate, was detected in females from both Sapp and Altai samples, but not from the Novo sample. The frequency of females bearing this trait was much greater in the Sapp sample than in the Altai sample ($\chi^2_1 = 27.98$, $P < 0.001$). In the 32 lines from Sapp showing the trait (excluding the ?SR lines that only produced females), only a single male resulted from 714 progeny, indicating the vertical transmission efficiency of the trait to be very high (0.9986) in this population. The vertical transmission efficiency in the one Altai SR line was 100% ($n = 21$).

The inheritance of the male-killing trait

The progeny of females from lines classified as potentially infected (16 lines, 29 crosses), including one of the Sapp ?SR lines, produced only daughters, whereas those from lines classified as normal (26 lines, 32 crosses), including five ?N, produced both sons and daughters in roughly equal numbers. Because of a lack of male progeny from lines classed as infected, we were unable to test whether surviving male progeny from infected females carried the trait (the only male produced from an infected line died shortly after eclosion and before mating).

All further crosses (numbering 74 SR and 55 N families, up to F_5) produced progenic sex ratios consistent with the sex ratio status of their mother. The mean egg hatch-rates for the SR and N lines were 0.47 and 0.81, respectively. The SR lines produced 963 female progeny with no males, confirming the high vertical transmission efficiency observed in the initially female-biased families.

Conclusion The criteria for recognizing females infected with the male-killing trait appear to be sound. The male-killing trait is maternally inherited with a vertical transmission efficiency in excess of 0.99.

Sensitivity to antibiotics

The proportion of grey eggs, egg hatch-rates and sex ratios of progeny from clutches produced by these females, both before and after treatment with golden syrup, or with golden syrup and antibiotic, are given in Table 4.

None of the SR lines produced any males prior to antibiotic treatment. Treatment with golden syrup on its own had no effect on progenic egg hatch-rates ($\chi^2_1 = 1.81$, $P > 0.05$) or sex ratio. Treatment of SR females with tetracycline or chloramphenicol in

Table 1 Egg hatch rates, progenic sex ratios and sex ratio status for families of *Harmonia axyridis* from Sapporo City, Japan

Line	No. of eggs laid	Egg hatch-rate	No. of progeny	Sex ratio	Sex ratio class
H1	163	0.43	22	0.00	SR
H3	159	0.26	7	0.00	SR
H4	73	0.88	26	0.50	N
H5	56	0.38	21	0.05	iSR
H6	193	0.47	29	0.00	SR
H8	104	0.90	35	0.43	N
H9	44	0.55	7	0.71	N
H10	76	0.89	17	0.65	N
H11	223	0.44	25	0.00	SR
H12	124	0.38	25	0.00	SR
H13	157	0.71	29	0.55	N
H14	325	0.42	40	0.00	SR
H15	144	0.75	22	0.64	N
H17	187	0.91	54	0.50	N
H19	119	0.33	20	0.00	SR
H21	102	0.23	6	0.00	SR
H22	63	0.56	21	0.33	N
H25	298	0.36	59	0.00	SR
H26	84	0.49	9	0.89	N
H27	132	0.75	8	0.25	N
H29	194	0.90	84	0.57	N
H30	181	0.94	61	0.57	N
f1	109	0.32	11	0.00	SR
f2	164	0.63	6	0.83	N
f4	147	0.97	32	0.56	N
f5	47	0.74	11	0.00	hhrSR
f6	142	0.39	14	0.00	SR
f7	203	0.47	47	0.00	SR
f8	156	0.38	22	0.00	SR
f11	399	0.42	52	0.00	SR
f12	185	0.40	26	0.00	SR
f13	203	0.50	47	0.00	SR
f14	275	0.36	32	0.00	SR
f16	141	0.64	35	0.00	SR
f17	203	0.40	8	0.00	SR
f18	201	0.54	32	0.00	SR
f19	148	0.70	21	0.38	N
f21	82	0.73	20	0.55	N
f22	107	0.83	6	0.83	N
f24	170	0.36	31	0.00	SR
f26	111	0.48	7	0.00	SR
f27	202	0.71	21	0.38	N
f30	66	0.42	11	0.00	SR
f31	110	0.32	12	0.00	SR
f33	83	0.49	6	0.67	N
f34	131	0.78	26	0.46	N
f37	73	0.96	19	0.42	N
f41	227	0.70	13	0.38	N
f43	80	0.76	14	0.43	N
f44	104	0.62	15	0.53	N
f45	108	0.87	11	0.27	N

Table 1 *Continued*

Line	No. of eggs laid	Egg hatch-rate	No. of progeny	Sex ratio	Sex ratio class
f46	51	0.43	8	0.00	SR
f50	106	0.55	19	0.00	SR
f51	71	0.34	9	0.00	SR
f53	62	0.34	6	0.00	SR
f55	80	0.88	8	0.38	N
f58	98	0.53	6	0.50	N
f62	34	0.94	15	0.60	N
f63	38	0.45	8	0.00	SR
f65	65	0.80	8	0.63	N
f67	54	0.44	10	0.00	SR
f69	88	0.90	9	0.44	N
f70	48	0.40	6	0.83	N
f72	66	0.79	10	0.50	N
f78	24	0.71	8	0.38	N

The sex ratio is given as the proportion of males in progeny. Sex ratio status classes are designated as described in the text.

golden syrup did not cause an increase in egg hatch-rates (over all families, pretreatment hatch-rate = 0.56; post-treatment hatch-rate = 0.44). However, the proportion of eggs showing signs of embryonic development (hatching + grey eggs) did increase significantly in SR lines treated with antibiotics ($\chi^2_1 = 38.47$, $P < 0.001$). This increase was not observed in the N lines treated with either antibiotic (for tetracycline $\chi^2_1 = 1.41$, $P > 0.05$; for chloramphenicol $\chi^2_1 = 2.49$, $P > 0.05$).

Four of the seven SR families treated with tetracycline and all four SR families treated with chloramphenicol produced at least one male after treatment. One of these families (A28) produced a sex ratio subsequent to antibiotic treatment that was no longer strongly female-biased.

Four post-tetracycline F_1 females of A28 were subsequently mated to unrelated males, each producing a normal sex ratio in their progeny (total of 71 males and 83 females), suggesting that, in this case, the cure effected was complete and heritable.

Conclusion Treatment with antibiotics produced a full heritable cure of the sex ratio trait in one line, with some alleviation of the symptoms in seven of the other 10 SR lines. Overall, egg hatch-rates in SR lines treated with antibiotics did not increase; however, the proportion of eggs that showed signs of embryonic development did increase, and some males were produced in eight of the 11 SR lines. It is concluded that the male-killers in both the Sapp and the Altai populations are probably bacteria.

Discussion

The analysis of sex ratios in *H. axyridis* from three populations from different geographical regions shows that some females in two of these produce only, or almost only, daughters. Observation of egg hatch-rates suggests that the bias in sex ratio in these females is the result of an early male-killer, similar to that reported in other coccinellids. Males are killed early in embryogenesis. The sex ratio trait is inherited maternally, again as are male-killers in other coccinellids, suggesting that the male-killer may be a cytoplasmic endosymbiont that has a very high vertical transmission efficiency. It is notable that in none of the original families or any of the subsequent crosses set up from them was there any substantial decrease in the sex ratio produced over time, indicating that the 'progressive SR' trait reported by Matsuka *et al.* (1975) was absent from our stocks.

Treatment with antibiotics failed to produce an increase in egg hatch-rates in SR lines, as has been observed in other studies on male-killers in coccinellids (Gotoh & Niijima, 1986; Hurst *et al.*, 1992, 1996). However, a significant increase in the proportion of eggs showing embryonic development was observed after antibiotic treatment, and 8 of the 11 SR females treated with antibiotics subsequently produced some male progeny. The small proportion of males produced after treatment, in all lines with the exception of A28, indicates that treatment with tetracycline or chloramphenicol does not usually effect a complete cure, contrary to previous reports (Gotoh & Niijima, 1986; Hurst *et al.*, 1992, 1996). A complete cure was produced in one only line (A28), the cure in this line being shown to be heritable.

Table 2 Egg hatch-rates, progenic sex ratios and sex ratio status for families of *Harmonia axyridis* from Altai Mountains, Mongolia

Line	No. of eggs laid	Egg hatch-rate	No. of progeny	Sex ratio	Sex ratio class
Alt1	93	0.92	5	0.6	N
Alt2	28	0.89	2	0.5	N
Alt3	72	0.90	8	0.5	N
Alt3a	141	0.86	0	—	?N
Alt4	126	0.88	4	0.5	N
Alt5	189	0.49	21	0	SR
Alt7	157	0.76	6	0.83	N
Alt8	244	0.87	4	0.25	N
Alt9	117	0.68	6	0.33	N
Alt10	181	0.64	5	0.6	N
Alt12	114	0.85	5	0.8	N
Alt13	207	0.87	4	0.25	N
Alt14	245	0.81	3	0.67	N
Alt15	364	0.85	10	0.4	N
Alt17	155	0.72	3	1	N
Alt18	85	1.0	4	0.5	N
Alt20	177	0.96	9	0.22	N
Alt21	266	0.93	4	0.75	N
Alt22	200	0.97	5	0.2	N
Alt23	226	0.92	6	0.67	N
Alt24	130	0.69	6	0.17	N
Alt25	133	0.75	6	0.33	N
Alt26	107	0.79	10	0.7	N
Alt27	113	0.90	4	0.5	N
Alt29	140	0.86	7	0.14	N
Alt31	103	0.92	16	0.56	N
Alt32	27	0.48	21	0.57	N
Alt34	90	0.67	8	0.75	N
Alt35	55	0.93	4	0.25	N
Alt36	190	0.66	2	0.5	N
Alt37	196	0.82	9	0.44	N
Alt38	153	0.61	5	0.4	N
Alt39	143	0.92	19	0.42	N
Alt40	49	0.49	4	0.75	N
Alt42	131	0.56	6	0.5	N
Alt43	113	0.73	6	0.17	N
Alt47	140	0.55	3	0.33	N
Alt48	91	0.77	8	0.38	N
Alt49	122	0.75	10	0.7	N
Alt50	80	0.75	8	0.5	N
Alt51	141	0.95	5	0.4	N
Alt52	179	0.79	7	0.86	N
Alt53	91	0.90	5	0.2	N
Alt54	23	0.78	3	0.33	N
Alt55	223	0.78	5	0.4	N
Alt63	72	0.72	5	0.6	N

The sex ratio is given as the proportion of males in progeny. Three sex ratio classes are designated as described in the text.

Table 3 Summary data for 11 families of *Harmonia axyridis* from Novosibirsk, Russia

No. of families	No. of eggs laid	Mean egg hatch-rate	No. of progeny	Sex ratio	Sex ratio class
11	1082 (28–217)	0.93 (0.73–1)	238 (14–35)	0.5 (0.385–0.625)	All N

Data ranges are given in brackets. All families were diagnosed as normal with respect to sex ratio.

The prevalence of infection found varied between the three populations sampled. In the Sapp sample, the prevalence was almost 50%, the highest prevalence of any male-killer reported in a coccinellid. In the Altai sample, only one female out of 46 produced a female-biased sex ratio. The prevalence in this population is thus likely to be less than 10%. None of the females from Novo produced a biased sex ratio; however, the number of families involved was too small to say with confidence that the Novo population lacks male-killers. Despite this, the available data show that the prevalence of the male-killer in both the Altai Mountains and Novosibirsk populations is substantially lower than that occurring in Sapporo.

Two questions arise from these findings. First, what factors might cause differences in the prevalence of male-killers in different populations? Secondly, how do different male-killing prevalence levels influence population sex ratio and demography?

Factors influencing the prevalence of male-killer infection

Crucial to the prevalence of male-killers in a population will be the vertical transmission efficiency of the male-killer and the benefit accruing to female progeny of infected females as a result of the death of their male siblings (Hurst *et al*., 1997b).

The vertical transmission efficiency of the *H. axyridis* male-killer is very high under laboratory conditions. If this rate is representative of the vertical transmission efficiency in the wild, the rate of production of uninfected progeny, either male or female, from infected females in a population, will be very low.

The advantage that accrues to infected female offspring from the death of their male siblings will depend upon two main factors (Hurst *et al*., 1992): first, the reduction in their probability of being cannibalized by siblings (Majerus, 1994); and, secondly, the reduction in the probability that neonate larvae starve to death in the period immediately after dispersing from their egg clutch, while they are seeking and attempting to subdue their first aphid prey. The latter will depend upon the aphid density, the size of aphids, the defences of aphids and, possibly, the presence of alternative foods in the form of honey-dew or batches of conspecific coccinellid eggs that can be cannibalized.

Do the factors that influence male-killer infection levels vary in the different populations? Extensive field studies to collect data on vertical transmission efficiencies, egg and neonate larval size, egg clutch size, egg hatch synchroneity and available prey species size and population demography, from populations differing in their male-killer prevalence, will be needed to answer this question.

The influence of male-killers on population demography

The presence of male-killing endosymbionts can have effects on population sex ratios, biasing them significantly in favour of females. For example, the male-killing rickettsid that infects 7% of *Adalia bipunctata* females in Cambridge, England, is associated with a significantly female-biased population sex ratio (Hurst *et al*., 1993). In the case of the Sapp sample, the high level of the male-killer trait appears to have led to a significantly female-biased population sex ratio (60.6% females, $n = 287$; assuming that the sex ratio in the overwintering population is representative). If the Sapp population is at equilibrium, there must be strong overall selection for nuclear resistance genes to the male-killing behaviour of this endosymbiont. This selection will act primarily through males (Werren, 1987; Hurst, 1991). If, on the other hand, the Sapp male-killer is still increasing in frequency, this could ultimately have a significant effect on the population as the sex ratio becomes increasingly biased.

In most sexually reproducing animals, the sex ratio is maintained at, or close to, 1:1 by frequencydependent selection (Fisher, 1930), and females are the limiting sex. In populations in which males are rare compared with females, it is possible that males become the limiting sex, with substantial evolutionary consequences for intersexual and intrasexual behaviour (Hamilton, 1967). Given the strong (and possibly increasing) female bias in the sex ratio in the Sapporo population of *H. axyridis*, coupled with reported temporally variable mating preferences by both females and males of this species (Osawa & Nishida, 1992), it is possible that the reproductive behaviour of these ladybirds has been, and is, affected by selective factors different from those that affect populations with more equitable sex ratios.

Table 4 The effect of treatment with antibiotics on the sex ratio trait in *Harmonia axyridis*

Line and treatment	Pretreatment				Post-treatment			
	Proportion of grey eggs	Hatch-rate	No. of progeny	Sex ratio	Proportion of grey eggs	Hatch-rate	No. of progeny	Sex ratio
(a)								
H31(Sapp)	0.01	0.42	2	0	0.14	0.64	14	0
H32 (Sapp)	0.27	0.63	2	0	0.19	0.71	24	0.04
H36 (Sapp)	0.03	0.34	9	0	0.19	0.30	18	0
H46 (Sapp)	0.02	0.53	14	0	0.21	0.62	18	0.06
H47 (Sapp)	0.03	0.42	22	0	0.17	0.37	22	0
M11 (Altai)	0.04	0.48	23	0	0.35	0.38	66	0.03
A28 (Altai)	0.02	0.43	13	0	0.12	0.51	36	0.31
(b)								
H40 (Sapp)	0.05	0.37	20	0	0.08	0.40	25	0
H42 (Sapp)	0.13	0.35	8	0	0.06	0.29	6	0
H45 (Sapp)	0.06	0.36	16	0	0.23	0.33	32	0
H53 (Sapp)	0.10	0.40	32	0	0.09	0.44	23	0
H56 (Sapp)	0.00	0.48	35	0	0.03	0.41	30	0
M13 (Altai)	0.07	0.41	9	0	0.04	0.53	16	0
A39 (Altai)	0.11	0.32	21	0	0.01	0.39	11	0
A41 (Altai)	0.04	0.51	16	0	0.09	0.41	28	0
(c)								
H33 (Sapp)	0.07	0.92	4	0.25	0.04	0.85	37	0.38
H35 (Sapp)	0.01	0.92	31	0.48	0.03	0.88	27	0.52
H39 (Sapp)	0.05	0.90	33	0.36	0.04	0.86	27	0.44
H55 (Sapp)	0.04	0.88	28	0.57	0.02	0.94	20	0.50
H58 (Sapp)	0.03	0.86	40	0.45	0.02	0.84	31	0.48
A38 (Altai)	0.08	0.82	15	0.6	0.08	0.83	26	0.58
A40 (Altai)	0.04	0.88	11	0.64	0.09	0.81	17	0.47
A42 (Altai)	0.03	0.86	10	0.50	0.02	0.87	25	0.36
(d)								
H40 (Sapp)	0.06	0.38	45	0	0.08	0.55	65	0.12
H45 (Sapp)	0.13	0.35	48	0	0.21	0.57	46	0.07
H53 (Sapp)	0.09	0.43	55	0	0.24	0.52	24	0.04
H56 (Sapp)	0.02	0.45	65	0	0.13	0.43	8	0.13
(e)								
H33 (Sapp)	0.06	0.90	41	0.37	0.04	0.88	14	0.57
H35 (Sapp)	0.02	0.90	58	0.5	0.08	0.82	17	0.59
H55 (Sapp)	0.03	0.91	48	0.54	0.05	0.89	22	0.5
H58 (Sapp)	0.02	0.85	71	0.46	0.04	0.92	15	0.4

Sex ratio lines were treated with a mixture of golden syrup and tetracycline (a), golden syrup alone (b) or golden syrup and chloramphenicol (d). Normal lines were treated with tetracycline in golden syrup (c) or chloramphenicol in golden syrup (e). The number of eggs that showed signs of development but failed to hatch (grey eggs), the egg hatch-rates and progenic sex ratios are given for before and after each treatment.

Studies of the copulatory behaviour and mating preferences of the Sapporo population, in comparison with others that are less female-biased, should be enlightening.

Acknowledgements

We wish to thank Alan Feast, Roger Day, Dennis Farrington and George Bennett for technical support. This work was carried out in a laboratory built with the support of the Wolfson Foundation. Gregory Hurst was funded via a Junior Research Fellowship from Christ's College.

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