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The value of an egg: resource reallocation in ladybirds (Coleoptera: Coccinellidae) infected with male-killing bacteria

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Abstract

Male-killing bacteria are thought to persist in host populations by vertical transmission and conferring direct and/or indirect fitness benefits to their hosts. Here, we test the role of indirect fitness benefits accrued from resource reallocation in species that engage in sibling egg cannibalism. We found that a single-egg meal significantly increased larval survival in 12 ladybird species, but the value of an egg (to survival) differed substantially between species. Next, we tested the impact of three male-killing bacteria on larval survival in one ladybird species, *Adalia bipunctata*. *Spiroplasma* reduced larval survival, whereas *Wolbachia* and *Rickettsia* had no effect. However, *Spiroplasma*-infected larvae showed the greatest response to a single-egg meal. The indirect fitness benefit obtained from a single egg is thus so large that even male-killing bacteria with direct fitness costs can persist in host populations. This study supports the hypothesis that fitness compensation via resource reallocation can explain male-killing bacteria persistence.

Introduction

Maternally inherited endosymbiotic bacteria, such as *Wolbachia*, *Rickettsia* and *Spiroplasma*, are common in arthropods, with estimates suggesting at least one-third of all insect species are infected (Werren *et al.*, 1995; Weinert *et al.*, 2007; Duron *et al.*, 2008). These bacteria are often reproductive manipulators that cause cytoplasmic incompatibility, induce parthenogenesis or kill male hosts early in their development ('early male-killers') (see O'Neill *et al.*, 1997; Engelstadter & Hurst, 2009 for reviews). Male-killing bacteria have attracted considerable interest in recent decades because they can lead to strongly female-biased sex ratios in their hosts and have important consequences for host fitness (e.g. Hurst & Jiggins, 2000; Montenegro *et al.*, 2006; Saridaki & Bourtzis, 2010), reproductive strategies (Jiggins *et al.*, 1998), demography (Hurst *et al.*, 1994) and evolutionary genetics (Randerson *et al.*, 2000; Schulenburg *et al.*, 2002; Jiggins & Tinsley, 2005; Majerus, 2006; Engelstadter & Hurst, 2007).

Several studies have discussed the invasion and persistence of male-killers in host populations (e.g. Hurst *et al.*, 1997, 1999a, 2003). If transmission of male-killing bacteria is strictly vertical and they confer a fitness advantage to their hosts, the bacteria will invade and spread through a novel host population (Hurst *et al.*, 1997). However, there is evidence that vertical transmission is not perfect (e.g. Hurst *et al.*, 1993), and the direct effect of infection on female fitness can be detrimental. For example, Hurst *et al.* (1994) reported a negative effect of *Rickettsia* infection on fecundity and survival in the two-spot ladybird, *Adalia bipunctata* (*L.*) (Coleoptera: Coccinellidae).

Three nonmutually exclusive hypotheses have been proposed to explain the invasion and persistence of male-killing bacteria in novel host populations if vertical transmission is less than perfect and/or there is a cost to infection (Hurst, 1991; Hurst *et al.*, 1997). The fitness of female progeny from infected hosts must be compensated through (i) reduced competitive interactions between siblings (i.e. local resource competition), (ii) reduced inbreeding and inbreeding depression and/or (iii) reallocation of resources through infected female larvae consuming the undeveloped eggs of their infected brothers (Hurst, 1991; Hurst *et al.*, 1997; Majerus & Hurst, 1997). In each case, fitness compensation is

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achieved through an increase in the lifetime fitness of female offspring of infected mothers, as a result of the death of their male siblings (Hurst *et al.*, 2003). Previous studies have highlighted the potential importance of male-killer infection in reducing local resource competition (Hurst *et al.*, 1997). By contrast, inbreeding is thought to be rare in wild coccinellids and inbreeding depression thus unlikely to be important in the dynamics of male-killers (Hurst & Majerus, 1993; Hurst *et al.*, 1996). Resource reallocation is regarded as a significant element of fitness compensation in species that engage in sibling egg cannibalism (Majerus & Hurst, 1997), but there have been few direct tests of the resource allocation hypothesis specifically in relation to male-killer persistence.

Aphidophagous coccinellids are ideal systems for testing hypotheses for male-killer persistence because certain aspects of their ecology and behaviour make them particularly prone to invasion by male-killers (Majerus & Hurst, 1997; Majerus, 2006), and male-killers from a variety of different clades have been described (e.g. *Rickettsia*, Werren *et al.*, 1994; Flavobacteria, Hurst *et al.*, 1999a; *Spiroplasma*, Hurst *et al.*, 1999c; *Wolbachia*, Hurst *et al.*, 1999b; and γ-Proteobacteria, Majerus, 1999). The ecological and behavioural aspects of particular importance are as follows: (i) they lay their eggs in tight clutches (Majerus, 1994), (ii) they exhibit sibling egg cannibalism (e.g. Banks, 1956), (iii) they feed on ephemeral aphid prey that are prone to rapid population crashes (Dixon, 2000), (iv) neonate larvae are not very efficient at finding and subduing aphid prey, and (v) survival of neonate larvae is low as a consequence of (iii) and (iv) (see the study by Majerus & Hurst, 1997 for a review). Previous studies have demonstrated that there is indeed a survival advantage to neonate coccinellid larvae in consuming undeveloped eggs. For example, both Kawai (1978) and Osawa (1992) found a positive effect of egg cannibalism on survival of *Harmonia axyridis* neonate larvae, but these studies did not consider the effect of male-killer infection. Hurst *et al.* (1994) showed that neonate larvae of *A. bipunctata* infected with *Rickettsia*, that were fed a single newly laid egg but no other food or water, survived 50% longer than larvae denied an egg meal. Moreover, *A. bipunctata* females in male-killer clutches survived longer in the absence of food than individuals from uninfected clutches (Hurst, 1993; Hurst *et al.*, 1997). In addition to surviving longer, larvae that consumed undeveloped eggs were larger when they dispersed and more capable of subduing their (considerably larger) aphid prey (Hurst *et al.*, 1997). Infected female offspring thus gain a high indirect fitness benefit by consuming the undeveloped male eggs in their clutch (Hurst *et al.*, 1994). More recently, Nakamura *et al.* (2006) compared the rate of sibling egg cannibalism between *Spiroplasma*-infected and uninfected clutches of *H. axyridis*. They found that *Spiroplasma* infection increases the opportunity for sibling egg cannibalism by approximately 4- to 14-fold, which (together with high vertical transmission) may explain the high prevalence of *Spiroplasma* in populations of *H. axyridis*. However, they correctly acknowledge that measurements of fecundity and survival are necessary before strong conclusions can be drawn.

Under the resource reallocation hypothesis, sibling egg cannibalism provides significant fitness compensation in male-killer infected families. This hypothesis has two major components: (i) the value of the benefit accrued from an egg meal, and (ii) the opportunity for cannibalism (females from male-killer clutches should have more opportunity for cannibalism and will therefore benefit more than uninfected females). In this study, we aimed to test the generality of the first component of the resource reallocation hypothesis in aphidophagous coccinellids. We hypothesize that (i) there is substantial nutritional value in a single-egg meal, and this is general among aphidophagous coccinellids, and (ii) the indirect fitness benefit to the host in consuming an egg meal is large enough to explain the persistence of male-killers in a population even if there is a direct cost to infection. To test these hypotheses, we first calculated the 'egg value' (i.e. the value of a single-egg meal, relative to no-egg meal, in terms of survival of a neonate larva given no other food or water) in laboratory cultures of 12 species of aphidophagous coccinellids, in which sibling egg cannibalism has been observed in the field. We then investigated the effect of different male-killer infections (*Rickettsia*, *Wolbachia* and *Spiroplasma*) on larval survival and egg value in a single host species (*A. bipunctata*), relative to uninfected larvae. Finally, we focused in detail on one male-killer, by investigating whether there is a difference in the value of *Spiroplasma*-infected and uninfected eggs for both uninfected and *Spiroplasma*-infected *A. bipunctata* larvae, again measured in terms of larval survival. Our results support the resource reallocation hypothesis and demonstrate that the value of consuming a single egg is so large that even male-killers that bear a direct cost on their host can persist in host populations.

Materials and methods

Coccinellid cultures and general procedures

Coccinellid cultures were maintained in Petri dishes at 21 °C with 14L : 10D artificial lighting and 35% humidity in a controlled environment room and were fed daily on pea aphids, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). When setting up mating pairs, individuals were chosen from different matrilines to minimize inbreeding depression, which has been described in laboratory cultures of coccinellids, although it is thought to be rare in the field (Lus, 1947; Hurst & Majerus, 1993; Hurst *et al.*, 1996). Eggs were harvested daily by removing adults to new dishes. First instar neonate larvae were removed from their egg clutch using a single bristle brush, before they had consumed any unhatched eggs in their clutch. Each neonate larva was placed in a new 5-cm Petri dish, with either one newly laid conspecific egg or with nothing. No other food or water was provided. Neonate larvae were monitored and their survival recorded (in h). A small number of larvae that were offered an egg meal failed to consume it and were excluded from analyses. In addition, larvae that died within 24 h after being removed from their egg clutch were assumed to have been injured during manipulation and were excluded from analysis.

Experiment 1: What is the value of a single egg?

In our first experiment, we tested the hypothesis that a single-egg meal increases larval survival relative to no-egg meal, in each of 12 coccinellid species (Table 1). Nine of these species are known hosts to male-killers (Majerus, 2003; Table 1). *Coccinella septempunctata* and *Coccinella septempunctata brucki* are suspected to host male-killers, but this awaits rigorous confirmation, whereas male-killers were not detected in *Eocaria muiri*, during assays of 138 matrilines (M.E.N. Majerus, personal observation). Larvae and eggs were from uninfected females, so the infection status did not influence the egg value, except in the case of *Coccinella undecimpunctata*, where only infected females were available. Number of larvae used in this experiment varied from 14 to 30 for each treatment (Table 2).

Table 1 Origins and infection status of coccinellid cultures of used in experiments.

Species	Male-killer	Origin
Adalia bipunctata (L.)	Uninfected	Moscow, Russia
A. bipunctata	*Rickettsia*	Moscow, Russia
A. bipunctata	*Wolbachia*	Moscow, Russia
A. bipunctata	*Spiroplasma*	St. Petersburg, Russia
Adalia decempunctata (L.)	*Rickettsia*	Bielfeld, Germany
Chilomenes sexmaculatus (Fabricius)	*γ-Proteobacterium*	Fuchu, Honshu, Japan
Coccinella septempunctata (L.)	Unconfirmed	Cambridge, England
Coccinella septempunctata brucki (Mulsant)	Unconfirmed	Fuchu, Honshu, Japan
Coccinella undecimpunctata (L.)	*Wolbachia*	Giza, Egypt
Coccinula crotchi (Lewis)	Flavobacterium	Fugi, Honshu, Japan
Coccinula sinensis (Weise)	Flavobacterium	Kofu, Honshu, Japan
Eocaria muiri (Timberlake)	None	Fuchu, Honshu, Japan
Harmonia axyridis (Pallas)	*Spiroplasma*	Fuchu, Honshu, Japan
Harmonia quadripunctata (Pontoppidan)	Flavobacterium	Provence, France
Hippodamia variegata (Goetze)	Flavobacterium	Crete, Greece
Propylea japonica (Thunberg)	*Rickettsia*	Fuchu, Honshu, Japan

The relative survival increment resulting from a single-egg meal ('egg value') was calculated as follows:

$$\frac{\bar{S}_{\mathrm{E}} - \bar{S}_{\mathrm{NE}}}{\bar{S}_{\mathrm{NE}}} \qquad (1)$$

where $\bar{S}_{\mathrm{E}}$ and $\bar{S}_{\mathrm{NE}}$ are the mean survival with a single-egg meal and with no-egg meal, respectively, for a given species. Ten single eggs and 10 adults from each species were weighed to test the prediction that greater value is obtained from larger eggs (relative to adult body mass). Statistical analyses were carried out using R (R Development Core Team, 2010) and PASW STATISTICS 18.0 (SPSS Inc., Chicago, IL, USA). Data were tested for normality using Kolmogorov–Smirnov (K–S) tests and for homogeneity of variances using Levene's test. We tested whether there was a difference in survival between treatments (egg vs. no egg) for each coccinellid species by (i) plotting Kaplan–Meier survival distributions and testing for differences between survival curves using chi-squared tests, and (ii) performing one-way analysis of variance (ANOVA) on natural log-transformed data (as data were not normally distributed, K–S $Z = 1.08$, $P > 0.05$ and Levene's $F_{1,597} = 0.20$, $P > 0.05$ after natural log transformation).

Experiment 2: What is the effect of different male-killer infections on egg value in a single species of host?

In our second experiment, we investigated the effect of *Rickettsia*, *Wolbachia* and *Spiroplasma* on egg value in a single host species (*A. bipunctata*) relative to uninfected hosts. Specifically, we asked whether being infected with a male-killer influences survival (i) with an egg meal ($\bar{S}_{\mathrm{E}}$), (ii) without an egg meal ($\bar{S}_{\mathrm{NE}}$) and c) overall ($\bar{S}_{\mathrm{E}} + \bar{S}_{\mathrm{NE}}$). Experimental procedures were the same as for Experiment 1; however, here the neonate larvae from females of a particular infection status were fed on eggs from females of the same infection status. Four cultures of *A. bipunctata* were established: one uninfected, one *Spiroplasma*-infected, one *Rickettsia*-infected and one *Wolbachia*-infected ($n = 22$–29, Table 3). We tested whether there was a difference between treatments (egg vs. no egg) for each infection status, again using (i) survival analyses and (ii) one-way ANOVAS on square-root transformed data (as data were normally distributed but Levene's test was significant even after natural log transformation, note that after square root transformation, K–S $Z = 1.20$, $P > 0.05$ and Levene's $F_{7,192} = 1.74$, $P > 0.05$) and *post-hoc* Fisher's least significant difference (LSD) tests to investigate pairwise differences between infections. In addition, a two-way ANOVA with *post-hoc* LSD tests was used to test for interaction between infection status and treatment.

Table 2 Experiment 1: the value of an egg.

Species	N_E	N_{NE}	Egg/adult mass	$\bar{S}_E$(SD)	$\bar{S}_{NE}$(SD)	Egg value	Chi-sq	One-way ANOVA (F)
Adalia bipunctata	26	28	1.06	90.77 (11.80)	62.71 (13.86)	0.45	41.6***	34.74***
Adalia decempunctata	22	25	1.08	81.91 (15.92)	55.36 (12.82)	0.48	29.9***	31.14***
Chilomenes sexmaculatus	23	24	0.72	102.26 (12.70)	62.17 (11.27)	0.64	49.4***	115.31***
Coccinella septempunctata	21	25	0.42	98.00 (24.44)	52.64 (13.94)	0.86	35.4***	53.69***
Coccinella septempunctata brucki	25	21	0.38	126.56 (22.03)	80.38 (16.28)	0.57	40.9***	54.54***
Coccinella unidecempunctata	30	29	1.07	120.20 (23.34)	69.31 (19.77)	0.73	48.5***	73.39***
Coccinula crotchi	16	17	2.5	135.13 (63.67)	76.82 (45.40)	0.76	8.2*	7.57*
Coccinula sinensis	18	22	2.5	168.89 (49.09)	46.36 (13.42)	2.64	34.2***	117.15***
Eocaria muiri	25	25	0.78	101.36 (21.34)	48.08 (10.26)	1.11	57.0***	172.29***
Harmonia axyridis	27	26	0.45	98.59 (18.56)	66.54 (17.31)	0.48	29.1***	32.34***
Harmonia quadripunctata	14	24	1.3	116.14 (19.65)	74.25 (8.41)	0.56	26.8***	71.54***
Hippodamia variegata	20	18	0.4	107.40 (26.23)	53.67 (13.96)	1.00	31.5***	55.02***
Propylea japonica	23	24	2	116.35 (29.85)	66.17 (16.97)	0.76	37.8***	40.74***

N, number of neonate larvae used in experiment; *E*, with egg; NE, no egg; egg/adult mass is the ratio of egg to adult mass; $\bar{S}$, mean survival (in h); SD, standard deviation; 'egg value' as calculated in eqn (1); 'chi-sq', chi-squared test statistic for differences between survival curves (see Fig. S1).

*P values, where *$P < 0.01$; ***$P < 0.0001$. All tests have 1 d.f.

Table 3 Experiment 2: the effect of male-killer infection status in a single host species (*Adalia bipunctata*).

Infection status (larvae and eggs)	N_E	N_{NE}	Overall ($\bar{S}_E + \bar{S}_{NE}$)(SD)	$\bar{S}_E$(SD)	$\bar{S}_{NE}$(SD)	Egg value	Chi-sq	One-way ANOVA (F)
Uninfected	26	28	76.22 (17.53)	90.77 (11.80)	62.71 (13.86)	0.45	36.8***	34.74***
Rickettsia	24	22	72.04 (20.12)	83.58 (19.21)	59.45 (12.02)	0.41	23.5***	25.28***
Wolbachia	22	23	72.84 (23.98)	86.00 (23.15)	60.26 (17.31)	0.43	16.8***	17.20***
Spiroplasma	26	29	60.55 (17.89)	75.46 (12.62)	47.17 (9.13)	0.60	45.0***	45.18***

N, number of neonate larvae used in experiment; *E*, with egg; NE, no egg; $\bar{S}$, mean survival (in h); 'egg value' as calculated in eqn (1); 'chi-sq', chi-squared test statistic for differences between survival curves (see Fig. S1).

*P values, where ***$P < 0.0001$. Note that larvae were fed on eggs with the same infection status. All tests have 1 d.f.

Experiment 3: What is the value of an infected egg?

In our final experiment, we investigated whether there was a difference in the value of *Spiroplasma*-infected and uninfected eggs for both uninfected and *Spiroplasma*-infected *A. bipunctata* larvae. Larvae and eggs of *A. bipunctata* used during this experiment were produced either by females infected with *Spiroplasma* (with a mean egg hatch rate of 0.41) or by uninfected females (with a mean egg hatch rate of 0.90). Neonate larvae from uninfected and *Spiroplasma*-infected *A. bipunctata* cultures were fed a single egg from either uninfected or *Spiroplasma*-infected cultures ($n = 26$–30, Table 4). In this case, we estimated the value of a single infected egg, as follows:

$$\frac{\bar{S}_{\mathrm{SE}} - \bar{S}_{\mathrm{UE}}}{\bar{S}_{\mathrm{UE}}} \tag{2}$$

where SE is *Spiroplasma*-infected egg UE is uninfected egg and $\bar{S}$ is mean survival, as in eqn (1). As before, we used survival analyses and one-way ANOVAS (in this case on untransformed data as data were normally distributed with homogeneous variances between groups: K–S $Z = 0.14$, $P > 0.05$, Levene's $F_{1,102} = 0.505$, $P > 0.05$) to test for differences between infected and uninfected eggs for each larval treatment, and *post-hoc* LSD tests to investigate pairwise differences in survival between treatments. Note that data from experiments 2 and 3 were obtained simultaneously to avoid introducing bias from unknown factors.

Results

Experiment 1: The value of an egg

As predicted, survival was always greater with an egg meal ($\bar{S}_{\mathrm{E}}$) than without ($\bar{S}_{\mathrm{NE}}$) (Fig. 1 and Fig. S1), and this difference was strongly significant for each species (Table 2) and overall (one-way ANOVA $F_{1,585} = 480.11$, $P < 0.0001$). However, egg value varied widely between species, ranging from 0.48 in *Adalia decempunctata* to 2.64 in *Coccinula sinensis* (Table 2). This was not explained by the difference in egg size between species, because there was no relationship between egg mass (corrected for adult body mass) and egg value (Table 2). For example, eggs of *A. decempunctata* are more than twice the mass

Table 4 Experiment 3: the value of an infected egg.

Larval infection status	N_{SE}	N_{UE}	Overall $(\bar{S}_{SE} + \bar{S}_{UE})$(SD)	$\bar{S}_{SE}$(SD)	$\bar{S}_{UE}$(SD)	Infected egg value	Chi-sq	One-way ANOVA (*F*)
Uninfected	30	26	84.39 (15.62)	78.87 (17.22)	90.77 (11.80)	−0.13	22.2***	11.90*
Spiroplasma	26	27	75.89 (12.79)	75.46 (12.62)	76.30 (13.18)	−0.01	0.1^{NS}	0.06^{NS}

N, number of *Adalia bipunctata* neonate larvae used in experiment; UE, with uninfected egg; SE, with *Spiroplasma*-infected egg; $\bar{S}$, mean survival (in h); 'infected egg value' as calculated in eqn (2).
**P* values, where ^{NS}not significant; **P* < 0.01; ****P* < 0.0001. All tests have 1 d.f.

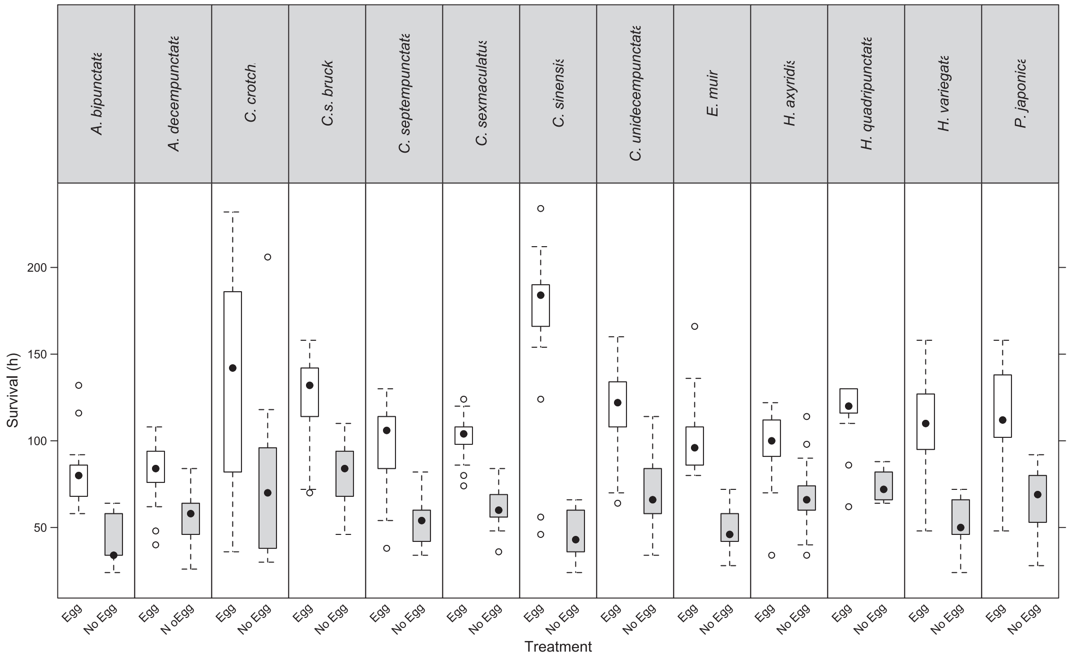

Fig. 1 Experiment 1: Survival of neonate ladybird larvae given no-egg or a single-egg meal. Boxplots summarizing survival data for each of 12 coccinellid species: survival is always greater with an egg meal (see also Table 2 and Fig. S1). The filled circles in the middle of the boxes corresponds to the median survival, boxes represent the 25th to 75th percentile, and whiskers represent the 95% confidence intervals. Open circles are outliers.

of those of *H. axyridis*, relative to adult mass, but the two species have the same egg value. Moreover, the two *Coccinula* species have the same egg/adult mass ratio, but *C. sinensis* has an egg value of 2.64, whereas *Coccinula crotchi* has an egg value of 0.76. In addition, plotting egg/adult mass against egg value gave a positive relationship ($R^2 = 0.187$, Fig. S2a), but this is driven by *C. sinensis*, which is a clear outlier. If this outlier is removed, the relationship is weakly negative ($R^2 = -0.019$, Fig. S2b).

Experiment 2: The effect of different male-killer infections on egg value in a single host species

As found in experiment 1, survival was always greater with an egg meal than without an egg meal (Fig. 2 and Table 3). Egg value was very similar for larvae that were uninfected or infected with *Rickettsia* or *Wolbachia* (0.41–0.45, Table 3), but substantially greater for larvae infected with *Spiroplasma* (0.60). Globally, survival was significantly different between infections (i) with an egg meal (one-way ANOVA $F_{3,94} = 3.20$, $P < 0.05$), (ii) without an egg meal ($F_{3,98} = 7.966$, $P < 0.0001$) and (iii) overall ($F_{3,200} = 5.42$, $P < 0.001$). Within treatment, survival was similar for *Rickettsia*, *Wolbachia* or uninfected larvae, but considerably lower for *Spiroplasma*-infected larvae (Fig. 2a,b; Table S1a,b, note though that with an egg, only the survival difference between *Spiroplasma*-infected and uninfected larvae remains significant in *post-hoc* LSD tests, Table S1c). The difference in response to an egg meal between *Spiroplasma*-infected and other larvae is illustrated in the estimated marginal means plot of survival (Fig. 2c). There was no significant interaction between infection status and treatment overall (two-way

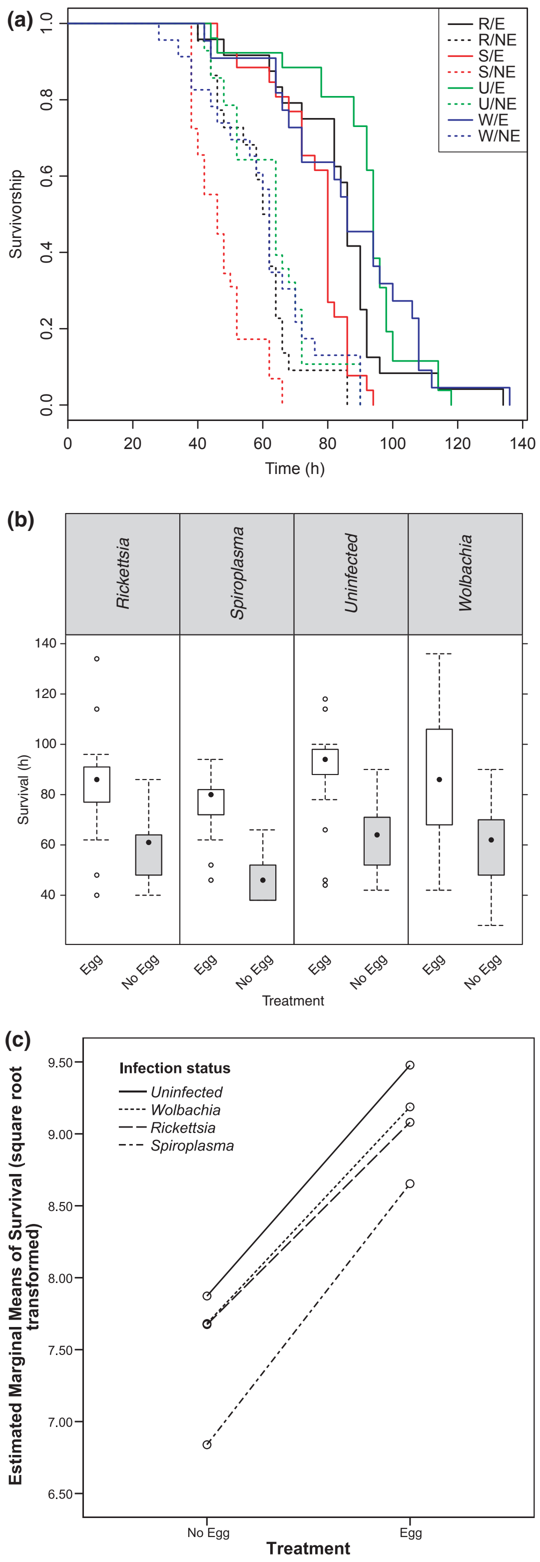

Fig. 2 Experiment 2: Survival of uninfected and male-killer infected *Adalia bipunctata* larvae given no-egg or a single-egg meal. (a) Kaplan–Meier survival curves: survival is similar within treatment for *Rickettsia*, *Wolbachia* or uninfected larvae, but significantly lower for *Spiroplasma*-infected larvae (with egg: chi-sq ranges from $\chi^2 = 7.1$, $P < 0.01$ for *Spiroplasma* vs. *Rickettsia*, to $\chi^2 = 24.4$, $P < 0.001$ for *Spiroplasma* vs. uninfected; no egg: chi-sq ranges from $\chi^2 = 10.8$, $P < 0.001$ for *Spiroplasma* vs. *Rickettsia*, to $\chi^2 = 23.6$, $P < 0.001$ for *Spiroplasma* vs. uninfected). R, *Rickettsia*; S, *Spiroplasma*; W, *Wolbachia*; U, uninfected; E, with egg; NE, no egg. (b) Boxplot summarizing survival data (see Fig. 1 legend for definitions and Table 3 for statistical results). (c) Estimated marginal means plot of survival data: *Spiroplasma*-infected larvae show the greatest response to an egg meal.

ANOVA $F_{3,192} = 0.425$, $P > 0.05$); however, comparisons between *Spiroplasma*-infected larvae and all others were strongly significant (LSD $P = 0.000$, Table S1d).

Experiment 3: The value of an infected egg

Uninfected *A. bipunctata* larvae had significantly lower survival when fed on *Spiroplasma*-infected eggs, compared to uninfected eggs (Fig. 3 and Table 4). However, survival was not significantly different for *Spiroplasma*-infected larvae fed either infected or uninfected eggs (Fig. 3 and Table 4). Consistent with this, infected-egg values (calculated as in eqn 2) were close to zero for *Spiroplasma*-infected larvae (Table 4). Survival was significantly lower in all three *Spiroplasma* treatments than when both larvae and eggs were uninfected ('U/U', Fig. 3, LSD U/U vs. others $P < 0.01$, Table S2). Global ANOVA was significant (one-way ANOVA $F_{3,94} = 6.934$, $P = 0.000$) and driven by the greater survival of uninfected larvae fed uninfected eggs.

Discussion

We quantified the benefit of an egg meal to neonate larvae in terms of survival (i.e. the 'egg value') and investigated the indirect fitness benefits accrued by sibling egg cannibalism in aphidophagous coccinellids. The nutritional value gained from a single-egg meal is substantial and general in the 12 coccinellid species studied here. Indirect fitness benefits conferred on the host appear sufficient to promote the establishment and persistence of male-killers in host populations, even when there is a direct *cost* to host survival from infection. Our results therefore support the resource reallocation hypothesis for male-killer persistence.

In our first experiment, we found that survival was much greater for neonate larvae given an egg meal, compared to those not given an egg meal. The fitness advantage of an egg meal is therefore general for coccinellids that indulge in sibling egg cannibalism. Interestingly, however, there was up to six-fold difference in egg value between species, and this did not relate

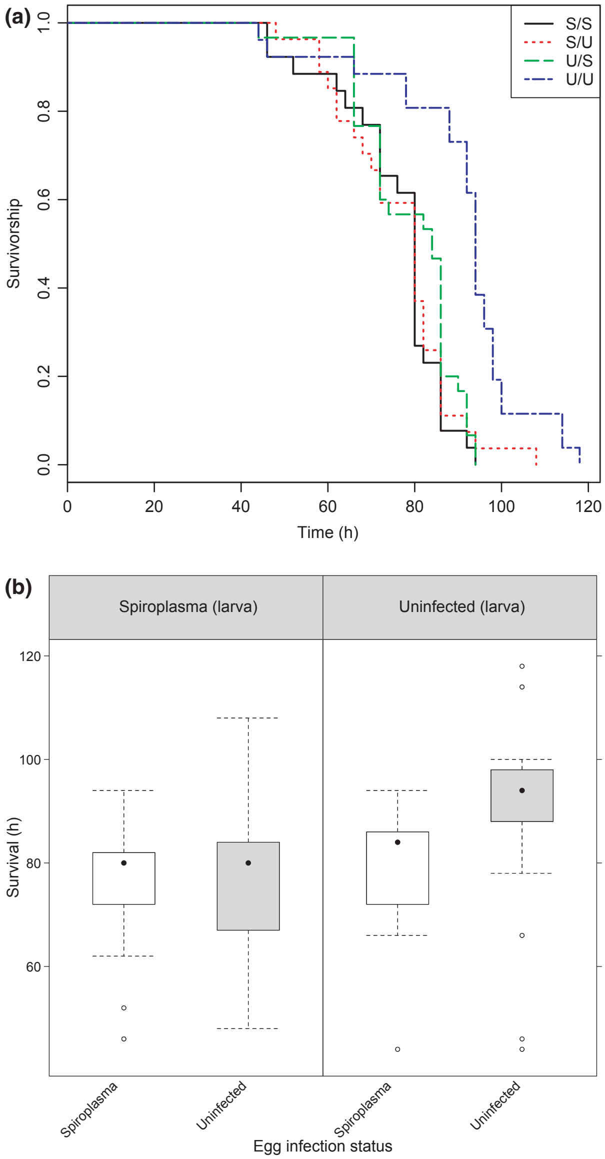

Fig. 3 Experiment 3: Survival of *Spiroplasma*-infected or uninfected *Adalia bipunctata* larvae given a *Spiroplasma*-infected or uninfected-egg meal. (a) Kaplan–Meier survival curves: Codes correspond to larval infection status/egg infection status, e.g. U/S, uninfected larvae fed *Spiroplasma*-infected egg. Comparisons between U/U and others are significantly different (U/U vs. U/S $\chi^2 = 22.2$, $P = 2.47 \times 10^{-6}$; U/U vs. S/U $\chi^2 = 17.8$ $P < 0.001$; U/U vs. S/S $\chi^2 = 24.4$, $P < 0.001$). (b) Boxplot summarizing survival data: survival is significantly higher for uninfected larvae fed on uninfected eggs, compared to *Spiroplasma*-infected treatments (see also Table 4 and Table S2).

to difference in egg size. Even congeneric species (e.g. *C. sinensis* and *C. crotchi*) respond very differently, suggesting the effect is independent of phylogeny, although more comparisons are required to formally test this. Notably, although these two species overlap in their habitat preferences and both produce large eggs (relative to their body size) in small clutches, they differ greatly in the palatability of their eggs. *Coccinula crotchi* eggs are much less palatable to other coccinellid species that engage in intraguild predation (IGP, e.g. *H. axyridis*) than are *C. sinensis* eggs, which is likely linked to toxins used in chemical defence (Ware *et al.*, 2008). Although chemical defence is obviously more important at the interspecific level in terms of deterring IGP, we speculate that the low egg value of *C. crotchi* eggs relative to those of *C. sinensis* could be linked to their low palatability (and greater toxin level), which could be adaptive in preventing sibling egg cannibalism in species that invest considerable resources in a few, large eggs. The reasons for this variation in egg value between species require further investigation.

In our second experiment, we investigated the effect of male-killer infection status on egg value in a single species of host, *A. bipunctata.* Mean survival was very similar for uninfected, *Rickettsia*-infected and *Wolbachia*-infected larvae that were fed on eggs with the same infection status, and all three treatments responded in the same way to an egg meal (i.e. egg value is almost identical). In contrast, *Spiroplasma*-infected larvae had lower survival than other larvae, but showed the greatest response to an egg meal. There appears to be, therefore, (i) no direct benefit (i.e. a neutral effect) of *Wolbachia* or *Rickettsia* infection and (ii) a direct cost of *Spiroplasma* infection, to neonate larvae in terms of their survival, relative to uninfected larvae. This cost to *Spiroplasma* infection was confirmed in our third experiment, in which we found lower larval survival in *Spiroplasma* treatments (i.e. infected larvae or eggs) relative to uninfected larvae fed uninfected eggs. At first glance, these results seem to contrast with those of Hurst (1993), who found that survival time of *A. bipunctata* females in male-killer clutches was greater in the absence of aphid prey compared to individuals from uninfected clutches (Hurst *et al.*, 1997). However, there is an important distinction between these studies. In Hurst's (1993) study, the neonate larvae were free to consume unhatched eggs from their own clutch, and the uninfected larvae ate significantly fewer eggs compared to infected larvae, because fewer eggs were available. Fitness of infected larvae was therefore indirectly compensated in Hurst's study, by the opportunity to cannibalize undeveloped male eggs.

Previous studies have demonstrated strict vertical transmission of *Spiroplasma* in coccinellids (e.g. Majerus *et al.*, 2000; Nakamura *et al.*, 2006). Therefore, to be maintained in the population, it must confer some advantage on its host. As noted previously, *Spiroplasma*-infected *A. bipunctata*, larvae obtain the greatest benefit from an egg meal. Sibling egg cannibalism is therefore likely to be particularly adaptive in *Spiroplasma*-infected individuals, which may explain how *Spiroplasma* is maintained in the population in spite of its apparent cost. In our third experiment, which examined the *Spiroplasma* infection in detail, we demonstrated that the cost of infection is linked to *both* the larva and the egg, but if the larva is already infected, there is no additional cost to consuming an infected (relative to uninfected) egg. This cost may be due to *Spiroplasma* activating the host's immune system, rather than due to lower nutritional value of *Spiroplasma*-infected eggs, however this warrants further investigation.

It is important to note that only one component of fitness (survival) has been studied here. In future studies, it will be crucial to investigate whether the cost to survival imposed by *Spiroplasma* is offset by increased fecundity. We also acknowledge that the true value of an egg to ladybird survival in the wild will depend on the dynamics of local aphid populations (Hurst *et al.*, 2003). The 'egg values' reported here should therefore be treated with some caution. Obtaining similar measures from wild coccinellids will be a worthwhile, if challenging endeavour. It would also be useful to investigate whether there is a relationship between egg value and male-killer prevalence, as one might expect a positive correlation between the two. Finally, we have focused here on just one major component of the resource reallocation hypothesis. The second major component – that females from male-killer clutches should have more opportunity for cannibalism and will therefore benefit more than uninfected females – warrants rigorous testing.

In conclusion, our results support a major component of the resource reallocation hypothesis that there is a substantial and general value in a single egg meal. The indirect fitness benefit obtained from a single egg meal is so large that even male-killers that impose a direct cost on their host can establish and persist. Sibling egg cannibalism could therefore be particularly adaptive in hosts infected with bacteria that impose direct costs on their hosts.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Kaplan–Meier survival distributions for Experiment 1: The value of an egg.

Figure S2 Relationship between egg value and egg mass (corrected for adult body size).

Table S1 Experiment 2: Pairwise *P* values from *post-hoc* LSD tests.

Table S2 Experiment 3: *P* values from *post-hoc* LSD tests for one-way ANOVA.

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