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**Parasitoid developmental mortality in the field: patterns, causes
and consequences for sex ratio and virginity**

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RUNNING HEAD: Developmental mortality and sex ratio

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Summary

1. Sex ratio theory predicts that developmental mortality can affect sex ratio optima under Local Mate Competition and also lead to ‘virgin’ broods containing only females with no sibling-mating opportunities on maturity.
2. Estimates of developmental mortality and its sex ratio effects have been laboratory based and both models and laboratory studies have treated mortality as a phenomenon without identifying its biological causes.
3. We contribute a large set of field data on *Metaphycus luteolus* Timberlake (Hymenoptera: Encyrtidae), an endoparasitoid of soft scale insects (Hemiptera: Coccidae), which has sex allocation conditional on host quality and female biased brood sex ratios. Developmental mortality within broods can be both assessed and attributed to distinct causes, including encapsulation by the host and larval-larval competition.
4. Thirty percent of *Metaphycus luteolus* offspring die during development with 65% of this mortality due to encapsulation and 28% due to larval competition. The distributions of mortality overall and for each cause of mortality separately were overdispersed.
5. The probability of an individual being encapsulated increased with clutch size while the probability of being killed by a brood mate declined with increasing clutch size and with increasing *per capita* availability of resources.
6. The sexual compositions of broods at emergence were influenced by both the degree and the type of mortality operating. At higher levels of mortality, single sex broods were more common and sex ratios were less precise. Overall, virginity was more prevalent than predicted and was more greatly affected by the occurrence of competition than by other sources of mortality, almost certainly because competition tended to eliminate males.
7. The reproductive and developmental biology of *Metaphycus luteolus* appears to be influenced by a complex interplay of maternal clutch size and sex allocation strategies, offspring-offspring developmental interactions, host defence mechanisms and post-emergence mating behaviour. Despite the great sophistication of sex ratio theory, it has not yet evolved to the point where it is capable of considering all of these influences simultaneously.

Key-words: Developmental mortality, encapsulation, facultative siblicide, facultatively gregarious parasitoid, field study, local mate competition

1 **Introduction**

2

3 Historically, sex ratio theory is one of the more thoroughly researched and important areas in
4 behavioural and evolutionary ecology (e.g. Seger & Stubblefield 2002; West 2009). A major
5 step was Fisher's (1930) development of a frequency-dependent selection explanation for the
6 sex ratio equality observed in many outbreeding species. Subsequently Hamilton (1967)
7 applied game-theoretic reasoning to explain the strongly biased sex ratios observed in many
8 inbreeding species. In Hamilton's model of local mate competition (LMC), offspring are
9 assumed to develop in groups in reproductively isolated and ephemeral patches, from which
10 mated daughters subsequently disperse. Under these assumptions, the optimal sex ratio
11 allocated by a 'foundress' to a patch depends on the number of contributing foundresses and
12 it is the best response in the presence of competing strategies, *i.e.*, an evolutionarily stable
13 strategy (ESS) (e.g. Freedberg 2002; West 2009); when small numbers of mothers contribute
14 offspring in a patch, sex ratio optima are strongly female biased. Extensions to Hamilton's
15 model under partial local mating (when mating occurs between, as well as within, offspring
16 groups) yield sex ratio optima that are less female biased for a given number of foundresses
17 (e.g. Nunney & Luck 1988; West 2009). When resource quality varies within a group and
18 fitness effects associated with resource quality are sexually differential, sex ratios are
19 predicted to be less female biased than under invariant resource quality conditions (Werren
20 1984). This prediction also holds when resources within an offspring group are uniformly
21 poor, provided there is inter-group variation in resource quality and partial local mating (e.g.
22 Ikawa *et al.* 1993). These latter predictions combine LMC theory with the third most
23 historically important development of sex ratio theory: sex allocation conditional on
24 environmental conditions such as resource quality (Trivers & Willard 1973; West 2009).
25 Responses to a combination of LMC and environmental quality are termed a 'mixed sex
26 allocation strategy' (Mayhew & Godfray 1997).

27

28 When just one foundress reproduces in a strictly isolated patch, Hamilton's (1967)
29 LMC model, which heuristically assumes that offspring group sizes are infinite (continuous
30 non-integer values), predicts an optimal sex ratio of 0.0 (proportion of offspring that are
31 male). This implies that a minimum number of sons are required to mate with the daughters.
32 With a single foundress, and under strictly local mating, a game-theoretic approach is not,
33 however, required to predict sex ratio optima because no competing foundress strategists are
34 present. A number of static optimality models have developed LMC theory for such cases,

1 assuming strictly local mating. Realistically, the offspring group sizes are relatively small
2 integers, and thus constrain the set of possible sex ratios. Models for a ‘discrete’ number of
3 offspring predict that sex ratios should be the reciprocal of group size, if a single male is
4 sufficient to mate all of his sisters (Green, Gordh & Hawkins 1982; Griffiths & Godfray
5 1988), and the sex ratios distributed across offspring groups manifest low (ideally zero)
6 variance, i.e., precise sex ratios (Green *et al.* 1982; Nagelkerke 1996). Limited mating
7 capacity of males or imperfect sex allocation control by foundresses each lead to greater
8 numbers of males expected in larger groups of offspring (Green *et al.* 1982, Nagelkerke
9 1996; Nagelkerke & Hardy 1994; Hardy *et al.* 1998; 2000).

10
11 A further consideration incorporated into static optimality models of single foundress
12 sex ratio optima has been that offspring may die between sex allocation and subsequent
13 maturity and mating. Foundresses are predicted to adjust their sex allocation decisions
14 according to the probability of developmental mortality and the offspring group size by
15 increasing the number of sons, as insurance against the production of unmated daughters, if
16 mortality (risk) and/or group size (value) increases (Green *et al.* 1982). A consequence of
17 such optimal sex allocation is that, at maturation, some offspring groups will contain no
18 males and the maturing females within these groups will remain virgin, with zero fitness if
19 mating is strictly local. Under a given mortality risk, the proportion of virgin broods is
20 expected to decrease with increasing offspring in a group (Heimpel 1994). Also, increasing
21 developmental mortality is expected to lead to increases in group sex ratio variance at
22 offspring maturity (Hardy *et al.* 1998). Optimal sex allocation is further predicted to be
23 influenced by the probability of male developmental mortality and its distribution across
24 offspring groups: under strict local mating, sex ratio optima are more greatly affected when
25 mortality variance is low and complete mortality of offspring in these groups has no effect
26 (Nagelkerke & Hardy 1994). In contrast, under partial local mating, complete mortality
27 influences sex allocation optima via its influence on the probability of non-local mating
28 (Freedberg 2002).

29
30 Much of the above sex ratio theory has been developed in conjunction with
31 experimental and comparative studies on parasitoid Hymenoptera. These have a haplo-
32 diploid sex determination system which allows maternal control of sex allocation, and a suite
33 of variations around a relatively simple core life-history (e.g. Godfray 1994). Locally mating
34 parasitoids manifest female biased sex ratios, approximating the reciprocal of group size and

1 low variance (Green *et al.* 1982; Griffiths & Godfray 1988; Hardy *et al.* 1998).
2 Developmental mortality also correlates with sex ratio variance and the proportion of virgin
3 broods across and within species (Hardy *et al.* 1998). The proportion of virgin broods within
4 species generally declines as offspring group size increases (Hardy & Cook 1995; Hardy *et*
5 *al.* 1998; Kapranas *et al.* 2009b).

6
7 Such studies of relationships between developmental mortality and sex ratios have,
8 however, had several limitations. First, most estimates of the sexual composition in
9 parasitoid offspring groups and their developmental mortality are laboratory-based whereas
10 those in the field may differ, thus limiting the degree to which laboratory evidence is relevant
11 (e.g. Kapranas *et al.* 2009b). More fundamentally, the static optimality models developed by
12 Green *et al.* (1982), Heimpel (1994) and Nagelkerke and Hardy (1994) do not consider
13 effects of environmental (host) quality, multiple-foundress cases or the possibility of partial
14 local mating; incorporating the latter two would require a game-theoretic approach (e.g.
15 Nagelkerke 1996; Freedberg 2002). Further, these models treat mortality in a
16 phenomenological manner, considering given means and distributions as fixed assumptions,
17 and predict a foundress' optimal sex allocation response to these conditions. This approach
18 may be appropriate for externally feeding (ectoparasitoid) species with scramble-type
19 competition between developing siblings, such as the members of the aculeate family
20 Bethylidae with which these models have been hitherto most tested (Hardy & Cook 1995;
21 Hardy *et al.* 1998). However, many parasitoid species develop inside their hosts
22 (endoparasitoids) and manifest contest-type inter-sibling interactions (e.g. Godfray 1987;
23 1994; Pexton *et al.* 2003; Tena *et al.* 2008; 2009; Segoli *et al.* 2010). The probability of
24 mortality due to encapsulation or within-host contests may not be independent of the initial
25 size and sexual composition of the offspring group (Godfray 1987; Rosenheim 1993;
26 Mayhew & Hardy 1998; Ode & Rosenheim 1998; Pexton *et al.* 2003; Tena *et al.* 2009).
27 Models exploring sex ratios under developmental mortality may thus require a more dynamic
28 interplay between sex allocation strategies and the subsequent offspring mortality.

29
30 Here we address some of the above limitations via a field-based evaluation of sex
31 ratio and developmental mortality in an endoparasitoid species, *Metaphycus luteolus*
32 Timberlake (Hymenoptera: Encyrtidae), in which the causes of developmental mortality that
33 operate within each offspring group can be identified. We show that mortality falls into at
34 least three biologically different classes; encapsulation by the host, larval-larval competition

1 and natural (intrinsic) causes, each occurring with a different frequency. Lastly, the sexual
2 compositions of broods are affected by both the degree and type of mortality operating.

3 4 *Biology of Metaphycus luteolus*

5
6 *Metaphycus luteolus* is a facultatively gregarious endoparasitoid of soft scale insects in the
7 genera *Coccus*, *Lecanium* and *Saissetia* (Hemiptera: Coccidae) and is considered native to
8 California (Bartlett 1978). The focal host of the current study, the brown soft scale, *Coccus*
9 *hesperidum* L., may have an African origin but is cosmopolitan and has been associated with
10 *M. luteolus*, one of its most important natural enemies in northern America, for at least a
11 century (Timberlake, 1913) and the host-parasitoid association is thus likely to be coevolved.
12 *Metaphycus* species account for *ca.*75% of brown soft scale parasitism and around 20% of
13 this is due to *M. luteolus* (Kapranas *et al.* 2007).

14 *Metaphycus luteolus* females are synovigenic, emerging from their hosts as adults
15 without mature eggs and then maturing eggs upon feeding on natural carbohydrate sources or
16 the haemolymph of their hosts (Kapranas & Luck 2008). This species is facultatively
17 gregarious and lays its eggs as clutches that range from one to nine eggs depending on host
18 size. Each deposited egg has a stalk-like structure that protrudes from the host's integument
19 and is thought to facilitate respiration of the egg and early instar larvae (Maple 1947). The
20 host's development is arrested upon parasitisation, as with other *Metaphycus* species
21 (Lampson, Morse & Luck 1996; Bernal, Luck & Morse 1998; 1999). Some *M. luteolus* eggs
22 are encapsulated by brown soft scale (Bartlett & Ball 1964; 1966; Kapranas *et al.* 2009a), as
23 is commonly observed in other encyrtid-soft scale associations (Blumberg 1997). The larvae
24 that hatch may then compete with conspecifics, including siblings, within a host and such
25 aggressive larval interactions result in the elimination and consumption of brood mates
26 (Bartlett & Ball 1964). Development from egg to adult takes 2-3 weeks. Adults of both sexes
27 are winged. Under laboratory conditions, females live 4–8 weeks when fed carbohydrate
28 (honey) and hosts whereas male longevity is 3-4 weeks, if fed carbohydrates only (Kapranas
29 & Luck 2008). Longevity and the possession of wings are likely to promote non-local and
30 non-sibling mating in the field while gregarious development is likely to promote local
31 mating (Kapranas *et al.* 2008).

32 33 **Materials and methods**

34

1 *Pre-exposure rearing of hosts*

2

3 We maintained a culture of brown soft scale on excised *Yucca recurvifolia* Salisbury
4 (Liliaceae) leaves grown hydroponically in the University of California, Riverside (UCR)
5 insectary. The leaves for the culture were obtained from plants grown in a plot at
6 Agricultural Operations, UCR.

7

8 *Field exposure of hosts*

9

10 Field experiments were carried out from February 2004 to March 2006 in eleven citrus
11 groves scattered throughout southern California, USA, generally following methods
12 described in Kapranas *et al.* (2007; 2008). We used yucca leaves infested with 20-28 day-old
13 brown soft scales; each leaf typically bore 200-300 individuals of variable size and
14 developmental stage. Because there were always substantially more soft scales present on
15 each of the yucca leaves than the numbers that were eventually parasitized (usually 15-20%
16 of hosts on any one leaf) our design minimises, rather than excludes, potential
17 superparasitism (see also Mackauer & Völkl 2002). Each leaf was placed in a water-filled
18 vial fixed on top of a one metre high stake, beneath the canopy of a citrus tree. Ants were
19 excluded from the yucca leaves by an insecticidal tape (chlorpyrifos) wrapped around the
20 stake mid-height above the ground. We placed three leaves in each citrus grove and replaced
21 them approximately biweekly.

22

23 *Post-exposure laboratory rearings*

24

25 Upon return to the laboratory, each yucca leaf was inspected for foraging adult wasps, which
26 were removed with an aspirator and killed. This precluded oviposition in the laboratory
27 environment. The leaves were maintained at ~25-28°C with naturally determined
28 photoperiod. The leaves were then inspected for the presence of parasitized scales, which can
29 be recognized by their mummification, i.e., their light yellow to golden colour and convex
30 shape. Also, the developing parasitoids are easily recognized and visible through the dead
31 scale's translucent cuticle. Each parasitized scale was isolated in a 3.0 × 0.8 mm glass vial
32 streaked with honey on the inside wall and stoppered with a cotton plug. Each sample leaf
33 was re-inspected for parasitized scales two to three times during the subsequent 15 days; the

1 period between inspections allowed immature parasitoids to develop to a stage that was
2 clearly visible.

3 4 *Parasitoid emergence*

5
6 We inspected vials with the developing parasitoids every 1-2 days for emergence and
7 emerged adult parasitoids were killed by freezing. The wasps (= brood) emerging from each
8 host was cold-stored separately in 95% alcohol. We verified that we had all of the emerged
9 brood, as each emerging parasitoid constructs a separate exit hole through the scale
10 integument. The number of exit holes always equalled the number of adults collected from
11 each host.

12 The species and sex of each emerged wasp was identified and recorded along with
13 the host scale's length and width to the nearest 0.01 mm, measured using an ocular
14 micrometer mounted in the eyepiece of a dissecting microscope. As scales are much more
15 closely discoid than spherical and because it was impractical to measure scale height, the
16 index of host size used was scale surface area, calculated as an ellipsoid ($\pi \times \text{width} \times \text{length} / 4$).

17 18 *Parasitoid developmental mortality*

19
20 Estimates of developmental mortality exclude cases of complete mortality of a clutch
21 because it is not possible to identify, to species level, dead immatures within a host. Each
22 host issuing *M. luteolus* adults was immersed in warm water for 5-10s to remove the
23 honeydew traces and other field debris from their cuticle and then inspected at
24 70×magnification. The number of stalks protruding through the integument indicated the
25 number of eggs that had been laid in the host (= clutch size) (Fig. 1). Visual inspection of the
26 integument of each host, followed by dissection, allowed us to determine the number of
27 encapsulated eggs (melanized capsules) per host (Fig. 1). A small proportion of dead larvae
28 was observed to have a melanotic appearance and these larvae were considered to have died
29 from probably 'intrinsic causes'. Cases in which the clutch size was greater than the sum of
30 the encapsulated eggs plus larvae dying of intrinsic causes plus the number of emerged
31 wasps (brood size) indicated direct physical competition between brood mates resulting in
32 the defeat and consumption of larvae (according to laboratory investigations on *M. luteolus*
33 and its congeners, larvae that lose fights are always consumed by the winners, Bartlett &

1 Ball 1964; Tena *et al.* 2008; 2009). We also recorded ‘mixed mortality’ when several causes
2 operated within a particular clutch.

3

4 *Statistical analysis*

5

6 Our general approach was to use Generalized Linear Models in which the assumed
7 distribution of residuals is matched, via initial assumptions and empirically estimated scale
8 parameters (which correct for over- or under-dispersion), to the structure of the data rather
9 than the ‘traditional’ approach of transforming data to fit standard Gaussian assumptions
10 (e.g. Crawley 1993; Wilson & Hardy 2002). Influences on clutch size, brood size and
11 numbers of males in a clutch were identified using log-linear analyses, which are appropriate
12 for small count data. Influences on sex ratios, the prevalence of all-female broods, and
13 developmental mortalities were explored using logistic analyses, which are appropriate for
14 proportional data. We report the percentage of deviance explained (%Dev) as an approximate
15 analogue of r^2 for log-linear and logistic models. We used backward stepwise procedures and
16 aggregation of factor levels to obtain the parsimonious ‘minimal adequate model’ by model
17 simplification (e.g. Crawley 1993; Wilson & Hardy 2002).

18 We used the variance ratio, R , as a descriptive statistic to quantify the variance of
19 brood sex ratios and developmental mortality across broods: $R = 1$ indicates a binomial
20 distribution while $R < 1$ and $R > 1$ indicate under- and over-dispersion, respectively (Krackow
21 *et al.* 2002). The Meelis test statistic, U , was used to assess the significance of any deviations
22 from binomiality (Krackow *et al.* 2002).

23

24 **Results**

25

26 Overall, we collected 2,758 parasitized scales; 494 of these scales were parasitized by *M.*
27 *luteolus*. The remaining scales were parasitized by other *Metaphycus* species or by species
28 belonging to other genera (Kapranas *et al.* 2007). A few scales yielded both *M. luteolus* and
29 another parasitoid species but this occurred rarely ($n = 7$). In some cases, scale size and/or
30 parasitoid mortality factors could not be assessed (see below) but each part of the analysis
31 was conducted using all suitable data available. There was a total data set of 395 clutches for
32 assessing mortality, of which 371 were gregarious (clutch size > 1).

33

34 *Pre-mortality clutch composition*

1
2 The number of eggs laid in each host (= clutch size) ranged between one and nine, although
3 95% of clutches contained 1-6 eggs, and clutch size increased with host size (log-linear
4 analysis corrected for underdispersion; $F_{1,403} = 289.72$, $P < 0.001$, %Dev = 41.9, Fig. 2). For
5 clutches of one egg (solitary clutches) and without developmental mortality (N = 24) the
6 probability of the offspring being a male (sex ratio) decreased as host size increased (simple
7 logistic regression; $G_1 = 11.20$, $P < 0.001$, %Dev = 51.8, Fig. 3). For clutches of more than
8 one egg (gregarious clutches) and with no developmental mortality (N = 127), the proportion
9 of offspring that were male (sex ratio) decreased significantly with clutch size (logistic
10 regression corrected for underdispersion: $F_{1,125} = 18.87$, $P < 0.001$, %Dev = 13.0) whereas
11 the number of males laid in each clutch increased (log-linear regression corrected for
12 underdispersion: $F_{1,125} = 12.25$, $P < 0.001$, %Dev = 9.0). These analyses were carried out
13 with the caveat that subsets of broods lacking developmental mortality may not be
14 representative of the overall primary sex ratio (Fiala 1980).

15

16 *Developmental mortality*

17

18 Thirty percent (428/1,449) of *M. luteolus* offspring developing in gregarious clutches died
19 before adulthood. Of these, 64% died due to egg encapsulation by the host, 27.6% died due
20 to intra-brood competition between larvae and 8.4% of other intrinsic causes.

21 In 34.23% (127/371) of gregarious broods all offspring survived (brood size at
22 emergence = initial clutch size), in 35.84% of clutches there was mortality solely due to
23 encapsulation (mean mortality within these clutches was 46.60%; in 57.14% [76/133] of
24 these clutches just one egg was encapsulated), in 16.98% of clutches there was mortality
25 solely due to larval competition (mean mortality within these clutches = 34%; in 73%
26 [46/63] cases just one larva was eliminated) and in 5.39% mortality was solely due to
27 intrinsic causes (mean mortality within these clutches = 41.55%; in 70% [14/20] of these
28 broods just one larva died of intrinsic causes). In some clutches (7.5%) two mortality sources
29 were evident, with encapsulation and competition co-occurring in about 71.4% of these cases
30 (mean mortality within these clutches = 53.1%). (All estimates of developmental mortality
31 necessarily excluded cases of complete mortality, see Methods, and are therefore likely to be
32 underestimates).

33 The overall distribution of mortality across clutches was significantly overdispersed
34 ($R = 1.569$, $U = 5.29$, $P < 0.001$) suggesting a tendency that broods members survived or

1 died collectively. Analyses of the distributions of each mortality source across all 371
2 clutches further showed consistent overdispersion (encapsulation mortality, $R = 2.351$, $U =$
3 15.5 , $P < 0.001$; competition mortality, $R = 1.532$, $U = 6.12$, $P < 0.001$; intrinsic mortality, R
4 $= 1.71$, $U = 8.87$, $P < 0.001$). Inclusion of broods with complete mortality would strengthen
5 all of these conclusions.

6 The probability of mortality affecting a brood was positively related to the initial
7 clutch size (logistic regression: $G_1 = 8.63$, $P = 0.003$, %Dev = 1.8): this overall relationship
8 was due to an increase in the probability of competition mortality ($G_1 = 13.74$, $P < 0.001$,
9 %Dev = 3.4) while there were no significant relationships for either encapsulation ($G_1 =$
10 3.43 , $P = 0.06$ [note marginal non-significance: the trend was higher encapsulation
11 probabilities in larger clutches], %Dev = 0.7) or for intrinsic mortality ($G_1 = 0.19$, $P = 0.77$,
12 %Dev = 0.001) (Fig. 4). We calculated an approximate index for resources available per
13 offspring within each host by dividing host size by clutch size (resource availability
14 correlated negatively with clutch size, Spearman' rank test: $r_s = -0.52$, $P < 0.001$) and then
15 repeated these analyses with resource availability as the explanatory variable. Overall, the
16 proportion of broods suffering mortality declined as resource availability increased ($G_1 =$
17 6.51 , $P = 0.011$, %Dev = 1.37). Again, the overall relationship was due solely to a decrease
18 in the proportion of broods manifesting competition mortality ($G_1 = 22.39$, $P < 0.001$, %Dev
19 $= 5.62$; encapsulation, $G_1 = 0.06$, $P = 0.81$, %Dev = 0.011, intrinsic mortality, $G_1 = 0.20$, $P =$
20 0.65 , %Dev = 0.10, Fig. 4). Because physical competition only occurs during the larval
21 stage, we explored the probability of competition mortality using host size divided by the
22 number of eggs that issued larvae. The result was similar but stronger ($G_1 = 63.38$, $P < 0.001$,
23 %Dev = 15.9). Across all broods with some mortality, the number of larvae that died
24 declined as resource availability increased (log-linear regression corrected for
25 underdispersion: $F_{1,241} = 43.37$, $P < 0.001$, %Dev = 16.4).

26 The likelihood of individuals dying increased weakly as the initial clutch size
27 increased (logistic regression corrected for overdispersion; $F_{1,369} = 4.78$, $P = 0.029$, %Dev =
28 1.27) but it differed depending on the cause of death within a clutch: encapsulation was
29 slightly more common among offspring in larger clutches whereas the proportion of
30 immature parasitoids dying from other sources declined with increasing clutch size (logistic
31 ANCOVA corrected for underdispersion: clutch size \times mortality category interaction: $F_{3,243} =$
32 3.24 , $P = 0.023$, %Dev = 3.5, Fig. 5). The increase in encapsulation as clutch size increased
33 was not due to larger hosts, which generally contained larger clutches, encapsulating a higher

1 proportion of parasitoids within broods (logistic regression corrected for underdispersion:
2 $F_{1,367} = 1.48$, $P = 0.228$).

3

4 *Developmental mortality and sex ratio*

5

6 Data from all gregarious clutches were used to explore effects of brood size and the
7 prevalence and type of developmental mortality on the brood sex ratio at adult emergence
8 (secondary sex ratio). Mortality type was assigned as a factor with five categorical levels
9 (none, intrinsic causes, encapsulation, competition or mixed) and the prevalence of mortality
10 was fitted as the proportion of eggs in a clutch that died (these analyses are unaffected by the
11 necessary exclusion of broods with complete mortality as such broods also have no
12 secondary sex ratios). Overall, the mean brood sex ratio was 0.262 (S.E. \pm 0.01), but sex
13 ratios decreased weakly as brood size and the prevalence of mortality increased (logistic
14 analysis of covariance corrected for underdispersion: brood size $F_{1,355} = 22.19$, $P < 0.001$,
15 %Dev = 5.55; mortality prevalence $F_{1,355} = 18.10$, $P < 0.001$, %Dev = 4.52, Fig. 6),
16 indicating that, overall, males have slightly higher developmental mortality than females.
17 The type of mortality affecting a clutch did not influence the brood's sex ratio significantly
18 ($F_{4,355} = 0.29$, $P = 0.88$, %Dev = 0.28). Also there were no significant interactions between
19 any of the fitted explanatory variables.

20

21 *Developmental mortality and sex ratio variance*

22

23 *Metaphycus luteolus* shows sex ratio precision: the sex ratios of gregarious broods were
24 significantly underdispersed (Table 1). Calculating variances for groups of broods separately
25 suggested that broods with no mortality had lower sex ratio variances than those affected by
26 any of the four types of mortality (Table 1). With the caveat that analysis of relationships
27 between variance ratios and other measured variables may not always be valid (Krackow *et*
28 *al.* 2002), we explored the effect of mortality on sex ratio variance in three ways. First we
29 correlated the variance ratio obtained within each mortality type with the mean mortality
30 experienced within that class (Table 1): these estimates were positively correlated
31 (Spearman's rank test: $r_s = 0.900$, $P = 0.004$). Second, we grouped broods by the degree of
32 mortality they experienced (0%, 0.1-10%, 10.1-20%, etc.), irrespective of mortality type. For
33 each group we calculated the variance ratio and regressed it against the mean mortality of the
34 group, initially including a quadratic term to allow for potential curvilinearity and weighting

1 each variance ratio according to the number of broods from which it was calculated (a proxy
2 for reliability). Variance ratios increased significantly and linearly with an increase in mean
3 mortality ($F_{1,5} = 70.26$, $P < 0.001$, $r^2 = 0.92$; quadratic term, $F_{1,4} = 2.03$, $P = 0.227$, $r^2 = 0.02$,
4 Fig. 7). We also observed that a weighted analysis gave a better fit to the assumptions of a
5 Gaussian distribution and constant errors variances compared with the equivalent un-
6 weighted regression. Both of these analyses lead to the same conclusion. Third, because a
7 high sex ratio variance may be associated with a preponderance of single-sex broods, we
8 carried out a logistic ANCOVA on whether or not broods contained just one sex of offspring
9 in relation to i) the proportion of offspring dying within those broods and ii) the category of
10 mortality operating (broods without mortality were excluded). Single sex broods were
11 significantly more common when mortality was prevalent ($G_1 = 79.34$, $P < 0.001$, %Dev =
12 24) and this depended on the category of mortality operating via a significant interaction
13 between proportion and category of mortality ($G_3 = 10.73$, $P < 0.013$, %Dev = 3.2). For
14 encapsulation and competition, sex ratio variances were less sensitive to the prevalence of
15 mortality than they were with intrinsic or mixed mortality. When overall mortality rates were
16 low, competition tended to generate single sex broods most frequently. Collectively, these
17 analyses show that sex ratio variance is affected both by the degree and the cause of
18 mortality operating.

19

20 *Developmental mortality and the optimal number of male eggs*

21

22 We calculated the maternal optimal number of male eggs at clutch oviposition assuming
23 single foundress clutches, strict local (within brood) mating and also that one adult male is
24 able to inseminate all emerging females (Green *et al.* 1982; Heimpel 1994). The optimal
25 number of male eggs, s , is predicted to depend on both the clutch size, c , and the probability
26 of mortality, m , and is the number that maximises the mean number of mated daughters, Dm ,
27 according to Heimpel's (1994) equation $Dm = (1-m^s)(c-s)(1-m)$. Calculations first used the
28 probability of developmental mortality estimated across all clutches and then, used clutch
29 size specific mortality estimates because we found a relationship between mortality and
30 clutch size (see above).

31 The mortality estimates derive from male and female mortality combined, although
32 only male mortality, which is likely to be higher in *M. luteolus* (see above), is predicted to
33 influence maternal optima (Nagelkerke & Hardy 1994). Further, Heimpel's (1994) equation
34 assumes a binomial distribution of individual mortality across clutches, while in *M. luteolus*,

1 mortality is overdispersed (see above), which is predicted to reduce the influence of
2 mortality on maternal sex ratio decisions (Nagelkerke & Hardy 1994). Our optimality
3 calculations are therefore approximate but these two ways in which *M. luteolus* mortality
4 does not match assumptions are expected to have opposing influences and thus may
5 effectively cancel. Using the estimate of overall mortality, the optimal number of male eggs
6 in clutches of 1 to 5 eggs was one, and in clutches of 6 to 9 was two. A similar result was
7 obtained using clutch size specific mortalities but the switch to laying two male eggs
8 occurred at clutch sizes of 7.

10 *Developmental mortality and the prevalence of virgin broods*

12 For each clutch size we calculated the expected proportions of ‘virgin broods’, containing
13 only females at emergence, using the optimal number of male eggs and the mortality
14 estimates as above (this proportion is equal to m^s , Heimpel 1994; Hardy & Cook 1995). The
15 expected proportion of virgin broods was 0.295 for clutches of 1-5 eggs, and 0.087 for
16 clutches of 6-9 using the overall mortality estimate. Predictions were similar when using
17 clutch size specific mortality estimates, except that virginity was predicted to be more
18 prevalent in broods developing from larger clutches (Fig. 8a). There was no relationship
19 between the probability of virginity and clutch size (simple logistic regression, with each
20 brood entered as virgin or non-virgin; $G_1 = 1.57$, $P = 0.211$, %Dev = 0.33, overall proportion
21 of virginity = 0.334, Fig. 7a). Observed virginity was significantly more prevalent than
22 expected (χ^2 -test: Using overall mortality, $\chi^2 = 43.2$, d.f. = 7, $P < 0.001$; Using clutch size
23 specific mortality, $\chi^2 = 33.3$, d.f. = 7, $P < 0.001$).

24 We next explored whether there was an effect of mortality category (see above) on
25 the proportion of broods that were virgin, using logistic ANCOVA, initially including
26 mortality as a factor with five levels and clutch size as a variate. The type of mortality
27 experienced had a significant effect on the probability of virgin broods ($G_4 = 32.10$, $P <$
28 0.001 , %Dev = 27.1) and there was a significant interaction with clutch size ($G_4 = 12.4$, $P =$
29 0.042 , %Dev = 2.1). Attempts at model simplification by progressively aggregating factor
30 levels showed that the ‘encapsulation’, ‘intrinsic’ and ‘mixed’ categories of mortality did not
31 differ significantly in their effects on virginity, but ‘competition’ and ‘no mortality’ could
32 not be merged with other categories. Virginity decreased as clutch size increased for
33 competition and other sources of mortality, but among broods without mortality, virginity
34 was almost absent irrespective of the clutch size (Fig. 8b).

1 Finally, using logistic ANCOVA, we explored the relationship between the
2 proportion of virgin broods and the proportion of offspring in those broods that died. Broods
3 were also classified by the type of mortality that was observed (i.e., intrinsic mortality,
4 encapsulation, competition, and mixed mortality; broods lacking mortality were excluded).
5 Virginity increased significantly with the prevalence of mortality ($G_1 = 32.81$ $P < 0.001$,
6 %Dev = 10.0) and aggregation of factor levels showed that virginity in broods with mortality
7 due to encapsulation, competition and intrinsic causes did not differ significantly in their
8 relationships with increasing mortality, but broods with ‘mixed’ causes of mortality were
9 significantly more sensitive to the prevalence of mortality (interaction term: $G_1 = 7.19$, $P <$
10 0.007), indicating a synergistic effect of mortality causes (Fig. 9).

11

12 **Discussion**

13

14 The observed reproductive biology of *M. luteolus* is typical of facultatively gregarious
15 endoparasitoids of soft scale insects. Their offspring generally develop in small broods, the
16 size of which depends on host size (e.g. Kapranas *et al.* 2008). In single egg clutches, sex
17 allocation was host size dependent, as has been observed in many solitary parasitoids,
18 including *Metaphycus* species (e.g. Lampson *et al.* 1996; Bernal *et al.* 1999; Godfray 1994;
19 West & Sheldon 2002; Kapranas *et al.* 2008; West 2009). However, this relationship was not
20 detected in a previous field study of *M. luteolus* sex allocation (Kapranas *et al.* 2008). The
21 sex ratios of larger broods lacking developmental mortality decreased with brood size, as
22 observed within and across other parasitoid species (e.g. Griffiths & Godfray 1988; Hardy *et*
23 *al.* 1998; Smart & Mayhew 2009; see Abe *et al.* 2009 for a recent exception) and also
24 showed low variance. Sex ratio precision is achieved by non-random sequences of sex
25 allocation, with one or two male eggs usually laid at the end of an oviposition sequence
26 (Kapranas *et al.* 2009b), again in common with many other species of parasitoids
27 experiencing LMC (e.g. Hardy 1992). Thus, *M. luteolus* females control clutch size and sex
28 allocation, with sex ratio optima apparently influenced by a combination of host-size
29 dependence and some degree of LMC, i.e., a ‘mixed sex allocation strategy’ (Mayhew &
30 Godfray 1997).

31

32 *Developmental mortality*

33

1 At least thirty percent of *M. luteolus* offspring died before maturity under field conditions.
2 This concurs with laboratory based-estimates for *M. luteolus* mortality ($\approx 40\%$, including
3 broods with complete mortality, Kapranas *et al.* 2009b) and alleviates concerns that
4 laboratory estimates of parasitoid mortality may be generally inaccurate (e.g. Hardy *et al.*
5 1998; Kapranas *et al.* 2009b). In particular, the relatively minor difference in these estimates
6 suggests that the complete mortality of broods is not extremely common in *M. luteolus* and
7 thus, that the exclusion of field data from such broods will not greatly affect our major
8 conclusions (note also that estimates of virginity and secondary sex ratio are unaffected by
9 exclusion of broods in which both males and females have all died).

10 Encapsulation was the most common cause of mortality ($\approx 70\%$ of all mortality) and
11 its rate increased slightly with increasing clutch size, although there was no significant
12 relationship. This might be due to the fact that larger clutches tend to be laid in larger hosts
13 but larger hosts are generally more resistant to parasitism via encapsulation (Blumberg
14 1997). The absence of any significant relationship between encapsulation rates and either
15 host size or clutch size could also be because data from broods with complete encapsulation
16 could not be included. Complete encapsulation was less frequent when clutch sizes were
17 larger in the solitary encyrtid *Comperiella bifasciata* Howard (Rosenheim & Hongkham
18 1996; Ode & Rosenheim 1998) and when clutch sizes were increased by superparasitism in
19 *Metaphycus flavus* (Howard) (Tena *et al.* 2008). From the host's perspective, complete
20 encapsulation is the only successful outcome of the post-parasitism host-parasitoid
21 interaction.

22 The second most common cause of mortality ($\approx 22\%$) was intra-brood larval
23 competition and intrinsic causes accounted for $\approx 8\%$. In contrast to trends for encapsulation,
24 mortality due to competition and intrinsic causes was less prevalent overall among offspring
25 developing from larger clutches. Although the likelihood that at least some competition
26 mortality occurred was greater among larger clutches, the number of larvae that were killed
27 was smaller. Competition mortality was clearly dependent upon the availability of resources
28 to developing offspring, only commonly occurring when resources were relatively scarce
29 (see also Tena *et al.* 2009). Intrinsic mortality showed no such pattern, suggesting that this
30 category of mortality was not due to a relatively cryptic form of inter-larval resource
31 competition such as physiological suppression.

32 Mortality was not randomly distributed across broods. Members of broods tended to
33 survive or die collectively, as observed in other parasitoid species and across a range of other
34 taxa (Hardy *et al.* 1998; Freedberg 2002). Although statistical overdispersion is commonly

1 caused by probability variation across categories within a data set (e.g. Krackow *et al.* 2002;
2 Wilson & Hardy 2002) and identified causes of *M. luteolus* mortality each had different
3 means, separate analyses for each mortality type showed that mortality was overdispersed
4 regardless of whether it was due to encapsulation, competition or intrinsic causes. This was
5 true even though the modal number of offspring dying was just one when mortality occurred.
6 These estimates further suggest that prior reports of overdispersed parasitoid mortality
7 represent biologically-based overdispersion rather than statistical mixtures of binomially or
8 sub-binomially distributed biological effects.

9

10 *Sex ratio consequences of developmental mortality*

11 Developmental mortality had a weak effect on mean brood sex ratio, indicating that mortality
12 is slightly more common among males than among female offspring. Candidate explanations
13 for sexually differential mortality include intrinsic differences between the sexes (e.g. the
14 expression of deleterious mutations in male haploids, Smith & Shaw 1980) and sexually-
15 asymmetric susceptibility to extrinsic factors such as competition or encapsulation (discussed
16 below).

17 Previous field-based estimates of parasitoid brood sex ratio variances have variously
18 reported under-dispersion, binomiality and over-dispersion but were either unable to provide
19 associated estimates of developmental mortality (Mackauer & Völkl 2002) or were
20 constrained to laboratory estimates of the mortality of broods collected in the field (Hardy *et al.*
21 1998). Laboratory estimates, within and across species of gregarious parasitoids, indicate
22 that sex ratio variances are increased by mortality, as would be expected if mortality has a
23 random component (Hardy *et al.* 1998). In *M. luteolus*, mortality clearly affected sex ratio
24 variance in the field; despite sex ratio precision (R values were consistently < 1), variances
25 generally increased with increasing mortality. The occurrence of single-sex broods (which
26 were mostly all-female broods, as also found by Kapranas *et al.* 2008) further suggests that
27 variance was not solely affected by random mortality because, for a given proportion of
28 developmental mortality, brood compositions at emergence were differentially affected by
29 the cause of mortality. In particular, when the overall proportion of mortality was low,
30 single-sex broods were observed with relatively high frequency when the mortality was due
31 to competition. This suggests that small numbers of male *M. luteolus* were eliminated by
32 females in these broods.

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34 *Virginity consequences of developmental mortality*

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Virginity (broods containing no males at emergence) is an aspect of variation in brood sex ratio for which optimality theory provides explicit predictions (Heimpel 1994). Virginity was virtually absent among those gregarious broods in which developmental mortality did not occur. Among broods with some mortality, the probability of virginity was higher when the proportion of mortality was higher (tallying with findings for the occurrence of single-sex broods). These findings indicate that mortality, rather than factors such as limited maternal control of sex allocation, is the predominant cause of virginity in *M. luteolus*, matching the conclusions of other recent studies (Kapranas *et al.* 2008; 2009b).

Under strict LMC and optimal sex allocation, the overall degree of developmental mortality observed in *M. luteolus* is predicted to lead to a lower proportion of virginity when offspring develop from larger clutches. In fact, the prevalence of virginity was independent of clutch size; this contrasts with laboratory data on *M. luteolus*, in which virginity declined with clutch size (explaining only 6.5% of the deviance) but not with results for two other *Metaphycus* species (Kapranas *et al.* 2009b). The prevalence of *M. luteolus* virginity was also greater than expected overall. The latter observation could be explained by a higher incidence of mortality among males than is assumed in our calculations.

Prior laboratory studies on species in other parasitoid families have, in contrast, generally found a qualitative fit to the expected overall prevalence of virginity and the predicted relationship with clutch size. However, in those species, sexually differential mortality appears to be absent (Hardy *et al.* 1998; 2000). Prior studies have not been able to explore the effects of different sources of mortality on the prevalence of virginity; doing this showed that in *M. luteolus*, virginity declined with increasing clutch size, provided some mortality occurred. The relationship was strongest for mortality due to larval competition; as above, this suggests that in small broods, initially containing high proportions, and small numbers, of males, these males tended to be eliminated when competition occurred (as found in *Metaphycus flavus* and other parasitoid species, Tena *et al.* 2009).

Conclusions and implications

Our findings indicate that *M. luteolus* foundresses laid larger clutches into larger hosts; this response to host size results in less resource per offspring in larger clutches and consequently, a higher probability of competition occurring between larvae within larger broods. The probabilities of other causes of mortality operating within a brood are unrelated

1 to clutch size or resource availability. Within broods with competition, the proportion of
2 offspring dying averaged *ca.* 0.3-0.4 and declined with increasing clutch size. Sex ratios
3 were initially female biased and precise but developmental mortality lead to a high
4 proportion of single-sex (including ‘virgin’) broods at offspring maturity. Virginity was more
5 prevalent than predicted from the overall mean mortality but this is at least partially
6 explained by higher male *versus* female mortality. Virginity did not decline with increasing
7 clutch size overall, but declined among broods experiencing some mortality and according to
8 the cause of mortality. When competition mortality occurred in small clutches, virginity
9 frequently resulted; in larger clutches all males initially present were rarely eliminated by
10 competition.

11 Our data, along with results from recent studies of *M. luteolus* and its congeners
12 (Kapranas *et al.* 2008; Tena *et al.* 2009), indicate that competition mortality is higher among
13 males than among females. Two candidate, and mutually non-exclusive, explanations for
14 sexually asymmetric competitive outcomes within small clutches are that: i) male larvae are
15 physically less able competitors than females or even that females are more aggressive than
16 males (Tena *et al.* 2009) and ii) females benefit more from siblicide than do males, due to the
17 genetic relatedness asymmetries between male haploids and female diploids (e.g. Godfray
18 1987; 1994; Mayhew & Hardy 1998; Ode & Rosenheim 1998; Giron *et al.* 2004; Gardner *et*
19 *al.* 2007). The latter requires the ability of immatures to assess relatedness within a host,
20 which has been demonstrated in other endoparasitic encyrtids (Giron & Strand 2004; Giron
21 *et al.* 2004) and seems likely in *M. flavus* (Tena *et al.* 2008; 2009). Eliminating brood mates
22 via competition increases the *per capita* resources available to survivors but carries inclusive
23 fitness costs which are higher when eliminated competitors are more closely related and
24 more numerous (e.g. Mayhew & Hardy 1998). The incidence of competition mortality is thus
25 expected to be brood-size dependent; in particular, siblicide should be rare when clutch sizes
26 are greater than *ca.* 3-4 eggs, especially when sex ratios are female biased (Godfray 1987;
27 Segoli *et al.* 2010). The vast majority of *M. luteolus* broods showed these characteristics and
28 while the occurrence of at least one contest per brood is greater among larger broods, the
29 proportion of individuals killed declines. The incidence of siblicide may also be reduced in
30 single sex broods with high intra-brood relatedness (Rosenheim 1993; Ode & Rosenheim
31 1998). In *M. luteolus*, single sex broods are common, but only as a result of (usually male)
32 mortality, as very few broods are single sex at oviposition (Kapranas *et al.* 2009b). Models
33 of siblicide, however, assume that offspring within a host are full siblings but
34 superparasitism may not be entirely absent in *M. luteolus* (see also Tena *et al.* 2008 for *M.*

1 *flavus*), which could explain a high incidence of competitive elimination in *M. luteolus*
2 (Godfray 1987) while attachment of early instar larvae to the hosts integument (Maple 1947)
3 could help to explain why several (usually female) members of a brood often survived when
4 competitive elimination of (usually male) brood mates occurred (Pexton *et al.* 2003; Tena *et*
5 *al.* 2009).

6 A further aspect of *M. luteolus*' life history that could promote within-host inter-
7 sexual competition is non-local mating (e.g. Gardner *et al.* 2007). Little is known about the
8 mating structure of *M. luteolus*: males are winged and long-lived so they are likely able to
9 disperse from the host from which they emerged and mate with females from other broods,
10 favouring less biased sex ratios than would be observed under strictly local (within brood)
11 mating (Nunney & Luck 1988; Hardy 1994; Gardner *et al.* 2007; West 2009). In our study,
12 developmental mortality resulted in 32.8% of gregarious broods being all-female. Under
13 strict LMC, 30.6% of the females developing from all gregarious broods would remain
14 unmated and would consequently be constrained to produce only male offspring. However,
15 production of all-male *M. luteolus* clutches is rare (4% of all gregarious clutches, see also
16 Kapranas *et al.* 2008), indicating that most ovipositing females are mated and thus, that
17 females from all-female broods mate non-locally. Non-local mating would reduce the cost of
18 all-female brood production, but would also decrease the advantage of female-biased sex
19 ratios and consequently the selective advantage of sex ratio precision (Nunney & Luck 1988;
20 Hardy 1994; Kapranas *et al.* 2008; West 2009).

21 The reproductive and developmental biology of *Metaphycus luteolus* appears to be
22 influenced by a complex interplay of maternal clutch size and sex allocation strategies,
23 offspring-offspring interactions and host defence. Despite considerable developmental
24 mortality and probable partial local mating, brood sex ratios have low variance yet, because
25 of considerable mortality, virgin broods are common, particularly due to sexually
26 asymmetric resource competition. Despite the great sophistication of sex ratio theory (e.g.
27 Godfray 1994; West 2009) it has yet to be developed sufficiently to consider all of these
28 influences simultaneously. A tangible next step might be to develop predictive models of sex
29 ratio strategies that take into account differences in the causes of mortality as well as its sex
30 specific means and distributions.

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33

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18 University Press, Cambridge.
- 19

1 **Table 1.** Variance of brood sex ratios among clutches with different classes of mortality
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Clutch types	Initial number of gregarious clutches	Mean egg to adult mortality (%)	Number of gregarious broods (≥ 2) for variance analysis	Sex ratio variance		
				<i>R</i>	U	p
All clutches	371	28.7	293	0.387	-7.4	< 0.001
Without mortality	127	0	127	0.090	-7.91	< 0.001
With some mortality	244	43.7	167	0.619	-3.42	< 0.001
Intrinsic mortality	20	41.6	12	0.373	-1.43	NS
Competition mortality	63	34.0	56	0.549	-2.65	< 0.01
Encapsulation mortality	133	46.6	78	0.640	-2.39	< 0.05

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1 **Figures legends**

2

3 **Fig. 1.** A brown soft scale parasitized by *Metaphycus luteolus*. The scale host has had 7
4 parasitoid eggs laid into it. Four eggs have been encapsulated (black arrows). Three eggs
5 have hatched and larval intestines are visible (light yellowish areas, white arrows).
6 protruding white-coloured stalks of parasitoid eggs are also visible.

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8 **Fig. 2.** Influence of host size on clutch size.

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10 **Fig. 3.** Influence of host size on the sex of offspring emerging from solitary clutches.

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12 **Fig. 4.** Influence of clutch size and resource availability on the probabilities of different
13 types of mortality. Each fitted line is the parsimonious description of the relationship derived
14 from a separate logistic regression of the probability of a particular source of mortality
15 occurring in a brood (non-significant relationships are plotted as means). Data are binary and
16 not shown.

17

18 **Fig. 5.** Influence of clutch size on the prevalence of different types of mortality. Regression
19 lines for each cause of mortality are shown separately; the categories ‘Intrinsic’ and
20 ‘competition’ did not, however, differ significantly ($F_{2,243} = 0.80$, $P = 0.45$). Data points are
21 shown slightly vertically displaced to indicate sample sizes. The fan shape of the data is due
22 to necessary exclusion of broods with complete survival and complete mortality, and the
23 limited range of proportions possible from small integer data.

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25 **Fig. 6.** Influences of brood size (panel a) and developmental mortality (panel b) on brood sex
26 ratio. Data points are shown slightly vertically displaced to indicate sample sizes.

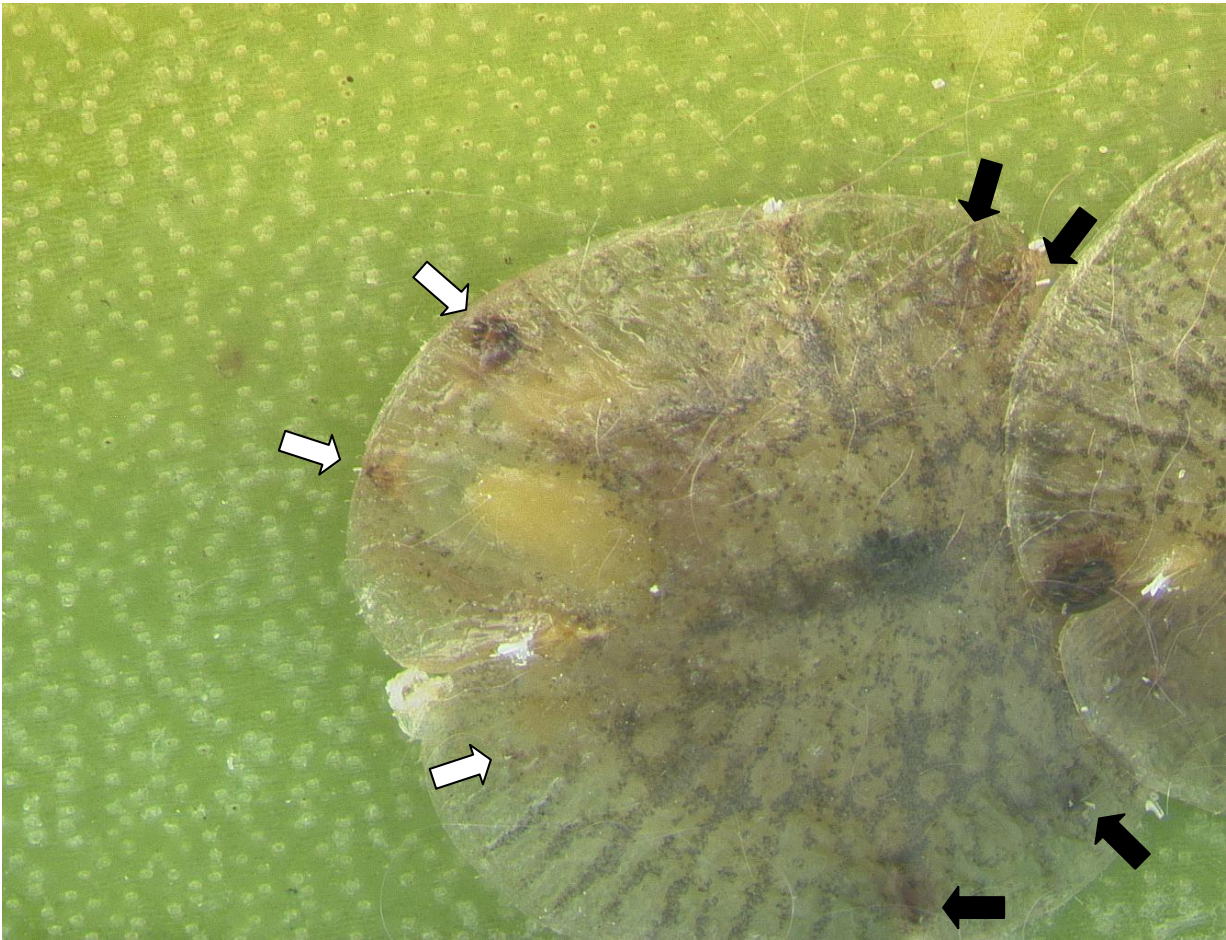
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Fig. 7. Relationship between brood sex ratio variance and developmental mortality. Numbers above data points indicate the number of broods from which each variance ratio is estimated.

Fig. 8. Relationships between the prevalence of virgin broods and clutch size. Panel a shows the proportion of virginity predicted using both the overall mean mortality and the clutch size specific estimates. Observed proportions are illustrated by the proportions of virginity at each clutch size (data points) and the overall mean (line with slope of zero, which is the minimal adequate regression model). Panel b shows relationships between the proportion of virginity (data are binary as individual broods either were or were not virgin, but are shown slightly vertically displaced to indicate sample sizes) and clutch size according to the class of mortality experienced by members of the brood.

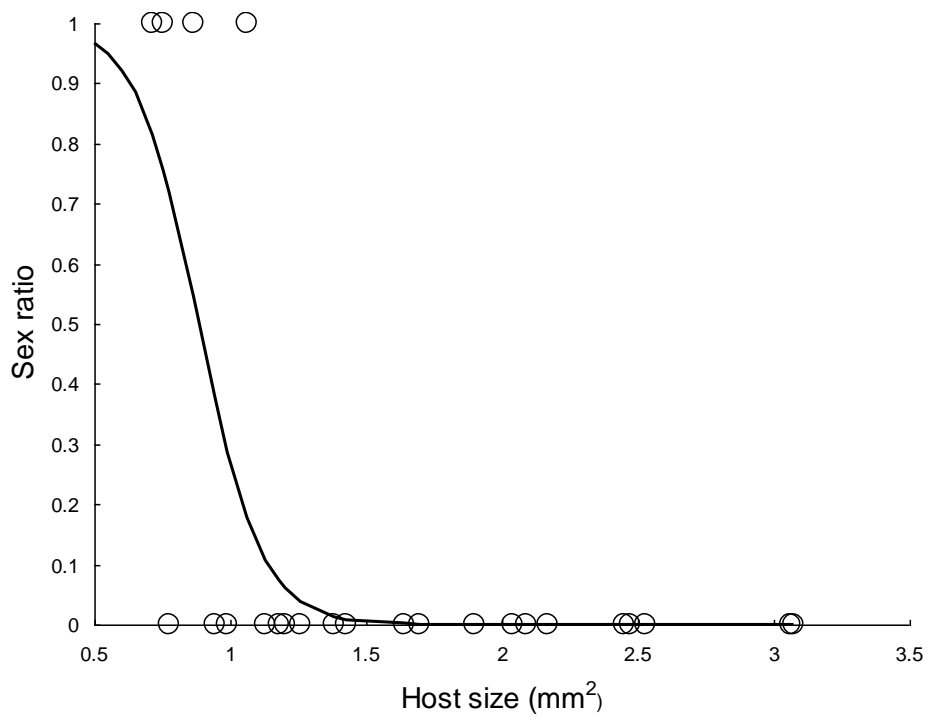
Fig 9. Relationships between the probability of a brood being virgin and the degree and source of developmental mortality experienced by brood members.

1 FIGURE 1



1 FIGURE 3

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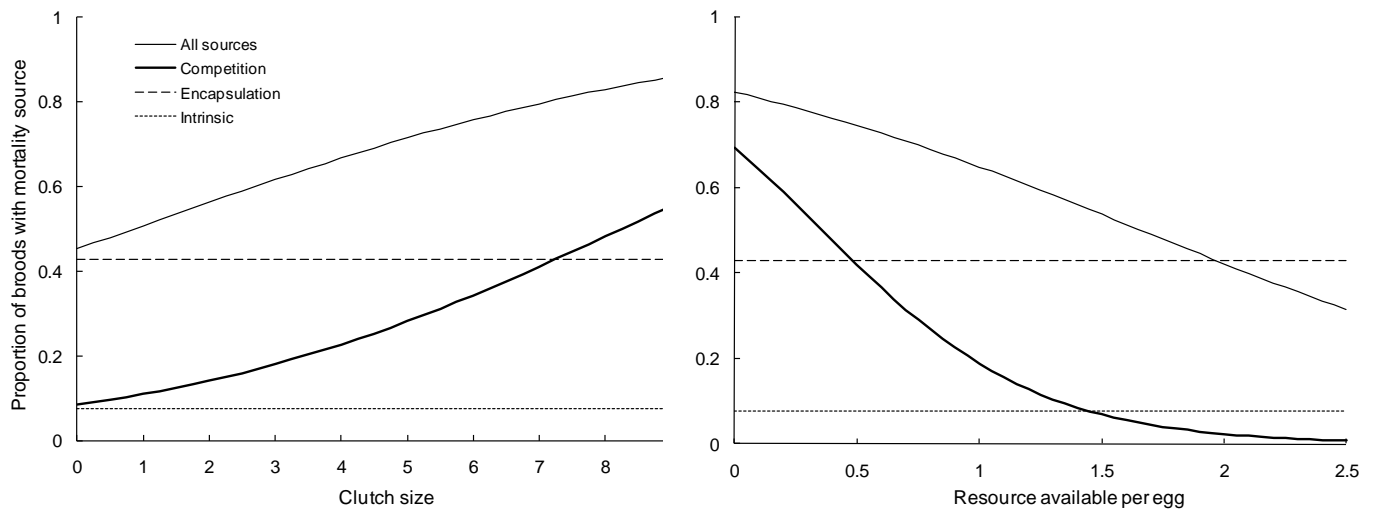
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1 FIGURE 4

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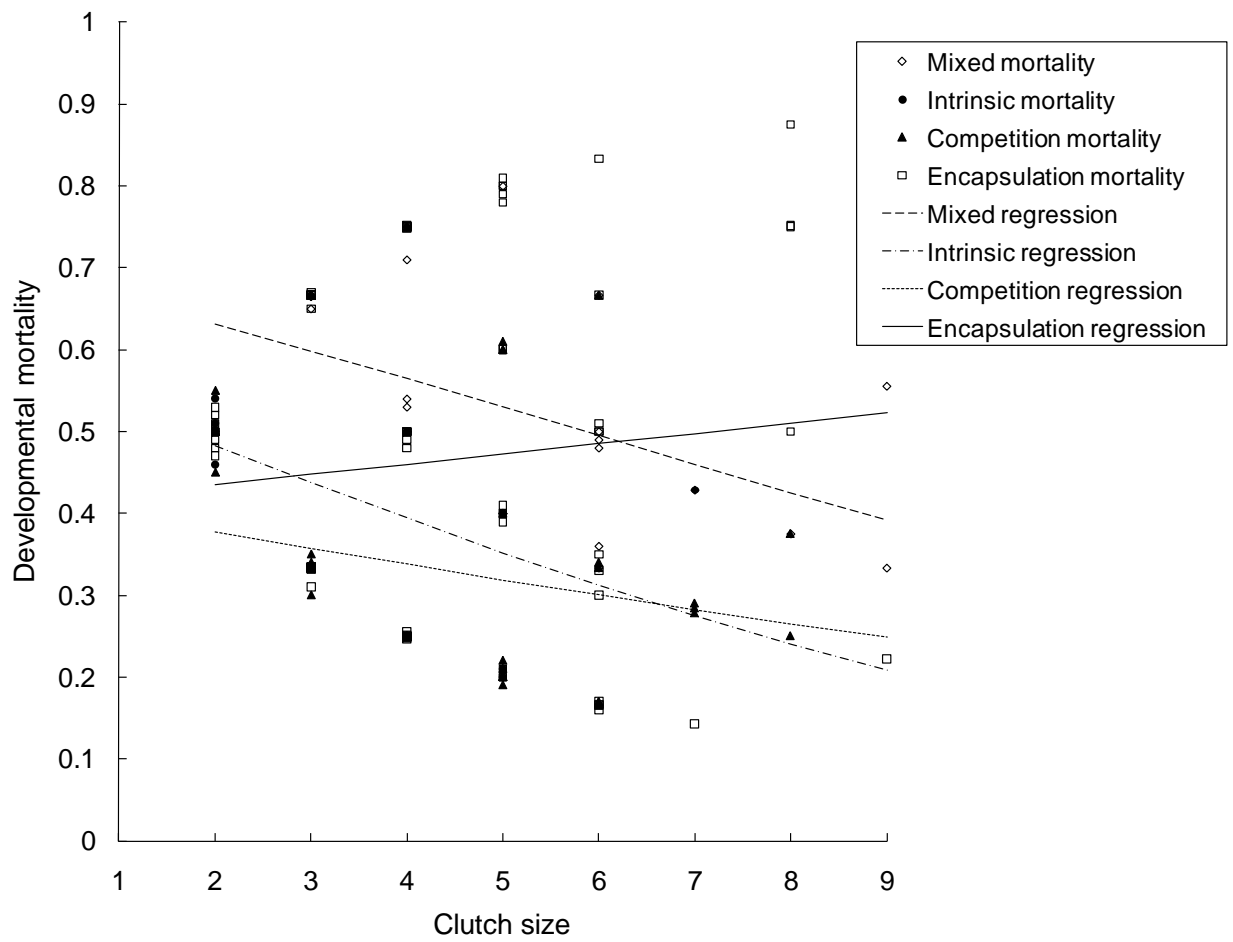
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1 FIGURE 5

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1 FIGURE 6

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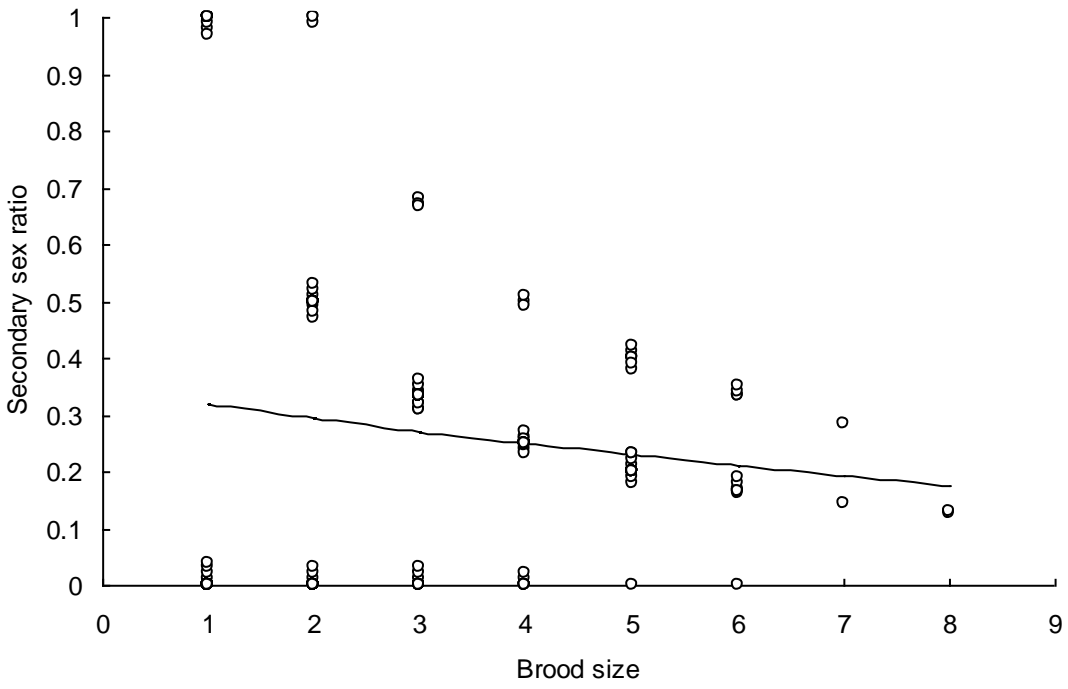
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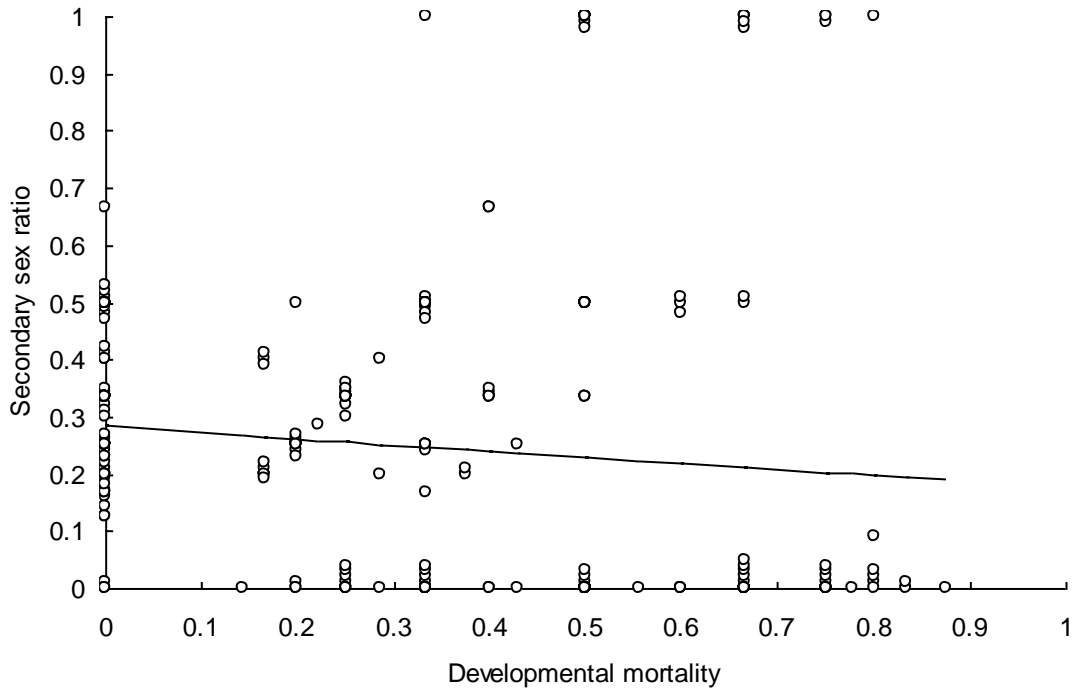
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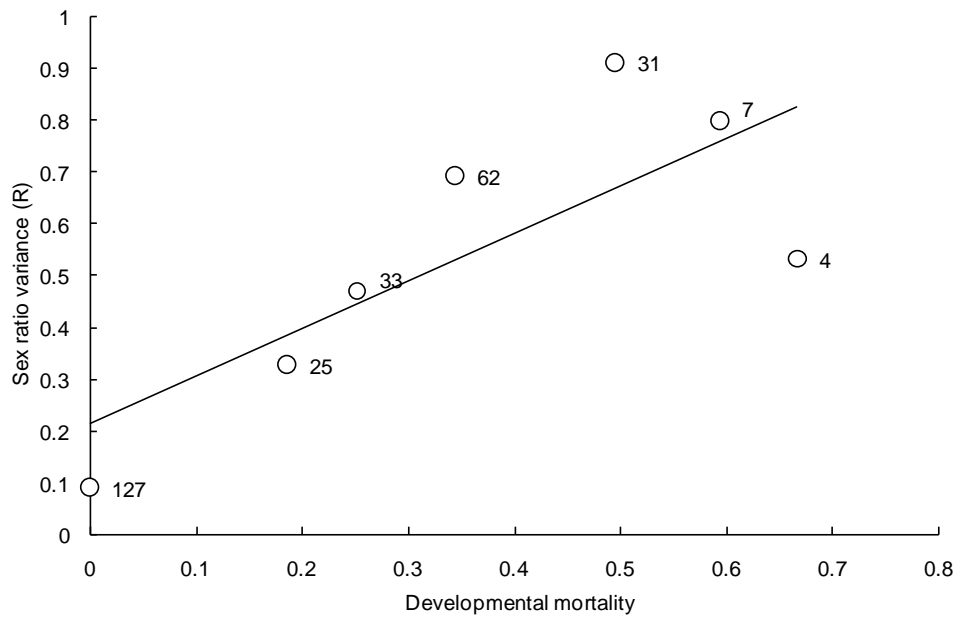
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1 FIGURE 7

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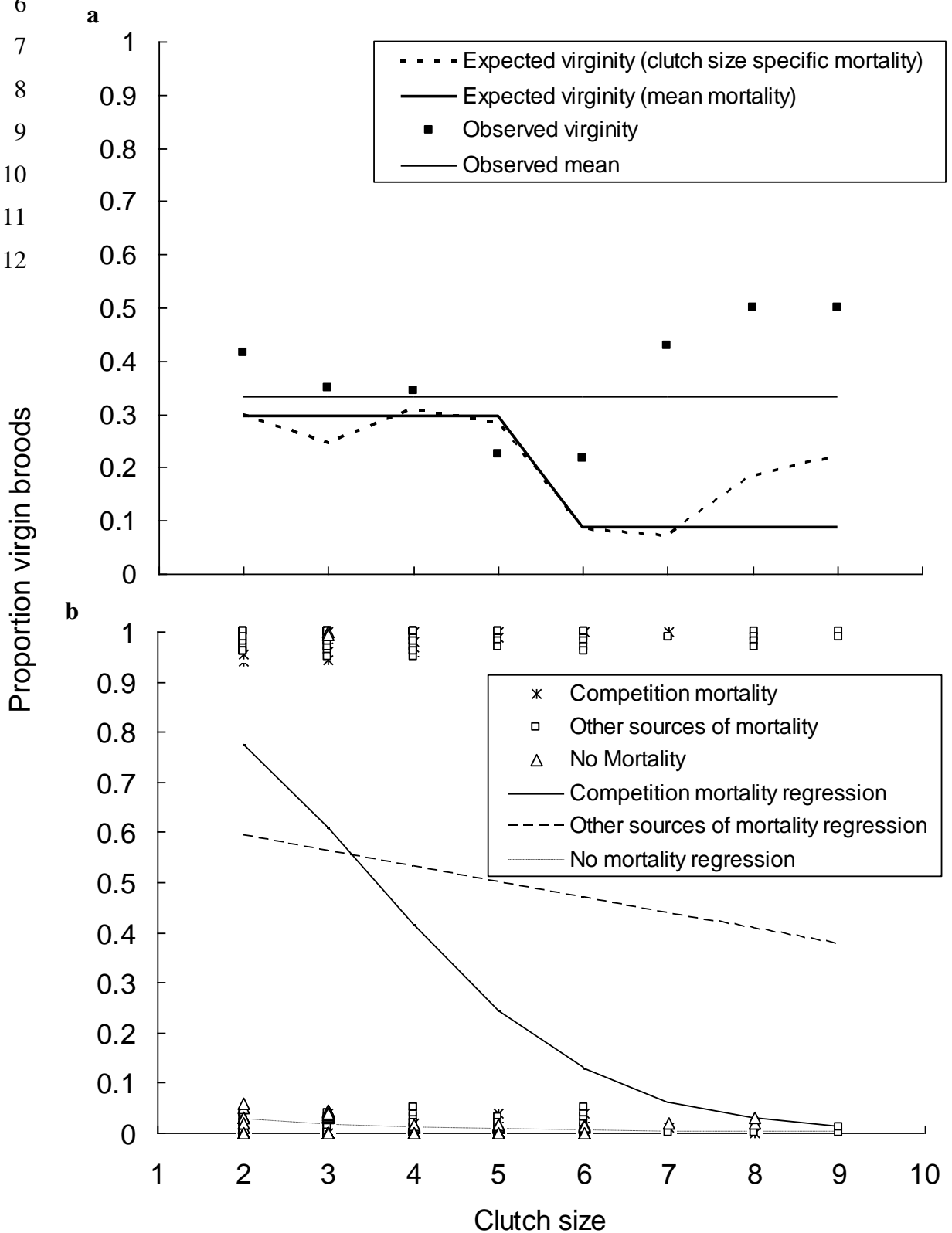
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1 FIGURE 8

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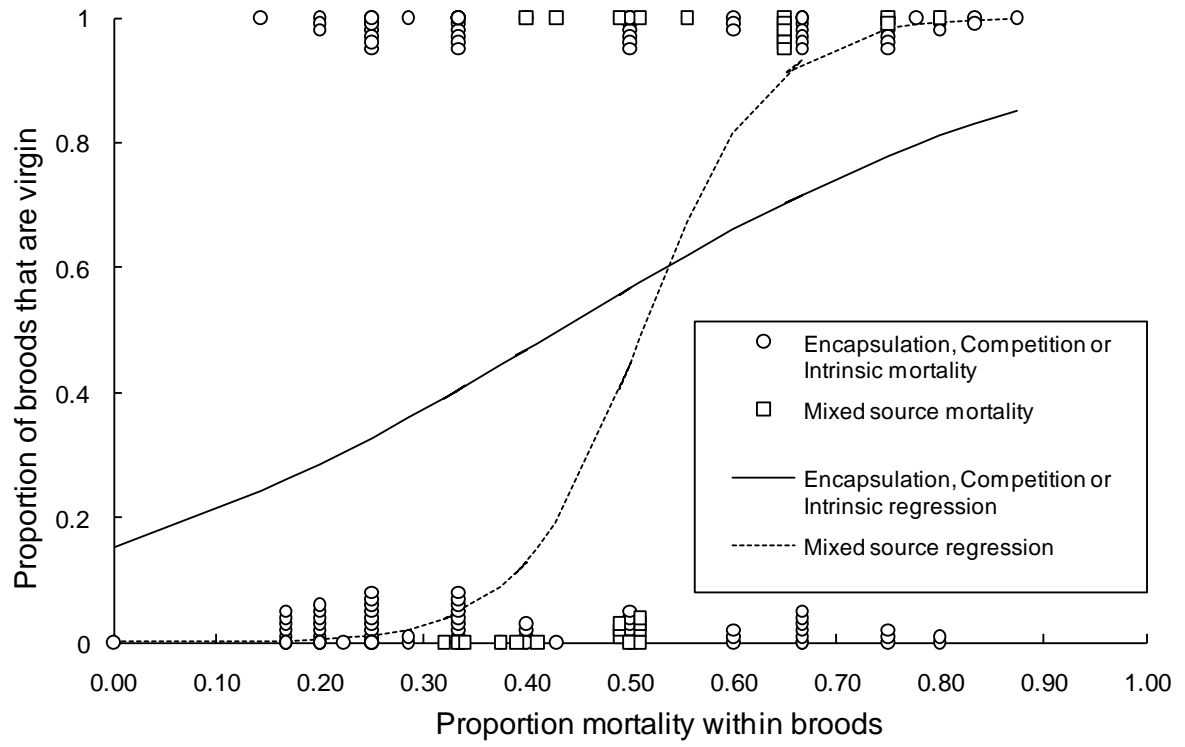


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2 FIGURE 9

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