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Testing predictions of small brood models using parasitoid wasps

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ABSTRACT

Question: How is variation in offspring size (between broods) related to brood size?

Hypotheses: Variance in offspring size (between broods) should decrease with increasing brood size as predicted by Charnov and colleagues' (Charnov and Downhower, 1995; Charnov *et al.*, 1995) small brood invariant. The range in resources put towards reproduction (for mothers producing a certain brood size) should be invariant over brood size (Downhower and Charnov, 1998). We also test assumptions underlying these predictions.

Data studied: We use previously collected data on six parasitoid wasp species.

Conclusions: As predicted, variance in offspring size among broods decreased with increasing brood size. However, this decrease did not follow closely the quantitative predictions of Charnov and colleagues (Charnov and Downhower, 1995; Charnov *et al.*, 1995). We found some support for the prediction that the range in resources invested in reproduction is invariant over brood size. The assumption that mean offspring size is constant over brood size was violated in three of six species. The assumption that resources are shared equally between individuals within a brood generally held.

Keywords: brood size, litter size, parasitoid wasps, resource allocation, trade-off.

INTRODUCTION

The trade-off between offspring size and offspring number is a classic problem in evolutionary biology (Smith and Fretwell, 1974; Schaffer and Gadgil, 1975; Parker and Begon, 1986; Godfray *et al.*, 1991; Stearns, 1992), but we still do not have a complete understanding of how and why offspring size varies

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among litters. The classic approach to this problem, as described by Smith and Fretwell (1974), predicts optimum offspring size by maximizing clutch (and therefore maternal) fitness, and this approach has been widely utilized to explore offspring size. However, the predicted number of offspring is generally not a whole-number, while the number of offspring produced by an organism is necessarily an integer value.

This integer problem has recently led to several theoretical models exploring offspring size in small broods (Ebert, 1994; Charnov and Downhower, 1995; Charnov *et al.*, 1995; Downhower and Charnov, 1998). These small brood models make several predictions about how the mean and range in offspring size is related to brood size. Here, we test three of the predictions, and two of the assumptions, of these models. Prediction 1 states that the range in offspring size (between different broods) should decrease with increasing brood size (Ebert, 1994; Charnov and Downhower, 1995; Charnov *et al.*, 1995). Prediction 2 describes this decrease, whereby the decrease in the range in offspring size is proportional to the brood size (Charnov and Downhower, 1995; Charnov *et al.*, 1995; West *et al.*, 2001). This is given formally by:

$$\frac{I_{max\_i} - I_{min\_i}}{I_{max\_j} - I_{min\_j}} = \frac{C_j}{C_i} \tag{1}$$

where I is offspring size, C is brood size, I_{max_i} and I_{min_i} are the maximum and minimum size of offspring in broods of C_i young, i and j are broods of different sizes, and $I = j + 1$. Figure 1 graphically depicts this relationship, which we refer to as Charnov and colleagues' (Charnov and Downhower, 1995; Charnov *et al.*, 1995) invariant rule.

Prediction 3 states that the range in resources invested in reproduction (for mothers producing a certain brood size) should be invariant over brood size (Downhower and Charnov, 1998). Put another way, the relationship between total resources devoted to a brood and brood size should be linear. We refer to this as Downhower and Charnov's (1998) invariant rule.

The study reported here is the first to test these invariants using multiple species, as we utilize previously published data on six parasitoid wasp species. The advantage of using published data is that it allows predictions to be tested in multiple species and ecological systems, and hence the generality of the models can be examined. Parasitic wasps are ideal for testing these models as there are a number of species that produce small, but variable, sized broods (Godfray, 1994; Mayhew and Hardy, 1998). The host organism provides the resources

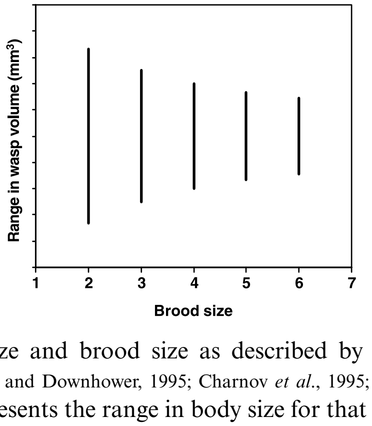

Fig. 1. Range in wasp body size and brood size as described by equation (1) and described by Charnov and colleagues (Charnov and Downhower, 1995; Charnov *et al.*, 1995; Downhower and Charnov, 1998). The solid line at each brood size represents the range in body size for that brood size.

for the wasp offspring until they mature to adulthood. Resources (measured as body size) and fitness are correlated in parasitoids (van den Assem *et al.*, 1989; Hardy *et al.*, 1992; Godfray, 1994; Visser, 1994; Petersen and Hardy, 1996; West *et al.*, 1996). We would expect wasp offspring to follow the invariant rules described above if: (1) female wasps can accurately assess host size (resources available to the developing brood) and adjust brood size accordingly; (2) average resources per offspring are constant over brood size; and (3) resources are shared equally between members of a brood. There is much evidence for assumption (1), reviewed by Godfray (1994), so we test assumptions (2) and (3) here.

Our specific aims are to test the predictions that: (1) variation in wasp size decreases with increasing brood size (Ebert, 1994; Charnov and Downhower, 1995; Charnov *et al.*, 1995); (2) the range of wasp sizes follows Charnov and colleagues' (Charnov and Downhower, 1995; Charnov *et al.*, 1995) invariant rule (see equation 1; Fig. 1); and (3) brood size scales linearly with resources devoted to reproduction as predicted by Downhower and Charnov's (1998) invariant rule. We also test two assumptions made by the small brood models, that: (4) mean offspring size is constant over brood size; and (5) resources are divided equally within a brood.

MATERIALS AND METHODS

Species

Two of the authors (S.A. West and M.A. Guinnee) collated data that allowed us to test the above predictions from parasitoid wasps that produce small, but variable, brood sizes. We located potentially useful studies by searching reviews of the subject (e.g. Godfray, 1994), and by directly contacting wasp researchers. Once potentially useful studies were located, we contacted the original authors to obtain the necessary raw data. Below, we briefly describe the species of wasps included in this study. We obtained data on six species. The type of data (field or experimental), sample sizes and how wasp body size was measured are summarized in Table 1. Detailed information on the biology of each wasp species, materials and methods are provided in the original publications. For two of the wasp species (*Achrysocharoides zwoelferi* and *Laelius pedatus*), the original publications contain similar and/or identical analyses testing some of the same hypotheses we test here. However, they are included here because we are looking for patterns that generalize over multiple species, and because we needed to standardize methods of analysis across species.

Achrysocharoides zwoelferi (Hymenoptera: Eulophidae) attacks members of the species *Phyllonorycter* that mine the leaves of *Salix* spp. The data reported here are both observational and experimentally manipulated field data with *Phyllonorycter salicicolella* as hosts (West *et al.*, 1996, 2001). *Metaphycus stanleyi* (Hymenoptera: Encyrtidae) is a parasitoid of many soft scales. The data reported here are laboratory data with the brown soft scales as hosts (Bernal *et al.*, 1999). *Aphaereta pallipes* (Hymenoptera: Braconidae) is a parasitoid of cyclorrhaphous Diptera. The data reported here are laboratory data using *Drosophila virilis* as hosts (Mayhew and van Alphen, 1999). *Laelius pedatus* (Hymenoptera: Bethylidae) is a parasitoid of the larvae of dermestid beetles. The data reported here are from the laboratory with larvae of the beetle *Anthrenus flavipes* as hosts (Mayhew, 1998). *Elachertus cacoeciae* (Hymenoptera: Eulophidae) attacks at least 13 species of Lepidoptera (Schauff, 1985; Huber *et al.*, 1996). The data reported here are experimentally manipulated field data with spruce budworm (*Choristoneura fumiferana*) larvae as hosts (Fidgen *et al.*, 2000). *Mastrus*

Table 1. Background information for each dataset

Species	Source of data	Type of data	Body size measurement	*N* (number of broods)	BS (a) mean (b) range	% female	Mixed-sex broods?	Are F or M bigger?	Sex	Change in body size with BS?
A. zwoelferi	West *et al.* (1996, 2001)	Field	Hind tibia length[3]	162	a. — b. 1–4	—	No	†F**	F M	†↓** —
A. pallipes	Mayhew and van Alphen (1999)	Lab	Hind tibia length[3]	33	a. 2.31 b. 1–5	19%	Yes	NS	F M	— ↓*
E. cacoeciae	Fidgen *et al.* (2000)	Lab and exp. field	Pupal length[3]	441	a. 3.20 b. 1–18	55%	Yes	F****	F M	↓NS ↑NS
L. pedatus	Mayhew (1998)	Lab	Mesosoma length[3]	164	a. 2.15 b. 1–5	68%	Yes	F****	F M	↑NS ↑NS
M. ridibundus	Bezemer and Mills (2003)	Lab	Hind tibia length[3]	358	a. 3.18 b. 1–9	50%	Yes	F****	F M	↓**** ↓****
M. stanleyi	Bernal *et al.* (1999)	Lab	Hind tibia length[3]	395	a. 1.67 b. 1–10	44%	Yes	F****	F M	↓NS ↓NS

Abbreviations: M = male; F = female; BS = brood size. 'Exp. field' = data from experiments that were carried out in the field but manipulated by the researchers (e.g. wasps were released into the area or hosts were placed on trees).

Significance: NS, $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. †Results first reported in original source (only included for columns 9 and 11); — indicates not applicable or sample size too small to test.

Note: As we are looking for general patterns in the data, we sometimes average over factors (such as host instar number or host size) that might affect mean brood size, % female, and change in body size with BS. Therefore, the results reported here do not necessarily reflect the patterns expected in nature, are specific to these datasets, and are meant to give an overall understanding of the data used.

ridibundus (Hymenoptera: Ichneumonidae) is a parasitoid of codling moth cocoons (*Cydia pomonella*). The data reported here are from the laboratory using codling moth cocoons as hosts (Bezemer and Mills, 2003). For all data, brood size is defined as the number of young found in/on, or emerging from, the host.

Statistical analyses

All reported P-values are for two-tailed tests, using the 0.05 level of significance. We calculated the sex ratio (% female) by dividing the total number of female wasps by the total number of wasps, irrespective of natal brood. We examined whether body size differed by sex for each wasp species using a mixed-model analysis with the model equation: wasp size = sex + brood size, with brood identity included as a random factor. Analyses were carried out using the SAS System, Release 8.0 (SAS Institute), except for tests of Charnov and colleagues' invariant rule and sampling within broods, which were carried out using Microsoft Excel 2000 and the Poptools add-in (Hood, 2002).

Variation in body size and brood size

We used an ordered heterogeneity (OH) test to evaluate whether variance in mean body size (averaged over brood, by sex) decreased with increasing brood size. The OH test combines the P-value from a Bartlett's homogeneity of variance test (Pc) with the Spearman's rank correlation coefficient (r_s) to calculate the OH statistic (r_sPc) as follows: $r_sP\text{c} = r_s \times (1 - P_{\text{Bartlett}})$ (Rice and Gaines, 1994). We chose the OH test because we wished to make a specific prediction of how variation in wasp body size would differ with brood size. We used wasp size averaged over brood (by sex) to avoid pseudo-replication (Hurlbert, 1984).

Charnov and colleagues' invariant rule

We used data on mean size of male and female wasps to determine if the range of wasp body sizes decreased with increasing brood size as predicted by Charnov and colleagues' invariant rule (Charnov and Downhower, 1995; Charnov *et al.*, 1995). We bootstrapped 10,000 re-samples of the left-hand side of equation (1), as described in West *et al.* (2001). This gives an estimate of the value and 95% confidence intervals of the ratio C to $C + 1$. We used the smaller sample size of the two brood sizes (i and j), because sample size can affect range, for both broods (i and j) being compared for each test. This test was only carried out when $n \geq 5$ for both i and j. We include brood sizes of 1 in our analyses, although it is more difficult to make precise predictions about variance when brood size is 1 than at other brood sizes (Charnov and Downhower, 1995; Charnov *et al.*, 1995).

Downhower and Charnov's invariant rule

We examined whether the amount of resources needed to increase from a brood size of C to a brood size of $C + 1$ is constant over all brood sizes as predicted by Downhower and Charnov's (1998) invariant rule. To do this, we used generalized linear regression to determine if brood size was positively related to host size. We tested for goodness of fit to non-linear models by testing the significance of adding a squared and a cubic term. The model equations are outlined in Table 2. We use host size in milligrams or length^3 as the measure of available resources.

Table 2. Relationship between host size and brood size

Species	Source	Host size measure	HS = BS*	HS = BS + BS^2*
A. pallipes	Mayhew and van Alphen (1999)	Length^3	HS = 7.6 BS + 66.0 $r^2 = 0.19$ BS: $F_{1,30} = 6.8$, $P = 0.014$	HS = −2.5 BS^2 + 20.5 BS + 51.7 $r^2 = 0.22$ BS^2: $F_{2,29} = 1.4$, $P = 0.24$
L. pedatus	Mayhew (1998)	mg	HS = 1.4 BS − 0.28 $r^2 = 0.68$ BS: $F_{1,162} = 136.7$, $P < 0.0001$	HS = −0.05 BS^2 + 1.6 BS − 0.52 $r^2 = 0.68$ BS^2: $F_{2,161} = 7.0$, $P = 0.0088$
M. stanleyi	Bernal *et al.* (1999)	Length^3	HS = 3.1 BS + 5.3 $r^2 = 0.22$ BS: $F_{1,393} = 109.6$, $P < 0.0001$	HS = −0.44 BS^2 + 6.6 BS + 1.6 $r^2 = 0.25$ BS^2: $F_{2,392} = 19.1$, $P < 0.0001$

* Best-fit equation, r^2, F-statistic and P-value for highest-order term. M = male; F = female; BS = brood size; HS = host size.

Mean body size and brood size

Wasp sizes were first averaged over brood. Because variance was heterogeneous for many of the species tested, we used Spearman's rank correlations to determine whether body size changed with brood size.

Are resources shared equally within a brood?

We examined whether resources were allocated equally within a brood by using the randomization procedure of West *et al.* (2001). We calculated the average within-brood variance for each sex and brood size combination where $n \geq 5$. We then randomly allocated wasps to broods, and calculated the within-brood variance of our randomized broods. We repeated this sampling procedure 10,000 times, and compared the observed within-brood variance to the sampled values. This procedure was carried out separately for each brood size.

RESULTS

We collated previously published data for six species of wasp. Four species include data on male and female wasps. The *A. zwoelferi* dataset contains data for females only. The *A. pallipes* dataset does not contain enough females for analyses on female wasps. For all species except *A. pallipes*, females were significantly larger than males (Table 1; $P < 0.01$). Therefore, all subsequent analyses were carried out separately for males and females. All species tested, with the exception of *A. zwoelferi*, contains mixed-sex broods.

Variation in body size and brood size

For every species–sex combination, a general pattern of decreasing variance (between broods) with increasing brood size was observed, confirmed by negative r_s-values, but not all were statistically significant (Appendix 1). It is highly unlikely that we would find the general pattern of decreasing variance with increasing brood size in all combinations studied by chance (two-tailed sign test, $n = 10$, $P < 0.01$). While some of the variance patterns resembled the predicted pattern of decreasing variance (compare *A. zwoelferi* females, *M. stanleyi* males and females in Fig. 2 with the predicted pattern in Fig. 1), other species–sex combinations did not closely follow the predictions of equation (1) (Fig. 1). The ordered heterogeneity (OH) test, which combines a test of variance (Bartlett's test) with a test of direction [Spearman's rank test (Rice and Gaines, 1994)], was statistically significant for 4 of the 10 species–sex combinations tested (Appendix 1). The OH test includes all brood sizes that contained at least two broods. To ensure that brood sizes containing few broods were not unduly affecting our results, we also carried out the OH test analyses only including brood sizes with ≥ 5 broods. The results were similar, except that one analysis became statistically significant (*L. pedatus* females), two analyses became (non-significantly) positive (*L. pedatus* males and *M. ridibundus* females) and one analysis showed no relationship (*E. cacoeciae* females).

In addition, using a random re-sampling technique, we found that the decrease in between-brood variance with brood size was, in general, slower than predicted. The observed ratio of C to $C + 1$ (equation 1) was generally ≤ 1 (where the ratio is ≤ 1 for over half of all C's tested for that species–sex combination) in all five species tested for females (17 of 21 brood size–species combinations, two-tailed sign test, $P < 0.01$; Appendix 1). For males this was only true for one of five species (10 of 21 brood size–species combinations, two-tailed sign test, $P > 0.10$; Appendix 1).

Charnov and colleagues' invariant rule

The observed ratio of C to $C + 1$ (equation 1) was generally greater than the ratio predicted by Charnov and colleagues' invariant rule (Charnov and Downhower, 1995; Charnov *et al.*, 1995) for both females (4 of 5 species; 19 of 21 brood size–species combinations, two-tailed sign test, $P < 0.01$; Appendix 1) and males (4 of 5 species; 17 of 21 brood size–species combinations, two-tailed sign test, $P < 0.01$; Appendix 1). The 95% confidence interval of the decrease in range included the ratio C to $C + 1$ predicted by equation (1) in most cases for both females (4 of 5 species, 15 of 21 brood size–species combinations; Appendix 1) and males (4 of 5 species, 14 of 21 brood size–species combinations; Appendix 1). However, the confidence intervals were large, and in most cases the 95% confidence intervals included 1 for both females (4 of 5 species, 16 of 21 brood size–species combinations; Appendix 1) and males (4 of 5 species, 19 of 21 brood size–species combinations; Appendix 1). Consequently, when considering each pair of brood sizes (i.e. comparing C to $C + 1$), individually there was no statistically significant change in range.

Downhower and Charnov's invariant rule

We tested Downhower and Charnov's (1998) invariant rule by looking for a linear relationship between resources devoted to reproduction and brood size. Brood size increased with increasing host size in all species tested using the linear model (Table 2; Fig. 3). For one of the three species tested (*A. pallipes*), no quadratic terms were statistically significant, while for two species (*L. pedatus* and *M. stanleyi*) the squared term was significant. The cubic term was not significant for any species ($P > 0.10$). Where the squared term was significant, it appeared that brood size increased rapidly with host size at small host sizes, but a few hosts contained broods that were much larger than the average for that host size. However, while the squared term is statistically significant for *L. pedatus*, the best-fit line is close

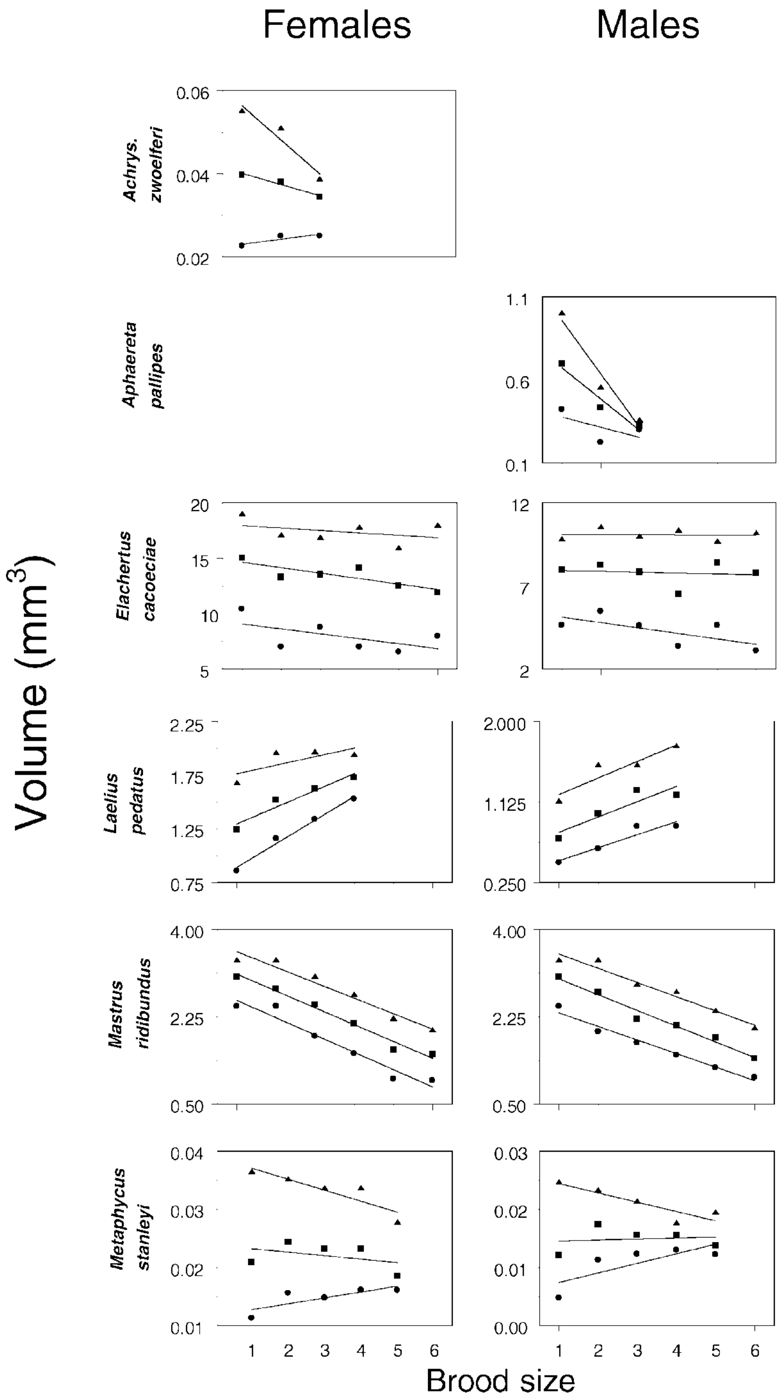

Fig. 2. The 90th percentile (triangles), median (squares) and 10th percentile (circles) wasp volumes for each of the species tested, separately by sex. For simplicity, only brood sizes up to six are included. Linear trend lines are included.

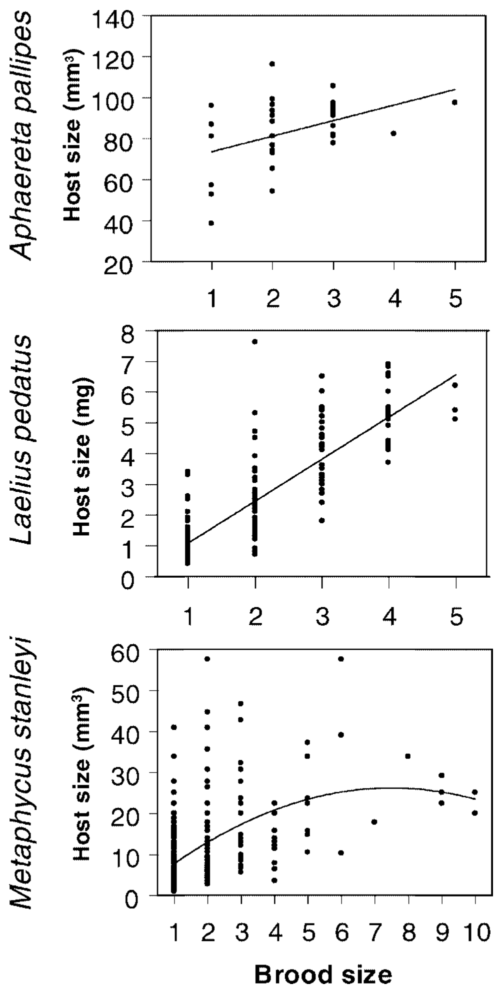

Fig. 3. Relationship between brood size and host size. Best fit lines are included. See Table 2 for trend line equations and r^2-values.

to linear (Table 2; Fig. 3), and including the squared term does not increase the r^2-value (Table 2).

Mean body size and brood size

For three wasp species (*A. zwoelferi* females; *A. pallipes* males; *M. ridibundus* males and females), wasp size decreased with increasing brood size (Table 1). For the other three species tested, wasp size did not vary (significantly) with brood size.

Are resources shared equally within a brood?

Our results support the assumption made by small brood models that each individual in a brood receives (relatively) equal resources. Our randomization procedure allowed us to test this statistically, by comparing variation in body size of wasps from the same brood to that of wasps from different broods (within the same brood size). The 95% confidence intervals obtained from our sampling procedure generally excluded the observed value for mean variance for three of five species of females (12 of 20 brood size–species combinations, two-tailed sign test, $P > 0.10$; Appendix 2) and two of four species of males (10 of 17 brood size–species combinations, two-tailed sign test, $P > 0.10$; Appendix 2). In addition, in most cases, the observed variance was less than the variance that is predicted if wasp size is randomly distributed in broods (females: 4 of 5 species; 18 of 20 brood size–species combinations, two-tailed sign test, $P < 0.01$; males: 3 of 4 species; 15 of 17 brood size–species combinations, two-tailed sign test, $P < 0.01$; Appendix 2). As the likelihood of this occurring by chance is low, this supports the hypothesis that wasps from the same brood are more similar in size than wasps from different broods.

DISCUSSION

We analysed previously reported data on parasitoid wasps to test the predictions of small brood (litter) models, and in particular their invariant rules (Ebert, 1994; Charnov and Downhower, 1995; Charnov *et al.*, 1995; Downhower and Charnov, 1998; West *et al.*, 2001). The predictions/assumptions tested and our findings are summarized as follows:

1. Our findings support prediction 1 that variance in offspring size among broods decreases with increasing brood size (Appendix 1).
2. In general, while our findings agree with the prediction of Charnov and colleagues' invariant rule (Charnov and Downhower, 1995; Charnov *et al.*, 1995) of decreasing variance in offspring size with increasing brood size, the observed decrease was slower than predicted (Fig. 2; Appendix 1).
3. Our findings offer some support for Downhower and Charnov's (1998) invariant rule, as brood size showed a linear or near-linear relationship to available resources for two of three species tested (Fig. 3; Table 2).
4. Generally, our findings do not agree with assumption 1 that offspring size is constant over brood size. This assumption was violated in half (3 of 6) of the species tested in this study. For those species that violated this assumption, offspring size decreased with increasing brood size (Table 1).
5. Our results support assumption 2 that resources are divided (relatively) equally within a brood (Appendix 2).

Life-history invariants have been described for such diverse topics as sex allocation, age at maturity and cellular maintenance rates, and for animals as varied as parasitic nematodes, mammals, fish, invertebrates and reptiles (Charnov, 1993; Gemmill *et al.*, 1999; Charnov and Skuladottir, 2000; Charnov, 2001; Allsop and West, 2003a,b). Here, we used Charnov and colleagues' invariants (Charnov and Downhower, 1995; Charnov *et al.*, 1995; Downhower and Charnov, 1998) to expand our knowledge specifically about the relationship between brood size and offspring size in parasitoid wasps, but these invariants should be useful in any organism that produces small (< 6) but variable brood sizes.

Charnov and Downhower (1995) calculated that Charnov and colleagues' invariant rule should hold for a variety of offspring size/offspring fitness functions. Previous studies in a fish (Charnov *et al.*, 1995), a *Daphnia* (Guinnee *et al.*, 2004) and parasitoid wasps (Mayhew, 1998; West *et al.*, 2001) have found that variance in offspring size among broods decreases with increasing brood size. However, this decrease in variance has rarely followed closely the predictions of

Charnov and colleagues' invariant, and the decrease in variance was generally slower than predicted, as was the case here.

We know of three tests of Downhower and Charnov's invariant in the literature. Two are generally supportive of the invariant, in a fish (Downhower and Charnov, 1998) and in *Daphnia* (Guinnee *et al.*, 2004). Interestingly, Mayhew (1998), using the data discussed here for *L. pedatus*, found that variance in host weights increased with increasing brood size, suggesting that larger broods have a greater size variation than expected. This could result from offspring being 'costlier' at larger broods. Our analyses offered some support for Downhower and Charnov's invariant rule (Fig. 3; Table 2), as two of three species showed a linear or near-linear relationship between brood size and host size. The species that did not fit the invariant pattern had a positive correlation between brood size and host size at small host sizes, but some larger hosts harboured broods that were much larger than the average brood size for that host size.

Examination of where the invariants do not hold can prove useful in expanding our knowledge about parasitoid wasp reproduction, and tell us more generally about resource investment in offspring. Possible reasons why we did not find a strong quantitative fit to the invariant rules are discussed below.

First, equation (1) assumes that the average amount of resources received by an offspring is constant over brood size. This assumption did not hold in this study, as body size was negatively correlated with brood size for half the species tested here (three of six). Under favourable conditions, it might pay females to increase number of offspring at the cost of offspring size (Hutchinson, 1951; Green, 1966) if host size is correlated with host abundance (or host finding rates) in the next generation. There is evidence that egg size is negatively correlated with clutch size in *Daphnia*, where mothers with the lowest food availability produce the largest offspring (see Guinnee *et al.*, 2004 and references therein). However, in parasitic wasps, resources for offspring do not come directly from the mother, but from the host. Why would a mother increase the number of offspring on one host when, if hosts are abundant, she can simply move on to the next host and lay more eggs there? One possibility is that the pattern reflects parent–offspring conflict – explicit theory would be useful to address this point (Godfray and Parker, 1991).

Second, in this study, we do not take into account variables such as host instar number or host size when examining the brood size–wasp body size relationship, as we are looking for general patterns. It might be that we have missed something important by glossing over these points. However, there are many explanations why offspring size might vary with brood size, most involving trade-offs (see Mayhew and Glaizot, 2001 and references therein). In other organisms (Westoby *et al.*, 1992; Rees and Westoby, 1997), and parasitic wasps (Mesterton-Gibbons and Hardy, 2004), complicated size–fitness relationships have been described, for example where fitness depends on an individual's size relative to competitors. In addition, for parasitic wasps, it has been demonstrated theoretically that multiple optimal brood sizes might exist (Mesterton-Gibbons and Hardy, 2004). It has also been demonstrated theoretically that variation in offspring size within an individual brood can be advantageous (Geritz, 1995).

Third, while it has been shown that female wasps assess host size and adjust brood size accordingly (Fig. 3; Table 2) (Godfray, 1994), their assessment might be imperfect. If host-size assessment is less accurate at larger host sizes, this would increase variability in wasp size at larger brood sizes. Alternatively, if there exists an asymmetric cost to producing too large a brood, the mother might favour the smaller brood size (Godfray and Ives, 1988). This could increase variability in offspring size at all brood sizes, in effect 'washing out' Charnov and colleagues' invariant. In addition, here brood size is defined as the number of young found on/in, or emerging from, a host, and therefore developmental mortality is not taken into account, and will likely affect body-size variance [although Mayhew (1998), using the *L. pedatus* data analysed here, performed similar analyses using only broods with no developmental mortality and found similar results for Charnov and colleagues' invariant as those reported here: variance in wasp size decreased with increasing brood size but more slowly than predicted by theory]. Similarly, if the level of competition between wasps is dependent on brood size, this would be a source of additional variation in wasp body size.

Fourth, with the exception of *A. zwoelferi*, the species analysed here produce mixed-sex broods. Different levels of optimum investment in male and female offspring might exist – the fact that females were bigger than males in five of six species here supports this. These differences have not been incorporated into theory, which assumes single-sex broods, and further modelling is required to examine the extent to which the invariant predictions are expected to hold with mixed-sex broods.

Fifth, while female wasps will attempt to avoid super-parasitism, multiple broods are sometimes laid on the same host; super-parasitism is more likely in data collected from the field. This might explain why *A. pallipes* and *M. stanleyi* both contained a few very large broods that were not on very large hosts (Fig. 3). It might also explain why, for some species, body size decreased with increasing brood size, as very large broods might in fact be multiple broods competing for the same host resources. Super-parasitism is not included in small brood theory and would increase variance in body size between broods at larger brood sizes.

CONCLUSIONS

We tested Charnov and colleagues' invariant predictions (Charnov and Downhower, 1995; Charnov *et al.*, 1995; Downhower and Charnov, 1998) for the relationship between brood size and variation in offspring size. We found qualitative support for the prediction that variance in offspring size should decrease with increasing brood size (Ebert, 1994; Charnov and Downhower, 1995; Charnov *et al.*, 1995) (Appendix 1; Fig. 2). Quantitative lack of fit to the invariant predictions has led to several hypotheses about resource allocation, highlighted above. These observations require further investigation.

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APPENDIX 1

Results of OH test and test of equation (1). The predicted, observed (mean of the 10,000 re-samples) and 95% confidence intervals for $(I_{max\_i} - I_{min\_i})/(I_{max\_j} - I_{min\_j})$ obtained by re-sampling the wasp body-size data (averaged over brood)

Species	Source	Sex	How variance changes with BS	BS	N	Pred.	Obs. (mean)	Confidence interval
A. zwoelferi	West *et al.* (1996, 2001)	F	↓****, $r_s = -1.00$, $Pc < 0.0001$, $k = 3$	2 vs 1	60	0.500	†0.759	†0.601–0.954
				3 vs 2	35	0.667	†0.503	†0.358–0.681
		M	—	—	—	—	—	—
A. pallipes	Mayhew and van Alphen (1999)	F	—	—	—	—	—	—
		M	↓****, $r_s = -1.00$, $Pc = 0.0011$, $k = 3$	2 vs 1	6	0.500	1.011	0.257–4.804
				3 vs 2	10	0.667	0.392	0.149–0.828
E. cacoeciae	Fidgen *et al.* (2000)	F	↓NS, $r_s = -0.10$, $Pc = 0.88$, $k = 10$	2 vs 1	45	0.500	0.951	0.696–1.392
				3 vs 2	34	0.667	0.693	0.532–0.900
				4 vs 3	30	0.750	1.425	1.057–1.832
				5 vs 4	17	0.800	0.809	0.598–1.267
				6 vs 5	17	0.833	0.966	0.761–1.133
				7 vs 6	10	0.857	0.916	0.423–1.565
				8 vs 7	8	0.875	1.374	0.639–2.584
		M	↓NS, $r_s = -0.15$, $Pc = 0.87$, $k = 10$	2 vs 1	55	0.500	0.788	0.598–1.138
				3 vs 2	40	0.667	1.152	0.849–1.454
				4 vs 3	27	0.750	1.102	0.793–1.585
				5 vs 4	22	0.800	0.896	0.578–1.253
				6 vs 5	18	0.833	1.068	0.666–1.685
				7 vs 6	7	0.857	0.806	0.184–1.794
				8 vs 7	5	0.875	1.120	0.227–2.329
L. pedatus	Mayhew (1998)	F	↓NS, $r_s = -0.40$, $Pc = 0.05$, $k = 5$	2 vs 1	40	0.500	0.792	0.561–1.340
				3 vs 2	36	0.667	0.886	0.657–1.150
				4 vs 3	20	0.750	0.849	0.406–1.305
		M	↓NS, $r_s = -0.30$, $Pc = 0.18$, $k = 5$	2 vs 1	20	0.500	1.332	0.895–2.023
				3 vs 2	34	0.667	0.818	0.606–1.014
				4 vs 3	20	0.750	1.224	0.782–1.918
M. ridibundus	Bezemer and Mills (2003)	F	↓NS, $r_s = -0.26$, $Pc = 0.086$, $k = 8$	2 vs 1	20	0.500	1.340	0.931–1.867
				3 vs 2	60	0.667	1.659	1.170–2.158
				4 vs 3	49	0.750	0.875	0.654–1.212
				5 vs 4	33	0.800	0.834	0.619–1.093
				6 vs 5	8	0.833	0.668	0.384–1.581
		M	↓NS, $r_s = -0.54$, $Pc = 0.77$, $k = 7$	2 vs 1	15	0.500	1.264	0.755–2.037
				3 vs 2	50	0.667	1.150	0.730–1.654
				4 vs 3	56	0.750	1.104	0.640–1.615
				5 vs 4	37	0.800	0.719	0.491–1.097
				6 vs 5	10	0.833	0.854	0.510–1.345
M. stanleyi	Bernal *et al.* (1999)	F	↓****, $r_s = -1.00$, $Pc = 0.019$, $k = 8$	2 vs 1	60	0.500	0.862	0.009–1.026
				3 vs 2	23	0.667	0.883	0.065–1.252
				4 vs 3	12	0.750	0.846	0.611–1.280
				5 vs 4	7	0.800	0.617	0.155–1.219
		M	↓**, $r_s = -0.71$, $Pc < 0.0001$, $k = 8$	2 vs 1	60	0.500	0.630	0.416–0.896
				3 vs 2	21	0.667	0.907	0.489–1.417
				4 vs 3	14	0.750	0.630	0.268–1.130
				5 vs 4	7	0.800	1.280	0.240–3.264

Significance: NS, $P > 0.05$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. *Note:* r_s is Spearman's rank correlation coefficient; Pc is the P-value from a Bartlett's homogeneity of variance test; k is the number of groups. M = male; F = female; BS = brood size. † Result first reported in original source; — indicates not applicable or sample size too small to test. The number of brood sizes included in the OH test (k) is sometimes greater than the number of brood sizes tested directly (columns 5–9) because all brood sizes with ≥2 brood were included in the OH test while only brood sizes with ≥5 broods are included in columns 5–9.

APPENDIX 2

Observed mean within-brood variance in wasp body size volume compared with sampled mean within-brood variance in wasp body size volume by brood size (95% confidence intervals are for sampled values)

Species	Source	Sex	BS	*N*	Obs. (mean) variance	Sampled (mean) variance	95% confidence interval
A. zwoelferi	West *et al.* (1996, 2001)	F	2	34	†3.7×10^{-5}	†4.5×10^{-4}	†2.7×10^{-4} to 6.6×10^{-4}
		M	—	—	—	—	—
A. pallipes	Mayhew and van Alphen (1999)	F	—	—	—	—	—
		M	2	7	0.00927	0.01952	0.00520–0.04018
			3	10	0.00456	0.00411	0.00193–0.00683
E. cacoeciae	Fidgen *et al.* (2000)	F	2	28	4.088	12.500	7.152–18.856
			3	28	5.890	11.265	6.503–17.345
			4	26	2.628	12.250	7.775–17.734
			5	16	3.659	13.024	8.239–18.757
			6	18	3.986	13.539	10.097–17.627
			7	8	6.405	8.706	5.487–12.646
			8	8	5.900	17.749	12.342–24.055
		M	2	65	1.173	3.837	2.647–5.203
			3	37	2.066	5.947	4.198–7.948
			4	24	1.953	6.024	4.067–8.376
			5	18	2.724	3.653	2.467–4.988
			6	15	2.901	4.839	3.285–6.593
			7	6	2.207	4.805	2.586–7.996
L. pedatus	Mayhew (1998)	F	2	9	0.0515	0.0895	0.0261–0.1800
			3	36	0.0527	0.0929	0.0502–0.1496
			4	20	0.0518	0.0672	0.0299–0.1246
		M	—	—	—	—	—
M. ridibundus	Bezemer and Mills (2003)	F	2	31	0.157	0.198	0.108–0.322
			3	59	0.197	0.292	0.201–0.404
			4	39	0.172	0.324	0.228–0.436
			5	27	0.107	0.227	0.137–0.344
			6	7	0.121	0.236	0.108–0.430
		M	2	21	0.050	0.192	0.096–0.318
			3	57	0.252	0.286	0.206–0.381
			4	36	0.079	0.201	0.137–0.279
			5	33	0.104	0.244	0.170–0.332
			6	8	0.049	0.153	0.090–0.235
M. stanleyi	Bernal *et al.* (1999)	F	2	15	5.6×10^{-5}	5.1×10^{-5}	2.1×10^{-5} to 9.5×10^{-5}
			3	13	2.7×10^{-5}	6.8×10^{-5}	3.5×10^{-5} to 10.8×10^{-5}
			4	9	1.9×10^{-5}	5.6×10^{-5}	2.3×10^{-5} to 9.5×10^{-5}
			5	7	4.2×10^{-5}	3.8×10^{-5}	1.4×10^{-5} to 7.2×10^{-5}
		M	2	15	2.4×10^{-5}	2.6×10^{-5}	1.1×10^{-5} to 4.6×10^{-5}
			3	15	1.4×10^{-5}	1.7×10^{-5}	7.0×10^{-5} to 3.0×10^{-5}
			4	10	9.1×10^{-5}	5.6×10^{-5}	2.3×10^{-5} to 9.5×10^{-5}
			5	5	1.7×10^{-5}	3.8×10^{-5}	1.4×10^{-5} to 7.2×10^{-5}

Abbreviations: M = male; F = female; BS = brood size.

† Result first reported in original source; — indicates not applicable or sample size too small to test.