



## A sex-specific size–number tradeoff in clonal broods

Yoriko Saeki, Philip H. Crowley, Charles W. Fox and Daniel A. Potter

Y. Saeki (yoriyoripp@uky.edu) and P. H. Crowley, Dept of Biology, Univ. of Kentucky, 101 T H Morgan Building, Lexington, KY 40506-0225, USA. – C. W. Fox and D. A. Potter, Dept of Entomology, Univ. of Kentucky, USA.

Polyembryonic parasitoids producing single-sex broods of clonal offspring provide an unusually clear window into the classic tradeoff between the number and size of offspring. We conducted a laboratory study of the encyrtid parasitoid *Copidosoma bakeri* parasitizing the noctuid *Agrotis ipsilon* to examine the way that size and number of offspring tradeoff in broods of each sex and to determine how the fit between host and parasitoid brood is achieved. We found that brood mass (wasp body mass  $\times$  brood size) was proportional to host mass, independent of brood sex, indicating a tight fit between brood and host and ensuring a size–number tradeoff. By correcting brood size and body mass of each brood for host mass, we demonstrated the expected inverse relationship between wasp variables. We postulated that the wasp brood might achieve the fit to the host by (1) adjusting brood size based on information available early in host development before and during division of the embryo, (2) manipulating host size late in host development after completion of embryo division, or (3) simply adjusting individual wasp mass to fill the host. We evaluated host responses to parasitism – and correlations between brood size and host growth early and late in development – for broods of each sex. The data are consistent with adjustment of brood size to the amount of host growth early in host development and with manipulation of host mass late in host development. The tight link between host mass and brood mass also suggests a final adjustment by parasitoid growth to achieve complete filling. Within the tight fit, female broods were smaller but contained larger individuals than male broods. The sex-specific balance point of the tradeoff and sex differences in balancing mechanisms and responses to host size suggest different selection pressures on each sex requiring future investigation.

The number and body size of progeny within broods are among the most studied traits in life history tradeoffs (Smith and Fretwell 1974, Stearns 1989) and should generally be inversely related when resources are limited (Sibly and Calow 1986). This number–size tradeoff has been documented in plants (Stuefer et al. 2002), invertebrates (reviewed by Fox and Czesak 2000), and vertebrates (Mappes and Koskela 2004). However, attempts to detect a size–number tradeoff may prove unsuccessful because of (1) the absence of a tradeoff (e.g. when brood size is genetically fixed), or because of masking of the tradeoff by (2) resource variation (van Noordwijk and de Jong 1986, Roff and Fairbairn 2007), (3) an interaction between resource variation and parental reproductive behavior (Mayhew 1998, Mayhew and Glazier 2001), (4) effects of another trait mediating the tradeoff (e.g. development time: Klingenberg and Spence 1997), (5) other fitness components influencing the tradeoff (e.g. future reproduction: Lack 1947), (6) continuous acquisition of resources during reproduction (Fox and Czesak 2000), or (7) competition among offspring (Godfray and Parker 1991).

In this study, we were able to by-pass many of these issues to address possible sex-differences in the size–number tradeoff. Sex-specific tradeoffs have been documented in previous work (Fischer and Fiedler 2000) but not to our knowledge for the size–number tradeoff, though two studies

have demonstrated declines in parasitoid body size with greater brood size. The usual genetically diverse and mixed-gender broods, involving parent–offspring and sibling conflicts (Godfray 1986) and sometimes differential provisioning of offspring by gender (West and Sheldon 2002, Young and Badyaev 2004), can obscure or render moot any such gender differences in the size–number tradeoff.

Here, we focus on the polyembryonic parasitoid *Copidosoma bakeri*, which produces large broods of clonally identical (and thus all male or all female) offspring. For endoparasitic wasps in general, food intake and biomass of the host constitute the resources used to produce a wasp brood. Interactions between endoparasitoids and their hosts affect parasitoid fitness through survival to the adult stage, development time, and adult body mass (Mackauer and Sequeira 1993, Godfray 1994). To increase their fitness, koinobiont endoparasitoids, which develop inside a host that continues to grow, may actively manipulate host growth ('host regulator'; Vinson and Iwantsch 1980) or may passively adjust their own development to the host's growth ('host conformer'; Lawrence 1986). Parasitoids may interfere with the host's endocrine system (Beckage and Riddiford 1982), increase the host's dietary assimilation efficiency (Slansky 1978), alter its nutritional status (Vinson and Iwantsch 1980, Slansky 1986), and ameliorate or neutralize host defenses (Beckage 1997). Also, parasitoids

may adjust their own development and/or emergence in response to host physiology (Lawrence 1986, Harvey 1996), allowing these wasps to maximize the available host resource (Mackauer and Sequeira 1993).

With these strategies, parasitoids may generally be able to shape various key traits to their advantage, especially development time, body mass and growth rate (Mackauer and Sequeira 1993). For gregarious parasitoids, in which multiple offspring of different genotypes emerge from one host, brood size may be adjusted by the ovipositing female (Charnov and Skinner 1985) or by siblicide or larval competition within a host (Godfray 1987). In these cases, selection may often favor increased body size due to the typical advantages of greater size in competition for resources (Mayhew and Glaizot 2001). In contrast, for polyembryonic broods that produce multiple genetically identical offspring arising from a single egg, these complexities of gregarious development do not apply. In polyembryonic species, brood size and body size are presumably adjusted by the brood itself and not by the ovipositing female (Godfray 1994). Also, when broods are exclusively of genetically identical offspring, selection favors cooperation among larvae rather than competition (Godfray 1994) and should tend to maximize reproductive success of the clonal brood as a whole (Godfray and Parker 1991). Multiple genetically identical offspring may provide advantages when a female wasp cannot predict host quality at the time of oviposition, because the brood itself may more accurately assess the host's ultimate resource value later in the development of brood and host – and without generating the parent–offspring conflict typical of gregarious parasitoids (Godfray 1994).

In the present study, we examined the way that wasp brood size and body mass trade off in *C. bakeri* and how the fit between host and parasitoid brood is achieved. We postulated that male and female broods would achieve similar total masses in hosts of similar size but that males and females may differ in how the tradeoff is balanced between individual wasp body mass and brood size. We also postulated that wasps might fit the brood to the host by adjusting brood size based on host characteristics early in development, manipulating host size to accommodate the brood size late in host development, or adjusting wasp body size to fit the brood into the host at its maximum mass.

## Material and methods

### Focal system

*Copidosoma bakeri* is a polyembryonic egg–larval parasitoid that oviposits in eggs of the host. The range of *C. bakeri* covers the central and northern United States into Canada, parasitizing at least 19 lepidopteran (Noctuidae) species (Schaaf 1972, Byers et al. 1993).

The genus *Copidosoma* (Hymenoptera: Encyrtidae), especially the species *Copidosoma floridanum*, has been extensively used to explore the biology of polyembryony (Hunter and Stoner 1975, Jones et al. 1982, Strand 1989a, 1989b). The development of a polyembryonic egg to produce hundreds to thousands of clonal individuals is synchronized with host development (Strand 1989a). After *Copidosoma* parasitizes a host egg, the host hatches and starts

to develop in the same way as an unparasitized larva, while the wasp egg divides multiple times (Ivanova-Kasas 1972). During the first several host instars, some portion of the parasitoid embryo develops into precocious larvae (~10; unpubl. data) that may perform defense functions but die before the embryo matures. When the host reaches the penultimate larval stadium, the wasp larvae start morphogenesis and growth (Baehreke and Strand 1990). Divisions of eggs in *C. bakeri* seem to be consistent with those of *C. floridanum* as indicated by the presence of the same-sized eggs of both species at the beginning of the penultimate stadium but not thereafter (unpubl.). During the host's final larval stadium, the wasp larvae devour the host and pupate (Strand 1989b). When the wasps form cocoons, the host is mummified and dies (Strand 1989a). Thus, the features of host development before the cessation of egg formation by the start of the host's penultimate stadium may cue or otherwise influence wasp brood size, whereas host development during and after the host' penultimate stadium may respond in part to the active influence of wasp brood size on the host.

### Empirical methods

*Copidosoma bakeri* parasitizing the black cutworm *Agrotis ipsilon* were collected at the Univ. of Kentucky Turfgrass Research Facility (Fayette County, KY; 38°0'41"N, 84°31'18"W) from July to September 2005, and maintained for about three generations before the start of the experiments. The black cutworm hosts were reared on a pinto-bean based diet in an environmental chamber maintained at 27 ± 0.1°C (daytime) and 25 ± 0.1°C (night time) and light:dark regime L14:D10.

Thirty newly emerged and mated female wasps were individually introduced into a petri dish that contained host eggs (<24 h old) and were left until they parasitized one to four eggs. Oviposition behavior was observed, and there was no superparasitism. Non-parasitized eggs were prepared in a petri dish in the same manner, without wasps. Both parasitized and non-parasitized eggs were placed singly on pre-weighed diet in a plastic cup [3.8 (top)–2.8 (bottom) cm diameter, 3.5 cm depth] with a cardboard lid. The cutworms were reared as described above and fed ad libitum.

Head capsule widths of cutworms were measured at every molt under a dissection microscope (25 ×). Larvae were weighed to the nearest 0.1 mg every 24 h beginning in the 3rd stadium, until the parasitized larvae formed mummies or non-parasitized larvae pupated. Black cutworms cease feeding about 2 days before pupation, expel their gut contents, and wander to find a place to pupate (wandering phase). Therefore, the last stadium was considered finished at the start of the wandering phase. Frass was removed and the uneaten diet was weighed to determine the amount eaten after estimating the amount of water loss from the artificial diet. Diet mass was corrected for water loss by weighing diet maintained without larvae under the same condition.

Wasps emerging from the host mummy were frozen within 24 h after emergence. All wasps, including adults, undeveloped larvae, and pupae that remained inside the mummy (hereafter total brood size) were counted. The adults found inside appeared to have eclosed, because they

occupied the middle of the cross section of the mummy with appendages liberated; individuals that had not eclosed were found between the layers of the integument of the host, the site of pupation (Strand 1989b), and their appendages were not liberated. The total number of wasps produced from an egg (including larvae that did not survive to adult) is the 'total brood size'. The sum of the number of adult wasps from both inside and outside the host is our measure of 'secondary brood size' (wasps inside the host had eclosed but were trapped inside the host). Secondary brood size has been termed 'secondary clutch size' in some earlier literature (Strand 1989c); however, in this paper brood size refers the number of larvae that hatched from a single egg. Size of the adult wasps was estimated from 40 randomly chosen wasps per brood by collectively weighing them, 20 at a time, to the nearest 0.1  $\mu\text{g}$ .

## Data analysis

All data were analyzed with SPSS (SPSS Inc., ver 15.0) unless otherwise indicated.

### *Tradeoff between the number and mass of progeny*

Differences in secondary brood size and wasp body mass between male and female broods were tested with ANOVA. We also examined whether there is a difference in brood mass (total brood size  $\times$  wasp body mass) between male and female broods. To examine whether the number of undeveloped larvae was related to total brood size, we regressed the number of undeveloped larvae on total brood size. Then, to evaluate whether wasp body mass and secondary brood size vary with sex and have an inverse relationship overall, we first adjusted the two variables for differences in host mass (Appendix 1). With these standardized data, we conducted a MANOVA of brood size and wasp body mass versus sex and then examined the brood size-body mass regression. Since both brood size and wasp body mass were variables measured with error and since neither was obviously causal with respect to the other, we used model II regression to test for an inverse relationship by log-transforming both variables and determining whether the slope was close to  $-1$  (Warton et al. 2006).

Also, using model II regression, we tested whether relationships between host mass and brood mass (brood size  $\times$  wasp mass), brood size, or wasp mass differ between males and females. When neither slopes nor intercepts differed between sexes, data were combined, and confidence intervals of slopes and intercepts were calculated for the statistically distinct relationships (Warton et al. 2006). To determine whether brood mass was proportional to and correlated with host mass, we examined whether the model II confidence interval for the slope included one. We calculated the coefficient of determination ( $r^2$ ) and the confidence interval for the intercept using model II on untransformed data, predicting that  $r^2$  should be high ( $>0.5$ ) and the intercept should approximate zero.

### *Interaction with host resources*

Survival rates of unparasitized and parasitized larvae were analyzed with Cox regression (failure time analysis). Broods

from hosts with supernumerary stadia ( $>6$ ) were excluded from analyses described in this section, since we did not know when the parasitoids' egg clutch was fully formed within the host undergoing supernumerary instars. The remaining analyses distinguished between the developmental interval through host stadium 4 (before brood size was set) and the interval including stadia 5 and 6 (after brood size was set).

To evaluate effects of parasitism and parasitoid sex on hosts, we conducted a repeated measures ANOVA, with these two developmental intervals constituting the time variable; a brood treatment reflecting whether the host was parasitized by a male brood, a female brood, or neither; and the mass gained by the host during each developmental interval as the response variable. Statistical significance of time, treatment, and time  $\times$  treatment then justified separate analyses by developmental interval to test two orthogonal contrasts: parasitized versus unparasitized and male versus female broods.

We investigated six different host characteristics as response variables: mass gain during the developmental interval, duration of the interval, total food consumption during the interval (natural log-transformed to improve normality), assimilation efficiency (mass gain divided by total food), growth rate (mass gain divided by duration), and feeding rate (total consumption divided by duration). We protected the statistical power of the mass gain contrasts, because the experiment was designed to focus on this variable most closely linked to available parasitoid resources. To test the other contrasts, we used the Bonferroni correction, dividing the critical p-value of 0.05 by the total number of contrasts (including those based on the mass gain response) to obtain a smaller and more conservative critical p-value.

To examine whether brood size might be influenced by host characteristics early in development or whether brood size might influence host mass, we conducted bivariate correlation analyses between brood size and each of the six host characteristics used in the previously described analysis. Once again we separated the analyses by developmental interval and protected the power to detect the mass gain relationship. Relationships between brood size and each of the other host characteristics were again evaluated using the Bonferroni correction for the total possible number of comparisons. For each developmental interval and host characteristic, we tested first for a significant difference between relationships for male and for female broods. If that difference was significant, then we tested the relationship separately by sex; otherwise, we tested the relationship for the data pooled across sexes.

## Results

### Basic brood data

Wasps from 25 broods successfully emerged as adults (Fig. 1). Eight broods produced all females, 16 broods were all males, and one brood was a mixture of males and females with a male fraction of 0.44. The sex ratio among broods did not differ from 1:1 ( $\chi^2 = 2.67$ ,  $p = 0.10$ ). There were two outliers showing unusually small brood sizes (258

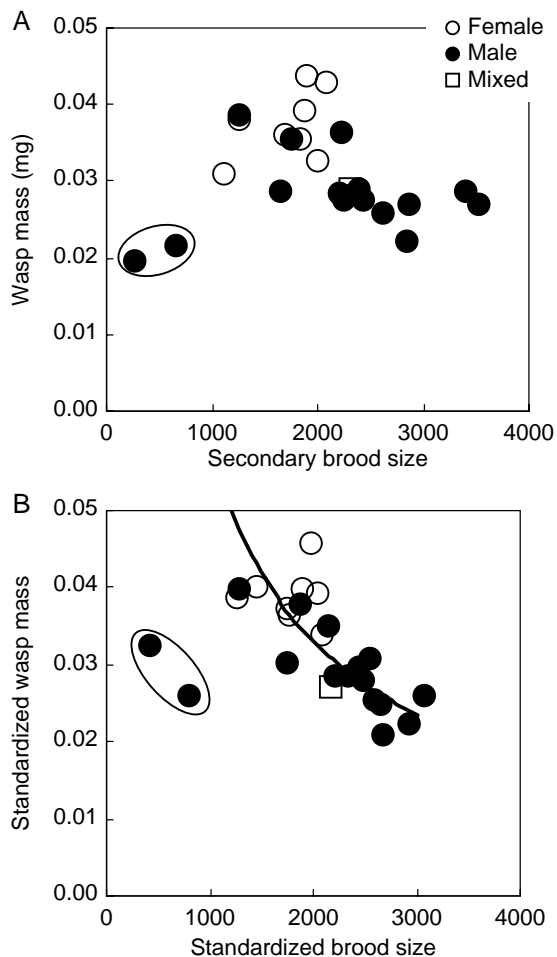


Figure 1. Relationship between secondary brood size and wasp body mass. The two outliers circled on the left side of the graphs and the mixed brood were not included in any of the analyses or curve fits because of high mortality. (A) unstandardized data, (B) data standardized to remove the effect of host mass. The best-fit line, drawn without the two outliers and mixed brood,  $Y = aX^b$ , where  $Y$  is standardized wasp mass,  $X$  is standardized brood size,  $a = 14.8$  (95% CI [3.15, 69.7]) and  $b = -0.86$  (95% CI [-1.15, -0.65]).

and 666; both male broods) with unusually low survival of wasp larvae (<50%; larval survival for all other broods was >80%), and we eliminated them from all analyses. The single mixed brood was eliminated from analyses as well.

There was no difference in brood mass (brood size  $\times$  wasp body mass) between male and female broods (unstandardized:  $F_{1,20} = 0.34$ ,  $p = 0.57$ ; standardized:  $F_{1,20} = 0.04$ ,  $p = 0.85$ ). Secondary brood sizes range from 1119 to 3522 wasps, averaging (mean  $\pm$  SE)  $1719 \pm 124$  ( $n = 8$ ) wasps for female broods and  $2411 \pm 168$  ( $n = 14$ ) for male broods. Total brood sizes (which include undeveloped pupae/larvae) range from 1171 to 3743 wasps, averaging  $1862 \pm 41$  wasps ( $n = 8$ ) for female broods and  $2530 \pm 46$  ( $n = 14$ ) for male broods. The percentage of adults emerging successfully [(the number of adults/(numbers of undeveloped larvae + pupae + adults))  $\times$  100] averaged  $94.1 \pm 1.4\%$  ( $n = 22$ ).

Total brood size was not correlated with the number of undeveloped larvae or pupae ( $r^2 = 0.01$ ,  $p = 0.60$ ).

Male broods were significantly larger (contained more offspring) than female broods in both total brood and secondary brood size (total brood:  $F_{1,20} = 7.33$ ,  $p = 0.01$ , female =  $1862 \pm 116.7$  [ $n = 8$ ], male =  $2530 \pm 172.9$  [ $n = 14$ ]; secondary brood:  $F_{1,20} = 8.16$ ,  $p = 0.01$ , female =  $1719 \pm 123.7$  [ $n = 8$ ], male =  $2411 \pm 167.6$  [ $n = 14$ ]). Female wasps were significantly larger than males ( $F_{1,20} = 16.7$ ,  $p < 0.01$ , females =  $37.4 \pm 1.6$   $\mu\text{g}$ , males =  $29.3 \pm 1.2$   $\mu\text{g}$ ). The MANOVA indicated that standardized brood size (the number of emerging adult wasps corrected with host mass) and individual wasp mass for males and females were significantly different ( $F_{2,19} = 8.97$ ,  $p < 0.01$ ); in other words, males and females had different combinations of brood size and offspring size, with females having smaller brood size and larger body mass but males having larger brood size and smaller body mass.

### Tradeoff between the number and size of progeny

There was an inverse relationship between standardized secondary brood size and wasp body mass (Fig. 1B). Model II was used for all of the remaining regression analyses reported below. This regression yielded a negative linear relationship between natural log-transformed standardized secondary brood size and wasp body mass (slope =  $-0.86$ , 95% CI = [-1.15, -0.65], intercept = 2.70, 95% CI = [1.15, 4.24],  $r^2 = 0.61$ ); back-transforming produced the power function illustrated in Fig. 1B. These results are consistent with an inverse relationship between the variables.

Brood mass (brood size  $\times$  wasp mass) was proportional to host mass (Fig. 2;  $r^2 = 0.60$ , slope = 0.054, 95% CI = [0.040, 0.072], intercept =  $-16.0$ , 95% CI = [-40.9, 8.98]). Neither slopes nor intercepts differed significantly between males and females (slopes:  $\chi^2 = 0.01$ ,  $p > 0.05$ ; intercepts:  $\chi^2 = 0.50$ ,  $p > 0.05$ ). Slopes of relationships

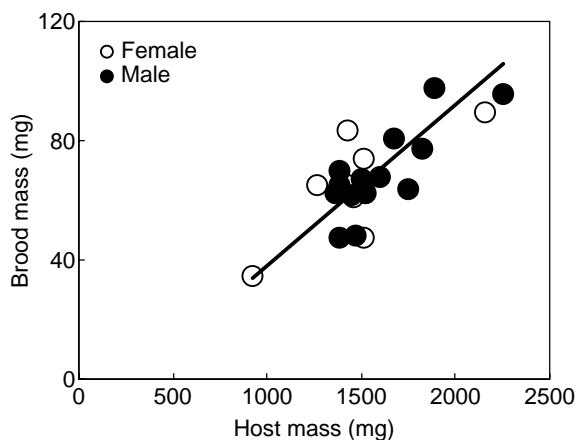


Figure 2. Relationship between host mass and brood mass (brood size  $\times$  wasp mass). The line fit to the data by model II regression is  $Y = aX + b$ , where  $Y$  is brood mass,  $X$  is host mass,  $a = 0.054 \times 10^{-2}$  (95% CI = [0.040, 0.072]) and  $b = -16.0$  (95% CI = [-40.9, 8.98]).

between secondary brood size and host mass differed significantly between males and females (Fig. 3A;  $\chi^2 = 4.62$ ,  $p = 0.03$ ; male: slope = 2.46, 95% CI = [1.73, 3.51], intercept =  $-1.54 \times 10^3$ , 95% CI = [ $-2.98 \times 10^3$ ,  $-0.10 \times 10^3$ ],  $r^2 = 0.67$ ,  $p < 0.05$ ; female: slope = 1.02, 95% CI = [0.50, 2.11], intercept =  $2.24 \times 10^2$ , 95% CI = [ $-9.84 \times 10^2$ ,  $14.3 \times 10^2$ ],  $r^2 = 0.38$ ,  $p > 0.05$ ). Slopes of relationships between wasp body mass and host mass also differed significantly between males and females (Fig. 3B;  $\chi^2 = 122.2$ ,  $p < 0.001$ ; male: slope =  $-1.74 \times 10^{-5}$ , 95% CI = [ $-3.07 \times 10^{-5}$ ,  $-0.98 \times 10^{-5}$ ], intercept =  $5.72 \times 10^{-2}$ , 95% CI = [ $4.02 \times 10^{-2}$ ,  $7.42 \times 10^{-2}$ ],  $r^2 = 0.09$ ,  $p > 0.05$ ; female: slope =  $1.32 \times 10^{-5}$ , 95% CI = [ $0.69 \times 10^{-5}$ ,  $2.55 \times 10^{-5}$ ], intercept =  $1.81 \times 10^{-2}$ , 95% CI = [ $0.42 \times 10^{-2}$ ,  $3.20 \times 10^{-2}$ ],  $r^2 = 0.51$ ,  $p < 0.05$ ). Therefore, brood mass in both sexes was proportional to host mass; however, males significantly increased only brood

size, whereas females significantly increased only body mass to fit to the host.

### Interaction with host resources

The probability of surviving to the final larval instar did not significantly differ between parasitized and non-parasitized hosts ( $p = 0.67$ ,  $\chi^2 = 0.19$ , 30.3% overall mortality). Five out of 57 surviving black cutworm larvae had supernumerary stadia (>6), of which four were parasitized and one was unparasitized. Five out of the 30 total host larvae presumed to have been parasitized pupated and emerged as a moth. Since it is unclear whether they were resistant to parasitism or wasps had actually failed to parasitize them, these larvae were eliminated from all analyses.

Repeated measures analysis of the brood data based on stadia (through stadium 4, and from stadium 5 through 6) as the time variable, parasitoid sex (female, male or unparasitized) as the treatment variable, and mass gain as the response variable, yielded statistical significance for time ( $p < 0.001$ ,  $F_{1,42} = 2.53 \times 10^3$ ), treatment ( $p < 0.001$ ,  $F_{2,42} = 66.0$ ), and time  $\times$  treatment ( $p < 0.001$ ,  $F_{2,42} = 68.5$ ). Orthogonal contrasts for female versus male broods and parasitized versus unparasitized hosts for each stadium are shown in Table 1. By the end of stadium 4, parasitized host larvae were significantly larger (on average by 15%), and had longer development time (on average by 9%) than unparasitized larvae, but no other significant differences among host responses were detected. In particular, male and female broods seemed to have similar effects on and responses to their hosts in these early stages of development. But for stadia 5 through 6, male broods were associated with significantly larger hosts than were female broods (on average by 15%). During that interval, parasitized larvae consumed more food (on average by 36%) and accumulated more mass (on average by 50%) while taking longer to develop than did unparasitized larvae (on average by 33%).

Both total and secondary brood sizes were larger in hosts that underwent supernumerary instars than in hosts that did not (ANCOVA: total brood size:  $F_{1,22} = 33.1$ ,  $p < 0.001$ ; secondary brood size:  $F_{1,22} = 27.2$ ,  $p < 0.001$ ). We thus eliminated the broods whose hosts underwent supernumerary instars from analyses of the relationship between host development and brood size to avoid introducing another factor that could obscure the focal relationship.

Table 2 summarizes correlations and slopes of model II regression relationships between brood size and host responses. Through stadium 4, only the positive correlation between brood size and mass gain pooled over sexes was statistically significant. For stadia 5 through 6, males and females differed significantly in the correlation between brood size and three of the host responses: mass gain, duration and growth. Only for the protected variable mass gain was there sufficient power to detect significant correlations within sex. For both sexes, the correlation was strongly positive, but mass gain increased more rapidly with brood size for male broods than for female broods.

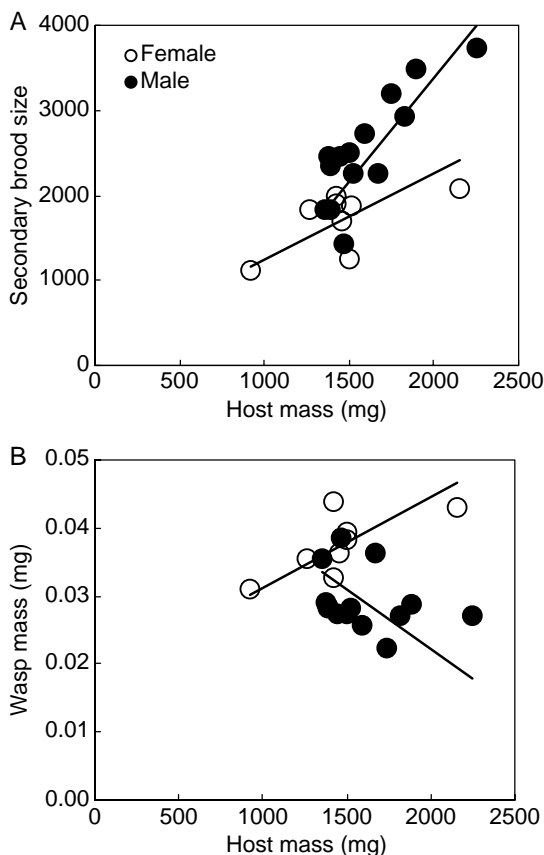


Figure 3. (A) relationship between host mass and brood size. The line fit to the data by model II regression is  $Y = aX + b$ , where Y is brood size, X is host mass. Female:  $a = 1.02$  (95% CI = [0.50, 2.11]),  $b = 2.24 \times 10^2$  (95% CI = [ $-9.84 \times 10^2$ ,  $14.3 \times 10^2$ ]); male:  $a = 2.46$  (95% CI = [1.73, 3.51]),  $b = -1.54 \times 10^3$  (95% CI = [ $-2.98 \times 10^3$ ,  $-0.10 \times 10^3$ ]). (B) relationship between host mass and wasp mass. The line fit to the data by model II regression is  $Y = aX + b$ , where Y is wasp mass, X is host mass. Female:  $a = 1.32 \times 10^{-5}$  (95% CI = [ $0.69 \times 10^{-5}$ ,  $2.55 \times 10^{-5}$ ]),  $b = 1.81 \times 10^{-2}$  (95% CI = [ $0.42 \times 10^{-2}$ ,  $3.20 \times 10^{-2}$ ]); male:  $a = -1.74 \times 10^{-5}$  (95% CI = [ $-3.07 \times 10^{-5}$ ,  $-0.98 \times 10^{-5}$ ]),  $b = 5.72 \times 10^{-2}$  (95% CI = [ $4.02 \times 10^{-2}$ ,  $7.42 \times 10^{-2}$ ]).

Table 1. Effects of sex and parasitism on host characteristics. Mean  $\pm$  SE of each host response at each host developmental stage. We emphasize that mass gain was consistently uncorrelated (through stadium 4) or negatively correlated (for stadium 5 through 6) with duration, based on analyses not presented here; this indicates that neither significant duration contrast can be explained by correlation with mass gain. Similarly, total food consumption was uncorrelated with mass gain (for stadium 5 through 6). Significance levels are \* = 0.05 > p  $\geq$  0.01, \*\*\* = 0.001 > p, and † = 0.0021 > p for the Bonferroni corrected contrasts (equivalent to 0.05 > p); no symbol = not significant.

Host response	Through stadium 4		Stadia 5 and 6	
	females vs males	parasitized vs unparasitized	females vs males	parasitized vs unparasitized
Mass gain (g)	0.075 $\pm$ 0.004	0.085 $\pm$ 0.005	0.081 $\pm$ 0.003	* 0.071 $\pm$ 0.002
Duration (d)	8.86 $\pm$ 0.14	9.17 $\pm$ 0.17	9.05 $\pm$ 0.12	† 8.27 $\pm$ 0.10
Growth rate (g day <sup>-1</sup> )	8.44 $\times 10^{-3}$	$\pm$ 0.48 $\times 10^{-3}$	9.00 $\times 10^{-3}$	$\pm$ 0.40 $\times 10^{-3}$
	9.33 $\times 10^{-3}$	$\pm$ 0.57 $\times 10^{-3}$	8.56 $\times 10^{-3}$	$\pm$ 0.30 $\times 10^{-3}$
Total food consumption (g)	0.725 $\pm$ 0.033	0.656 $\pm$ 0.026	0.682 $\pm$ 0.022	0.647 $\pm$ 0.025
Daily food consumption (g day <sup>-1</sup> )	0.304 $\pm$ 0.012	0.278 $\pm$ 0.012	0.288 $\pm$ 0.009	0.319 $\pm$ 0.007
Food assimilation efficiency (g g <sup>-1</sup> )	0.086 $\pm$ 0.004	0.108 $\pm$ 0.006	0.100 $\pm$ 0.005	0.090 $\pm$ 0.004
			1.28 $\pm$ 0.08	* 1.48 $\pm$ 0.05
			10.00 $\pm$ 0.69	9.58 $\pm$ 0.19
			13.35 $\times 10^{-2}$	$\pm$ 1.27 $\times 10^{-2}$
			15.54 $\times 10^{-2}$	$\pm$ 0.63 $\times 10^{-2}$
			7.06 $\pm$ 0.73	7.21 $\pm$ 0.60
			0.724 $\pm$ 0.082	0.757 $\pm$ 0.066
			0.191 $\pm$ 0.019	0.219 $\pm$ 0.016
			1.41 $\pm$ 0.05	*** 0.94 $\pm$ 0.02
			9.74 $\pm$ 0.27	† 7.31 $\pm$ 0.15
			14.74 $\times 10^{-2}$	$\pm$ 0.64 $\times 10^{-2}$
			13.02 $\times 10^{-2}$	$\pm$ 0.40 $\times 10^{-2}$
			7.15 $\pm$ 0.45	† 5.27 $\pm$ 0.17
			0.745 $\pm$ 0.050	0.725 $\pm$ 0.024
			0.208 $\pm$ 0.012	0.184 $\pm$ 0.007

## Discussion

In a species with many advantages for studying the size–number tradeoff within broods of offspring, we were able to demonstrate the tradeoff and clarify how the tradeoff is balanced. Wasp brood mass was proportional to host mass with a relatively high coefficient of determination, indicating a tight fit of the polyembryonic wasp brood to the size of its host, and thus establishing the basis for the tradeoff.

Of the three ways we considered that wasps might achieve a fit to host mass, data were most consistent with host manipulation by the wasp brood. Both sexes extended late development by the host, resulting in greater total food consumption and mass gain, but there was a stronger relationship between brood size and host mass and a greater host mass gain for male broods than for females. The inverse relation between brood size and wasp body mass after correcting for host size suggested body mass adjustment to fill the host, particularly for males, with their apparently greater variation in brood size. Moreover, brood size was significantly correlated with host mass gain before the time of brood size fixation independent of brood sex, which could mean that mass gain by the host cues or otherwise leads to larger brood size, in anticipation of a larger final host mass. This interpretation seems consistent with transplant experiments on *Copidosoma floridanum*, in which brood embryos transplanted early in development to new hosts increased their ultimate brood sizes with the new host's age, but those transplanted late (apparently after brood size fixation) did not (Corley et al. 2005). However, the combination of greater mass gain and a longer early developmental interval in parasitized versus unparasitized hosts could alternatively be explained as an early onset of host manipulation by the developing brood.

The two sexes clearly differed in the ways they balanced the size–number tradeoff. Females tended to produce smaller broods of larger individuals, and they responded to different final host sizes mainly by adjusting the mass of individual offspring. In contrast, males tended to produce larger broods of smaller individuals, and they responded to different final host sizes primarily by adjusting brood size. Our results thus provide strong evidence of differences in balancing mechanisms, balance points, and adjustments of balance points to resource levels between broods of the two sexes.

That parasitism increased total food consumption by hosts appears to be a typical consequence of parasitism by polyembryonic wasps (Hunter and Stoner 1975, Jones et al. 1982, Strand et al. 1990, Byers et al. 1993). Parasitized hosts may grow larger by extending the feeding phase (Rahman 1970), by increasing the efficiency of metabolism (Hunter and Stoner 1975), or because the parasitoids have a lower metabolic rate than their host (Slansky 1978; but see Jones et al. 1982). In contrast to the patterns for early instars, parasitized black cutworms had a prolonged late developmental interval and more total food consumption but did not have significantly greater daily food consumption or food utilization than non-parasitized larvae. This suggests that *C. bakeri* does not manipulate food processing by the host at this stage but does prolong the host's last stadium and thus ensures greater mass gain by the host. In *Trichoplusia ni* parasitized by *Copidosoma floridanum*, the

Table 2. Correlations between host responses and brood size. Correlation coefficients ( $r$ ) and slopes ( $b$ ) from model 2 regression for each developmental interval and host response. For the slopes, brood size was on the abscissa and host response on the ordinate. Significance levels are \* = 0.05 >  $p$  ≥ 0.01, \*\* = 0.01 >  $p$  0.001, \*\*\* = 0.001 >  $p$ , † is equivalent to 0.05 >  $p$  following Bonferroni correction, and no symbol = not significant.

Host response	Through stadium 4		Stadia 5 and 6
Mass gain (g)	F vs M, NS $r = 0.47$ , $b = 3.83 \times 10^4$ , *	***	F: $r = 0.85$ , $b = 1.46 \times 10^3$ , * M: $r = 0.75$ , $b = 3.25 \times 10^3$ , **
Duration (d)	F vs M, NS $r = 0.42$ , $b = 1.11 \times 10^3$	†	F: $r = -0.91$ , $b = -1.63 \times 10^2$ M: $r = 0.52$ , $b = 8.37 \times 10^2$
Growth rate (g day <sup>-1</sup> )	F vs M, NS $r = 0.32$ , $b = 3.32 \times 10^5$	†	F: $r = 0.89$ , $b = 8.89 \times 10^3$ M: $r = 0.33$ , $b = 2.57 \times 10^4$
Total food consumption (g)	F vs M, NS $r = -0.21$ , $b = -6.17 \times 10^3$		F vs M, NS $r = 0.31$ , $b = 2.94 \times 10^2$
Daily food consumption (g day <sup>-1</sup> )	F vs M, NS $r = -0.11$ , $b = -1.49 \times 10^4$		F vs M, NS $r = 0.30$ , $b = 2.66 \times 10^3$
Food assimilation efficiency (g g <sup>-1</sup> )	F vs M, NS $r = 0.55$ , $b = 2.78 \times 10^4$		F vs M, NS $r = 0.15$ , $b = 1.09 \times 10^4$

normal decline in juvenile hormone titer and corresponding increase in ecdysteroid hormone titer are delayed by 24 h, resulting in delayed onset of the host's wandering phase (Strand et al. 1990).

Immediately before pupating and emerging from the host, the wasp brood consumes almost everything inside the cuticle of the caterpillar. This is consistent with the relatively tight proportional relationship between (dry) brood mass and (wet) maximal host mass in Fig. 2. From our preliminary data of dry host and brood mass, we estimate that the emerging brood accounts for roughly 53% of the host mass. The other 47% of the host mass may constitute an essentially irreducible residual: the few larvae that died shortly before pupation and pupae unable to emerge, host cuticle, wasp molting waste products, frass (meconium), and perhaps a very small amount of uneaten material inside the host. Thus, selection may have nearly maximized the mass of the emerging brood, which is the mathematical product of body mass and brood size.

The inverse relationship between standardized wasp body mass and wasp brood size implies that a larger number of offspring in the host yields smaller individuals because of less host resource available per individual wasp (Slansky 1986). In genetically heterogeneous gregarious parasitoids, larger wasp mass in a larger host is predicted as an adaptive response by the wasps to more intense intra-brood competition (sibling conflict; Godfray and Parker 1991). In addition, gregarious parasitoids that halt host development (idiobionts) tend to have both larger wasp adults and larger broods in larger hosts, due to under-compensation for larger host size providing more resource per offspring (Mayhew 1998, Mayhew and Glaizot 2001; see also Charnov and Skinner 1985). But in clonal broods, compensation via a decrease in wasp body mass at large brood size can be advantageous, since intense larval competition is counterproductive. Clonal broods may maximize brood fitness by increasing host size regardless of the way that brood size and body mass tradeoff.

Figure 2 strongly suggests that most of the variation in brood mass of *C. bakeri* (= brood size × wasp body mass), and thus much of the variation in wasp brood size and body mass, is attributable to differences in host mass. Variation in the precise way that each brood trades off brood size against body mass, notably including differences between the sexes, accounts for another component of variation among broods

in body mass and in brood size, which may be influenced by particulars of the host environment beyond host size alone. This is because brood size is almost certainly fixed when physiological and morphological cues to the ultimate host size could only be very rough and approximate (West and Sheldon 2002), with body mass providing the later adjustment to achieve the tight fit between brood mass and final host mass. Selection on adult traits seems likely to determine the typical or average balance point of the tradeoff (Mayhew and Glaizot 2001).

After correcting for differences in host mass, female broods tended to have fewer but larger wasp individuals, whereas males broods had more individuals of smaller body mass. Female wasps were about 32% heavier than males, in general accord with the typical pattern for insects (Teder and Tammaru 2005). Larger females are assumed to have more eggs or sometimes larger eggs (Stearns and Koella 1986). In parasitoids, the typically female biased sexual size dimorphism may arise from selection on host finding ability (Bennett and Hoffmann 1998) or on fecundity (Visser 1994). Since female *C. bakeri* are larger bodied than males, female broods must necessarily contain fewer individuals than male broods to allow females to attain larger mass (Godfray 1994) – in contrast to smaller male broods than female broods or no differences in some other polyembryonic parasitoids of the families Encyrtidae, Platygasteridae and Braconidae (Strand 1989c, Ode and Strand 1995).

Two patterns in the data that deserve further attention are the apparently higher variance in male than in female body mass and brood size (Fig. 1) and the increase in body mass with host mass in females but the increase in brood size with host mass in males (Fig. 3). Though the trend toward increasing brood size with host mass in females is not statistically significant, a positive relationship between these variables is consistent with the sub-proportional increase in female body mass with host mass. Taken together, these patterns suggest (1) weaker selection overall on the tradeoff balance point in males than females and (2) a difference between sexes in the relative benefits of body mass and brood size.

Effects of body size on fitness may differ between males and females, in which female body size has stronger positive association with their fitness than male body size does with male fitness (Charnov 1979, van den Assem 1989). Both males and females have a primary searching and handling

problem to solve as adults – males must rendezvous with females and females with host eggs. One possible difference between sexes is that the ability to find and oviposit into hosts is more positively related to body mass than is the ability to find and mate with females. Larger females may be more likely to find hosts than smaller females (Bennett and Hoffmann 1998). In contrast, the mating strategies of males may minimize size effects on mating success. For example, males of differing sizes may be equally able to locate females emerging in large aggregations, or may attract them by swarming (Thornhill and Alcock 1983), as documented for some encyrtids (Nadel 1987). However, larger females may be better able to insert the ovipositor into eggs more difficult or time-consuming to penetrate for smaller females. These factors may all yield more readily to larger female size, and larger females are able to produce more eggs as well. Thus, males may benefit more from increasing their numbers rather than their body size to maximize the number of females mated, while females may achieve more total oviposition by trading some brood size for body mass. These ideas need to be tested in additional laboratory, field, and perhaps greenhouse studies, which would also benefit from modeling work to assess the potential adequacy of proposed mechanisms and to sharpen the hypotheses to be tested.

Sex-specific balance points have been documented in other life-history tradeoffs (reviewed by Nylin and Gotthard 1998). For example, in species that are univoltine or territorial or both, males usually emerge earlier (protandry) with smaller body size than females. In the tradeoff between age at maturity and body size, males prioritize early maturation while females may be under greater selection pressure to be larger for greater fecundity (Fischer and Fiedler 2000). These tradeoffs may become more evident at high densities leading to intense sexual selection or under strong resource limitation environmental stress. Future studies will investigate how the sex-specific balance points of the tradeoff respond to these intensifying conditions.

Our results on the size–number tradeoff in *C. bakeri* highlight some significant advantages of working with clonal systems in identifying both the mechanisms and the selection pressures involved in establishing and balancing tradeoffs. Many other clonal species should prove amenable to this approach. But our results may also contribute to the analysis of genetically mixed broods and provide a way of measuring the cost of sibling conflict during development. For example, the sex of the eggs being laid within a host can sometimes be consistently determined from the female's oviposition behavior, and some females will lay multiple eggs into a host (Strand 1989b). Thus the size–number tradeoff in clonal broods of each sex can potentially be compared with the way the tradeoff works for sibling eggs of the same or different sexes and for unrelated eggs of the same or different sexes.

*Acknowledgements* – We thank E. C. Kane and M. E. Schauff (US Dept of Agriculture), and J. S. Noyes (British Museum) for kindly identifying wasps. We are especially grateful to M. Strand and D. Donnel for providing helpful advice on rearing and handling *Copidosoma*, and to S. Cilles, D. Donnel, M. Segoli, M. Strand and P. V. Switzer, who provided insightful comments on a previous version of the manuscript. We also would like to express

gratitude for P. Ode for useful comments and discussion. This study was funded by a grant from the US Golf Ass. (to DAP), a fellowship from the Univ. of Kentucky Graduate School, a Sigma Xi Grant-in-Aid of Research, and a Biology Dept Teaching Assistantship to YS.

## References

- Beckage, N. E. 1997. The parasitic wasp's secret weapon. – *Sci. Am.* 277: 50–55.
- Beckage, N. E. and Riddiford, L. M. 1982. Effects of parasitism by *Apanteles congregatus* on the endocrine physiology of the tobacco hornworm, *Manduca sexta*. – *Gen. Comp. Endocrinol.* 47: 308–322.
- Baehrecke, E. H. and Strand, M. R. 1990. Embryonic morphology and growth of the polyembryonic parasitoid *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae). – *Int. J. Insect Morphol. Embryol.* 19: 165–175.
- Bennett, D. M. and Hoffmann, A. A. 1998. Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). – *J. Anim. Ecol.* 67: 580–591.
- Byers, J. R. et al. 1993. Parasitism of the army cutworm, *Euxoa auxiliaris* (GRT) (Lepidoptera: Noctuidae), by *Copidosoma bakeri* (Howard) (Hymenoptera: Encyrtidae) and effect on crop damage. – *Can. Entomol.* 125: 329–335.
- Charnov, E. L. 1979. The genetical evolution of patterns of sexuality: Darwinian fitness. – *Am. Nat.* 113: 465–480.
- Charnov, E. L. and Skinner, S. W. 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. – *Environ. Entomol.* 14: 383–391.
- Corley, L. S. et al. 2005. Both endogenous and environmental factors affect embryo proliferation in the polyembryonic wasp *Copidosoma floridanum*. – *Evol. Dev.* 7: 115–121.
- Fischer, K. and Fiedler, K. 2000. Sex-related differences in reaction norms in the butterfly *Lycaena tityrus* (Lepidoptera: Lycaenidae). – *Oikos* 90: 372–380.
- Fox, C. W. and Czesak, M. E. 2000. Evolutionary ecology of progeny size in arthropods. – *Annu. Rev. Entomol.* 45: 341–369.
- Godfray, H. C. J. 1986. Models for clutch size and sex ratio with sibling interaction. – *Theor. Popul. Biol.* 30: 215–231.
- Godfray, H. C. J. 1987. The evolution of clutch size in parasitic wasps. – *Am. Nat.* 129: 221–233.
- Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. – Princeton Univ. Press.
- Godfray, H. C. J. and Parker, G. A. 1991. Clutch size, fecundity and parent–offspring conflict. – *Philos. Trans. R. Soc. B* 332: 67–79.
- Harvey, J. A. 1996. *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*: is the parasitoid a conformer or regulator? – *J. Insect Physiol.* 42: 1017–1025.
- Hunter, K. WJr. and Stoner, A. 1975. *Copidosoma truncatellum*: effect of parasitization of food consumption of larval *Trichoplusia ni*. – *Environ. Entomol.* 4: 381–382.
- Ivanova-Kasas, O. M. 1972. Polyembryony in insects. – In: Counce, S. J. and Waddington, C. H. (eds), *Developmental systems*, Vol. 2. Insects. Academic Press, pp. 243–271.
- Jones, D. et al. 1982. Effect of the parasite *Copidosoma truncatellum* on development of its host *Trichoplusia ni*. – *Ann. Entomol. Soc. Am.* 75: 7–11.
- Klingenberg, C. P. and Spence, J. R. 1997. On the role of body size for life-history evolution. – *Ecol. Entomol.* 22: 55–68.
- Lack, D. 1947. The significance of clutch-size. – *Ibis* 89: 302–352.
- Lawrence, P. O. 1986. Host–parasite hormonal interactions: an overview. – *J. Insect Physiol.* 32: 295–298.



Mackauer, M. and Sequeira, R. 1993. Patterns of development in insect parasites. – In: Beckage, N. E. et al. (eds), Parasites and pathogens of insects, Vol. 1. Parasites. Academic Press, pp. 1–23.

Mappes, T. and Koskela, E. 2004. Genetic basis of the tradeoff between offspring number and quality in the bank vole. – *Evolution* 58: 645–650.

Mayhew, P. J. 1998. Offspring size-number strategy in the bethylid parasitoid *Laelius pedatus*. – *Behav. Ecol.* 9: 54–59.

Mayhew, P. J. and Glaizot, O. 2001. Integrating theory of clutch size and body size evolution for parasitoids. – *Oikos* 92: 372–376.

Nadel, H. 1987. Male swarms discovered in Chalcidoidea (Hymenoptera: Encyrtidae, Pteromalidae). – *Pan-Pac. Entomol.* 63: 242–246.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. – *Annu. Rev. Entomol.* 43: 63–83.

Ode, P. and Strand, M. R. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. – *J. Anim. Ecol.* 64: 213–224.

Rahman, M. 1970. Effect of parasitism on food consumption of *Pieris rapae* larvae. – *J. Econ. Entomol.* 63: 820–821.

Roff, D. A. and Fairbairn, D. J. 2007. The evolution of tradeoffs: where are we? – *J. Evol. Biol.* 20: 433–447.

Schaaf, A. C. 1972. The parasitoid complex of *Euxoa ochrogaster* (Guenee) (Lepidoptera: Noctuidae). – *Quaest. Entomol.* 8: 81–120.

Sibly, R. M. and Calow, P. 1986. Physiological ecology of animals: an evolutionary approach. – Blackwell.

Slansky, F. Jr. 1978. Utilization of energy and nitrogen by larvae of the imported cabbageworm, *Pieris rapae*, as affected by parasitism by *Apanteles glomeratus*. – *Environ. Entomol.* 7: 179–185.

Slansky, F. Jr. 1986. Nutritional ecology of endoparasitic insects and their hosts: an overview. – *J. Insect Physiol.* 32: 255–261.

Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. – *Am. Nat.* 108: 499–506.

Stearns, S. C. 1989. Tradeoffs in life-history evolution. – *Funct. Ecol.* 3: 259–268.

Stearns, S. C. and Koella, J. C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. – *Evolution* 40: 893–913.

Strand, M. R. 1989a. Development of the polyembryonic parasitoid *Copidosoma floridanum* in *Trichoplusia ni*. – *Entomol. Exp. Appl.* 50: 37–46.

Strand, M. R. 1989b. Oviposition behavior and progeny allocation of the polyembryonic wasp *Copidosoma floridanum* (Hymenoptera: Encyrtidae). – *J. Insect Behav.* 2: 355–369.

Strand, M. R. 1989c. Clutch size, sex ratio and mating by the polyembryonic encyrtid *Copidosoma floridanum* (Hymenoptera: Encyrtidae). – *Fla. Entomol.* 72: 32–42.

Strand, M. R. et al. 1990. Alterations in the ecdysteroid and juvenile hormone esterase profiles of *Trichoplusia ni* parasitized by the polyembryonic wasp *Copidosoma floridanum*. – *Arch. Insect Biochem. Physiol.* 13: 41–51.

Stuefer, J. F. et al. 2002. A genotypic tradeoff between the number and size of clonal offspring in the stoloniferous herb *Potentilla reptans*. – *J. Evol. Biol.* 15: 880–884.

Teder, T. and Tammaru, T. 2005. Sexual size dimorphism within species increases with body size in insects. – *Oikos* 108: 321–334.

Thornhill, R. and Alcock, J. 1983. The evolution of insect mating systems. – IUniverse.com, Inc.

van den Assem, J. et al. 1989. Is being large more important for female than for male parasitic wasps? – *Behaviour* 108: 160–195.

van Noordwijk, A. J. and de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. – *Am. Nat.* 128: 137–142.

Vinson, S. B. and Iwantsch, G. F. 1980. Host regulation by insect parasitoids. – *Q. Rev. Biol.* 55: 143–165.

Visser, M. E. 1994. The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). – *J. Anim. Ecol.* 63: 963–978.

Warton, D. I. et al. 2006. Bivariate line-fitting methods for allometry. – *Biol. Rev.* 81: 259–291.

West, S. A. and Sheldon, B. C. 2002. Constraints in the evolution of sex ratio adjustment. – *Science* 295: 1685–1688.

Young, R. L. and Badyaev, A. V. 2004. Evolution of sex-biased maternal effects in birds: I. Sex-specific resource allocation among simultaneously growing oocytes. – *J. Evol. Biol.* 17: 1355–1366.

## Appendix 1.

### Standardizing brood size and body mass of parasitoids

Let  $n$  be brood size and  $y$  be body mass of parasitoids, and let  $h$  be maximum body mass of the host caterpillar. We assume that the total parasitoid brood mass  $ny$  is proportional to  $h$  (Fig. 5), and thus  $n \times y = k \times h$ , where  $k$  is a constant of proportionality (independent of  $n$ ,  $y$  and  $h$ ) representing the fraction of the maximum host mass attributable to the parasitoid brood. Let  $\bar{h}$  be the mean maximum host mass over the set of hosts and parasitoid broods to be analyzed, and let the subscript  $i$  identify a particular host and brood, for which  $n_i \times y_i = k \times h_i$ .

For the  $i$ th host and brood, we define the standardized

parasitoid brood size to be  $N_i = n_i \sqrt{\frac{\bar{h}}{h_i}}$ , and the standar-

dized parasitoid body mass to be  $Y_i = y_i \sqrt{\frac{\bar{h}}{h_i}}$ , where

standardization removes the effect of host mass from the magnitude of brood size and body mass. To see this, substitute for  $n_i$  and  $y_i$  in  $n_i \times y_i = k \times h_i$ , resulting in

$$\left[ \frac{N_i}{\sqrt{\frac{\bar{h}}{h_i}}} \right] \left[ \frac{Y_i}{\sqrt{\frac{\bar{h}}{h_i}}} \right] = kh_i, \text{ or } N_i Y_i = k\bar{h}.$$

This means that  $N_i$  and  $Y_i$  will be inversely related to each other across all  $i$  independently of host mass.