

A comparative study of body size and clutch size across the parasitoid Hymenoptera

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Across animal species, body size and clutch size often form part of a suite of associated life history traits, exemplified by the “fast–slow continuum” in mammals. Across the parasitoid Hymenoptera however, a major axis of life history variation is the development mode of the larva (koinobiosis versus idiobiosis), and body size and clutch size do not seem to form clear associations with this major axis. Here we use a large comparative data set and the latest phylogenetic information to explore hypotheses that might explain the variation in body size and clutch size across species in parasitoids. We find evidence for three novel evolutionary correlations: changes in the stage of host attacked by the parasitoid (i.e. egg, larva, pupa) significantly predict changes in both body size and clutch size, whilst in gregarious species changes to higher latitudes are associated with reduced clutch size. We also find a number of hypothesized cross-species (phenotypic) associations that, however, we cannot demonstrate are the result of evolutionary correlations: large bodied species in our data tend to lay small clutches; koinobionts are larger than idiobionts attacking the same host stage; tropical species are smaller than temperate species (Bergmann’s rule). Our results provide support for theoretical models of trait evolution in parasitoids, whilst the associations between latitude and life history may help explain why species richness in the family Ichneumonidae peaks at intermediate latitudes. Our results also show the continuing value of phylogenetically-based comparative analyses and demonstrate that recent work on parasitoid phylogenetics has produced significant benefits for our understanding of life history evolution.

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The explanation of life history variation across species remains one of the major challenges in evolutionary ecology. In recent years considerable progress has come from the interplay of interspecific comparative studies, which describe the associations between traits across taxa, and theoretical models that attempt to predict those associations. Perhaps the most notable studies have concerned mammals, where a fast-slow continuum of traits exists; large bodied species have long lifespans, suffer low adult mortality, mature late, have low fecundity, small litters, and large offspring that suffer low juvenile mortality. Small-bodied species have the opposite characteristics (Harvey and Purvis 1999). Some adaptive models (Charnov 1991, 1993, Kozłowski and Weiner

1996) have had notable success in predicting this suite of associations (Purvis and Harvey 1995, Harvey and Purvis 1999). Some of the mammalian associations have also been found in other organisms, such as parasitic nematodes (Gemmell et al. 1999), and angiosperms (Franco and Silvertown 1997), though other groups such as birds differ in substantial ways that demand alternative models (Charnov 2000, Bennett and Owens 2002).

Parasitoids are insects that develop to maturity by feeding on the body of another host arthropod, eventually killing it. The parasitoid Hymenoptera (wasps) are one of the most species-rich components of terrestrial ecosystems; about 77 000 species have been described (Mayhew 1998 using data from Brown 1982), and they

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may comprise 20% of all insect species (LaSalle and Gauld 1991). They also display enormous variation in life history traits that begs explanation (Godfray 1994). Despite much study on intraspecific variation, the associations between traits across species are still relatively poorly known, and as yet no general theoretical model has attempted to predict or describe such variation. In this study we describe novel interspecific associations between traits that should promote understanding of the causes of life history variation in this group and help the development of theory.

Previous comparative work on the parasitoid Hymenoptera has revealed that development mode is a major predictor of a suite of life history traits (Askew and Shaw 1986, Sheehan and Hawkins 1991, Godfray 1994, Quicke 1997, Mayhew and Blackburn 1999, Müller et al. 1999, Jervis et al. 2001). Specifically, koinobionts, which allow their host to continue to develop after parasitization, have long development times, tend to be endoparasitic, have short adult lifespans, a high fecundity, lay small eggs, emerge with many eggs matured, and have narrow host ranges. Idiobionts, which permanently paralyze their hosts, have the opposite traits. One could consider koinobionts to have “slow” larvae but “fast” adults, and idiobionts to have “fast” larvae but “slow” adults. Another useful distinction is that, unlike mammals and birds, parasitoids are not exclusively “income” breeders (Stearns 1992), meaning that the energy for reproduction is not only taken from current energy harvest. Instead, koinobionts more closely approximate “capital” breeders, where energy is stored as a larva and used during adulthood for reproduction. Idiobionts tend more towards income breeding (Jervis and Kidd 1986, Jervis et al. 2001). Unsurprisingly then the suite of inter-related traits seen in mammals is not found in parasitoids (Blackburn 1991a). Interestingly, not only is parasitoid body size not associated with most other traits, unlike in mammals (Blackburn 1991a), but it is also not strongly associated with development mode (Mayhew and Blackburn 1999). Correlates of clutch size have not yet been investigated in large-scale interspecific studies. This begs the question of what does control the variation in these two life-history traits that are such central components of variation in other groups.

Here we test hypotheses, derived both from knowledge of parasitoid biology and studies on other organisms, that have not yet received tests in a large cross-species comparative study in this group:

- 1) Body size and clutch size might be associated with the stage of host attacked (e.g. egg, larva, pupa). Theoretical models of interspecific variation (Mayhew and Glaizot 2001) have suggested that both clutch size and body size have the potential to increase with host size across species. In addition, interspecific comparative studies on two parasitoid

- taxa, the braconid genus *Apanteles* (le Masurier 1987) and the family Bethyilidae (Mayhew and Hardy 1998), have shown that both these trends occur. However, these studies could only examine relationships between close relatives: in addition all these parasitoids attack a single host stage (the host larva).
- 2) When controlling for host stage attacked, clutch size and body size might be negatively correlated. Intraspecific studies have shown that, when the size of the host is controlled, larger clutches result in smaller bodied individuals because offspring must compete for limited resources (Hardy et al. 1992). In addition, families that contain gregarious species (where more than one individual can develop from each host) also tend to be smaller bodied than their sister taxa that are exclusively solitary (Mayhew 1998). However, gregarious development is only a crude indicator of actual clutch size, and the latter study could not examine relationships at finer taxonomic levels.
- 3) After controlling for the stage of host attacked, koinobionts might have larger bodies or lay larger clutches than idiobionts. Since koinobionts allow their hosts to continue to develop for some time after parasitization, the host should in general be larger when the parasitoid completes development than for idiobionts. Koinobionts may exploit this advantage in two ways: by having more offspring per host (increased clutch size) or by increased individual offspring fitness per host (increased body size). Previous comparative studies have failed to support a direct relationship between development mode and body size (Mayhew and Blackburn 1999), but the host stage attacked is a possible confounding variable.
- 4) Body size and clutch size might be positively correlated with latitude. The positive correlation of body size with latitude (Bergmann's rule) has been described in a number of taxa (Gaston and Blackburn 2000), although there are significant exceptions amongst the insects. Clutch size increases strongly with latitude in birds (Cardillo 2002) but has not been extensively investigated in other taxa. Neither hypothesis has been tested in parasitoids, yet latitudinal effects on life history hold the potential to explain much of the variation across species.

Methods

Data

We used a data set that has been the material for three previous comparative studies (Blackburn 1991a, b, Mayhew and Blackburn 1999). The data comprise

information on 474 parasitoid wasp species derived from the published literature prior to 1990. Since initial investigations of this data set produced few positive results, it was criticized, quite rightly, as representing a 'very sparse representation of a very diverse group of wasps, with a rather poorly resolved taxonomy' (Godfray 1994, p. 320). The implication was that, if only the data had been more complete and standardized, more significant associations would have emerged. However, a further investigation of the data by Mayhew and Blackburn (1999) provided evidence for a number of hypothesized associations, suggesting that the data are at least good enough for major axes of life history variation to be identified. The implication now is that the associations hypothesized in the earlier studies were genuinely absent. Given that these data are now known to contain useful information, we feel it is now important to ask more questions from them, especially about variables that are so far unexplained.

In addition there has been much recent work on the phylogenetic relations of parasitoid wasps. In our comparative analyses we use both the traditional taxonomy used in the earlier analyses as well as information from recent phylogenetic studies. Differences in outcome allow us to judge the sensitivity of results to phylogenetic assumptions, as well as the added value of this recent phylogenetic work.

The variables investigated here are:

- Body length (mm) excluding antennae and ovipositor.
- Clutch size: the mean number of parasitoid offspring completing development per individual host.
- Solitary or gregarious development: solitary development is where the mean clutch size as defined above is one, gregarious development where it is greater than one.
- Development mode: idiobiont or koinobiont.
- Geographic distribution: temperate/ tropical.
- Host stage attacked. Species were classified as ovipositing into eggs, nymphs, larvae, pre-pupae, pupae, adults or any combination of these. In the independent contrast analyses (below) several of these categories were not sufficiently well represented to enable contrasts to be calculated, and we only considered species attacking a single host stage.

Analysis

Both continuous variables were \log_{10} transformed prior to analysis to meet statistical assumptions. Hypotheses were first tested by analysis of the raw data across species. Such "phenotypic associations" allow one to predict something about the value of one species trait in our data given knowledge of another trait. Phenotypic

correlations are the product of any evolutionary correlations between traits (associations between evolution in one trait and evolution in another). Phenotypic correlations can reflect evolutionary correlations relatively accurately if there is no phylogenetic dependence in the data, such that trait values in each species are relatively independent of those in others (Price 1997, Freckleton et al. 2002). However, if there is a degree of phylogenetic dependence in the data, phylogenetic information needs to be incorporated into the analysis to detect evolutionary correlations, and to allow this we used the phylogenetic regression (PR; Grafen 1989).

Phylogenetic regression is an independent contrast method that uses the raw species data and a phylogeny to calculate sets of contrasts that represent differences between sister-taxa in the phylogeny, and are evolutionarily independent. The raw data undergo two transformations prior to the calculation of the final F-ratio. First, a "long regression" is carried out, in which each datapoint represents the deviation of a species value from its parent, where parental values are themselves calculated as the average of their descendents (Grafen 1989, section 3a). This long regression is used to fit the regression parameters, simultaneously with a parameter ρ , both reported here in Table 1. The value of ρ is used to scale the branch lengths of the phylogeny prior to the calculation of the independent contrasts, from which the final "short regression" is derived, and the resulting F-ratio, from which significance is taken. The value of ρ is essentially used to adopt an appropriate evolutionary model, which is important if evolutionary correlations are to be detected (Price 1997, Harvey and Rambaut 2000, Freckleton et al. 2002), and it should ideally reflect the degree of phylogenetic dependence of the data. Recent simulation studies show that ρ is a relatively good estimator of phylogenetic dependence when the number of species analyzed is quite large, as it is in our study (Freckleton et al. 2002). Values of ρ close to zero represent phylogenetic independence, whilst values approaching unity represent much phylogenetic dependence.

When there is a significant phenotypic correlation but a non-significant evolutionary correlation, possible reasons include: a) reduction in power in the phylogenetic analysis b) an inappropriate evolutionary model in the phylogenetic analysis c) phylogenetic dependence in the data, meaning that cross species analysis does not accurately reflect evolutionary correlations (Mayhew and Pen 2002). In case (c), evolutionary changes deep in the phylogeny can exert a disproportionately large historical impact on extant phenotypes (Hardy and Mayhew 1998, West and Herre 1998). To examine whether such explanations apply, it is useful to examine individual contrasts, first to see which particular events have been historically influential in this way, and second to examine how some contrasts come to be in the opposite direction to that expected from the cross species

Table 1. Phylogenetic regression results (*P < 0.05 **P < 0.001). Numbers denote regression estimates or p values (italics). Estimates represent the slopes for continuous variables or the means of each factor level relative to the first factor level, arbitrarily set at zero. r – response variable, e – explanatory variable, c – control variable. E – egg, N – nymph, L – larva, Pr – prepupa, P – pupa, A – adult, Cs – clutch size, S – solitary, G – gregarious, I – idiobiont, K – koinobiont, Te – temperate, Tr – tropical.

Analyses	Taxonomy	Conservative cladogram	Resolved cladogram
Body size (r) and host stage attacked (e) (full data set)	0.379* 0.000 E –0.101 N 0.120 L 0.205 Pr 0.109 P 0.027 A	0.370** 0.000 E –0.070 N 0.268 L 0.364 Pr 0.261 P 0.065 A	0.389** 0.000 E –0.182 N 0.156 L 0.289 Pr 0.179 P 0.046 A
Clutch size (r) and host stage attacked (e) (full data set)	0.082* 0.000 E –0.435 N –0.125 L 0.504 Pr 0.047 P –0.133 A	0.045* 0.000 E –0.384 N –0.106 L 0.550 Pr 0.039 P –0.169 A	0.082* 0.000 E –0.334 N –0.099 L 0.541 Pr 0.048 P –0.070 A
Body size (r) and clutch size (e) (full data set)	0.379 0.002 Cs	0.352 –0.006 Cs	0.476 0.002 Cs
Body size (r), clutch size (e), host stage attacked (c) (full data set)	0.419 –0.023 Cs	0.192 –0.023 Cs	0.500 –0.018 Cs
Body size (r), solitary/gregarious development (e) (full data set)	0.379 0.000 S –0.028 G	0.352 0.000 S –0.035 G	0.476 0.000 S –0.028 G
Body size (r), development mode (e), host stage attacked (c) (full data set)	0.526 0.000 I –0.040 K	0.260 0.000 I 0.022 K	0.526 0.000 I –0.046 K
Clutch size (r), development mode (e), host stage attacked (c) (full data set)	0.100 0.000 I 0.081 K	0.055 0.000 I 0.035 K	0.111 0.000 I 0.052 K
Body size (r), geographic distribution (e) (full data set)	0.334 0.000Te –0.022 Tr	0.288 0.000 Te –0.032 Tr	0.334 0.000 Te –0.018 Tr
Body size (r), geographic distribution (e) (Ichneumonidae only)	0.399 0.000 Te 0.050 Tr	0.003 0.000 Te 0.087 Tr	0.045 0.000 Te 0.084 Tr
Clutch size (r) and geographic distribution (e) (full data set)	0.045 0.000 Te 0.120 Tr	0.037 0.000 Te 0.292 Tr	0.082 0.000 Te 0.291 Tr
Clutch size (r) and geographic distribution (e) (gregarious species only)	0.009 0.000 Te 0.298 Tr	0.002* 0.000 Te 0.292 Tr	0.002* 0.000 Te 0.291 Tr

analysis. Phylogenetic regression does not easily allow us to examine the contrasts, so instead we re-ran the analysis through an alternative software package that does (CAIC, Purvis and Rambaut 1995). CAIC cannot run all the types of general linear model we wished to perform, and does not automatically scale the branch lengths of the phylogeny to search for appropriate evolutionary models, hence our overall preference for PR. Nonetheless it is useful at a basic descriptive level. We applied the equal branch length option to generate contrasts, and then identified contrasts of interest, the source of which we then investigated in the raw data. In all cases analyses that were non-significant using PR were also non-significant using CAIC.

Phylogenetic assumptions

We performed phylogenetic analyses using the traditional taxonomy used in earlier analyses of the data

(Blackburn 1991a, b), and also by constructing composite cladograms from recent analyses of phylogeny. If several phylogenetic analyses had been attempted on a group and there was a lack of consensus between them, we took two alternative approaches; we first constructed a “conservative” cladogram, representing only relationships that are considered robust, and collapsing uncertainties into soft polytomies. Second, we used only the most highly resolved tree available in an attempt to maximize power. In both cladograms, where no phylogenetic estimates were available for a group, we used the information from taxonomy.

The basal branches of the conservative tree are taken from Fig. 4 of Ronquist (1999a). Chrysidoidea are from Fig. 5 of Ronquist (1999a). Ceraphronoidea relationships are taxonomy based, as are the Evanoidea, the Platygasteroidea, and the Proctotrupoidea. Chalcidoidea relations are also taxonomy based, apart from the Eulophidae, which are based on Fig. 5 of Gauthier et al., (2000). The Cynipoidea are based on Fig. 2c of

Ronquist (1999b). Braconidae relationships are taken from Fig. 1a of Dowton et al. (2002). The Ichneumonidae are taken from Fig. 8 of Belshaw and Quicke (2002).

The more resolved tree differs from the conservative tree only in the following areas: the basal relationships are taken from Fig. 9 of Dowton and Austin (2001), the Chalcidoidea are based upon Noyes (1990), the microgastroids (Braconidae) are based on Fig. 5 of Dowton and Austin (1998).

The number of nodes in these different estimates of phylogeny are 166, 190, and 209 for the taxonomy, the conservative phylogeny, and the resolved phylogeny respectively. This represents the maximum number of contrasts that could be made in the data, if all variables were represented for all species.

Results

Body size, clutch size, and host stage attacked

There was a significant association between body size and host stage attacked across species (Kruskal–Wallis test, $\chi^2 = 88.61$, $df = 12$, $P < 0.001$), and in all the PR analyses (Table 1). Across species, parasitoids attacking eggs or nymphs had the smallest bodies whilst parasitoids attacking prepupal host stages had the largest bodies (Fig. 1a). The phylogenetic regressions produced values of ρ ranging from 0.370 to 0.389, indicating moderate phylogenetic effects. PR estimates also showed that parasitoids attacking eggs and nymphs had the smallest bodies, whilst those attacking pre-pupae had the largest bodies (Table 1).

Across species there was also a significant association between clutch size and host stage attacked (Kruskal–Wallis test, $\chi^2 = 26.20$, $df = 11$, $P = 0.006$). This relationship was also significant in all PR analyses (Table 1). Across species, parasitoids attacking nymphal host stages laid the smallest clutches and parasitoids attacking prepupal host stages laid the largest (Fig. 1b); egg, larval, pupal and adult parasitoids tended to lay similarly sized clutches. The PR analyses produced values of ρ ranging from 0.045 to 0.082 indicating very weak phylogenetic effects. PR estimates also showed that parasitoids attacking nymphs laid the smallest clutches, whilst parasitoids attacking prepupae laid the largest clutches (Table 1).

Across species, there was a significant negative association between clutch size and body size (Spearman $R = -0.211$, $n = 221$, $P = 0.002$). However, none of the PR analyses were significant (Table 1). Values of ρ (ranging from 0.352 to 0.476) indicate moderate phylogenetic effects. Investigation of CAIC contrasts revealed a strongly negative contrast at the root of the tree. The latter is likely to have been historically influential on the cross-species result. Unexpected contrasts were found in the conservative phylogeny between *Mesochorus agilis*

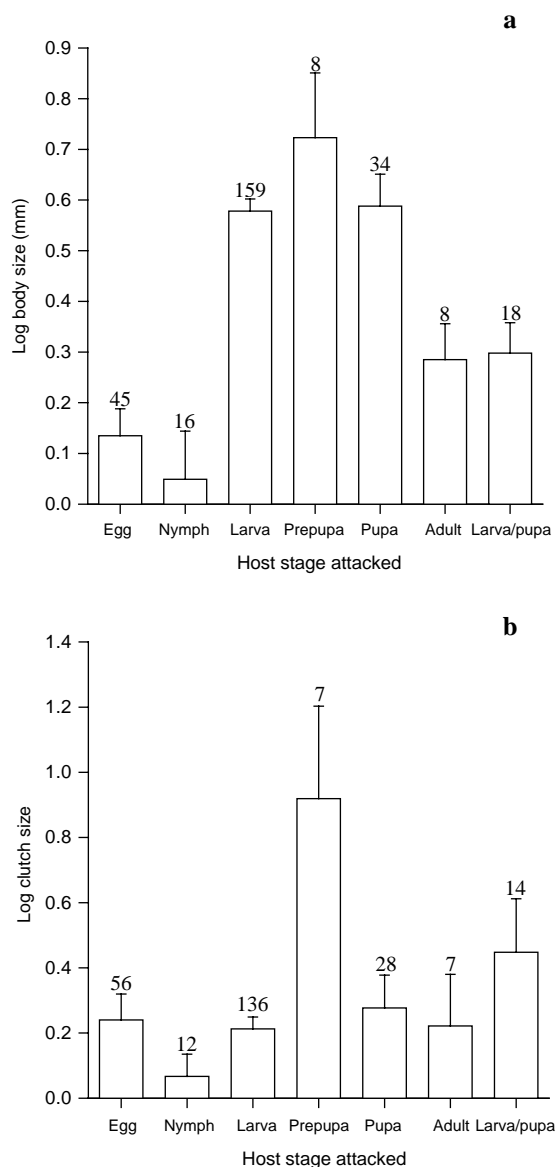


Fig. 1. Log body size (a) and log clutch size (b) against host stage attacked across species (means \pm SE). Numbers indicate sample sizes.

(Mesochorinae) and *Lophyrophlectus oblongopunctatus* (Scolobatinae) (Ichneumonidae), and between species of Cremastinae and Metopiinae (Ichneumonidae) within the resolved phylogeny. In both cases there have been large changes in body size without any change in clutch size (all species are solitary). Variation in the size of the host seems a likely reason. The above analysis was repeated controlling for host stage attacked, a possible confounding variable. Across species, there was a significant interaction between clutch size and host stage attacked on body size; relationships were more negative

for larval and pupal parasitoids than for others (Fig. 2). However, all phylogenetic regressions remained non-significant (Table 1). Again, phylogenetic effects were apparent, with values of ρ ranging 0.192 to 0.500.

Across species, solitary species had larger bodies than gregarious species (t-test, $t = 2.95$, $df = 219$, $P = 0.001$), but all PR analyses were non-significant, with ρ values ranging from 0.352 to 0.476. One contrast influential in the cross species result was found at the root of the tree; higher taxa with larger bodies tended to be solitary. One

unexpected negative contrast involved species within the subfamily Encyrtinae (Chalcidoidea: Encyrtidae). Some of the gregarious species within this subfamily have very large body sizes in comparison to the solitary species. This is likely due to variation in host size: the large bodied gregarious species attack larval/pupal host stages, which are amongst the largest host stages attacked. Across species, a significant interaction emerged between solitary and gregarious development and host stage attacked on body size (Fig. 3). Solitary parasitoids have much larger bodies than gregarious ones attacking larval and pupal host stages. However, solitary parasitoids attacking both larval and pupal host stages have smaller body sizes than gregarious ones. When controlling for host stage attacked, solitary/gregarious development now significantly affected body size in one (conservative cladogram, $F = 2.43$, $df = 5$, 83 , $P = 0.041$), but not the other two PR analyses, although probabilities were higher in all cases.

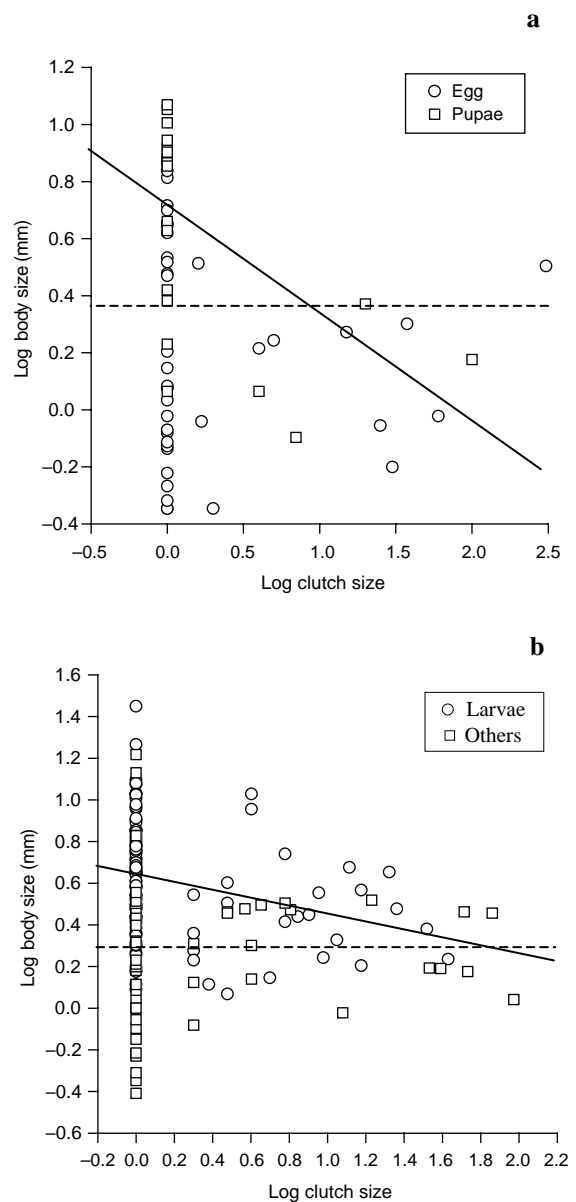


Fig. 2. Log body size against log clutch size across species. Solid lines are regressions for (a) pupal parasitoids, and (b) larval parasitoids. Dotted lines are regressions for (a) egg parasitoids, and (b) parasitoids attacking other host stages.

Body size, clutch size and development mode

Across species, when controlling for host-stage attacked, there was a significant effect of development mode on body size ($F = 6.32$, $df = 1$, 194 , $P = 0.013$) and also a significant interaction ($F = 3.42$, $df = 6$, 194 , $P = 0.003$), with koinobionts being larger bodied than idiobionts on a given host stage (Fig. 4) but mainly in egg and larval–pupal parasitoids. However, there is no significant effect in any of the PR analyses, with ρ values ranging from 0.260 to 0.526 (Table 1). Within the egg parasitoids, influential positive CAIC contrasts were

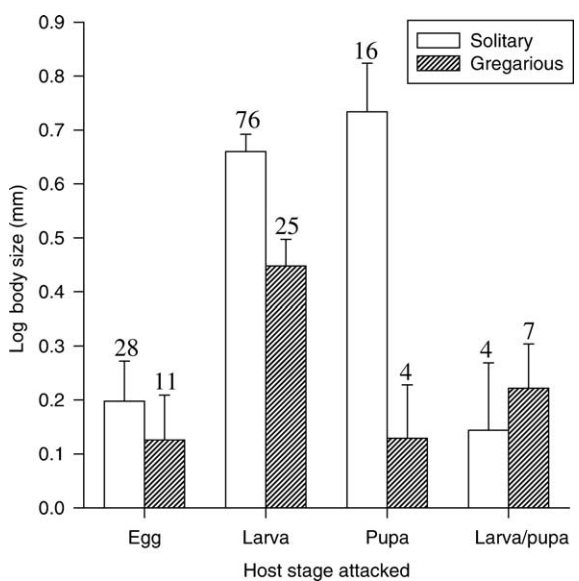


Fig. 3. Log body size against host stage attacked, for solitary and gregarious wasp species (means + SE). Numbers indicate sample sizes.

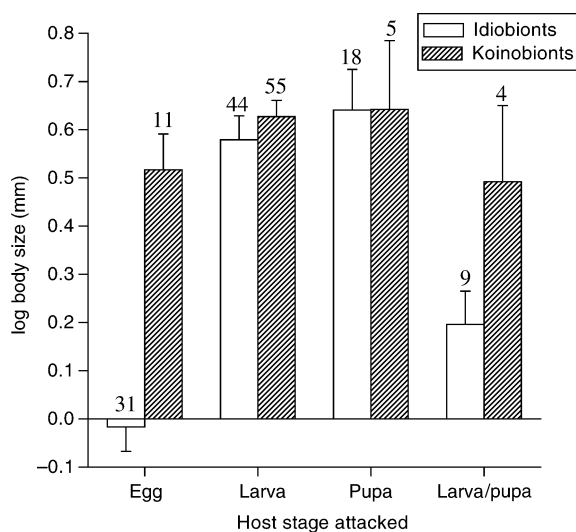


Fig. 4. Log body size against host stage attacked, for idiobiont and koinobiont wasp species (means+SE). Numbers indicate sample sizes.

found at the root, with koinobionts having larger body sizes than idiobionts. An unexpected contrast, in which idiobionts increased in size relative to koinobionts, was found in the family Encyrtidae. The only koinobiont within this group has a very large clutch size, which may account for it having such a small body size in comparison to the idiobionts. For larval parasitoids, influential contrasts in the expected direction were found between the Rogadinae species and also between species of Braconinae (Ichneumonidae: Braconidae). A contrast in the opposite direction is found within a subsection of the Ichneumonidae (subfamilies Mesochorinae, Porizontinae and Scolobatinae). The koinobionts have smaller bodies than the only idiobiont species (*Olesicampe ratzeburgi*), which is very large (11.50 mm). This wasp is known to attack very large bodied hosts. A similar contrast is found between the Pimplinae and Cryptinae (Ichneumonidae). Two idiobiont species (*Rhyssa persuasoria* and *Pseudorhyssa maculicoxis*) are influential here, both using very large insect larvae as hosts. For pupal parasitoids an influential contrast was found between species of Chalcidoidea. Two species within the tribe Entedontini (Chalcidoidea: Eulophidae: Entedoninae) provided the first unexpected contrast. The *Chrysocharis* species (1.49–1.89 mm) and *Pediobius acanthi* (1.62 mm) are idiobionts and a koinobiont respectively, all with similar body sizes. Another unexpected contrast was found in the subfamily Cryptinae (Ichneumonidae). Here *Agrothereutes adustus* has a slightly larger body size (8.25 mm) than the koinobiont species (4.25–7.50 mm). There was no effect of biosis on clutch size after controlling for host stage attacked, either across species ($F=0.21$, $df=1$, 183 , $P=0.641$) or in the PR analyses, where p values range from 0.055 to 0.111 (Table 1).

Body size, clutch size, and latitude

Across species there was a significant association between body size and latitude, with larger bodies associated with temperate as opposed to tropical species (Kruskal–Wallis test, $\chi^2=9.08$, $df=1$, $P=0.003$) (Fig. 5a). The relationship was however not significant in any PR analyses, with a range of p values from 0.288 to 0.334 (Table 1). A contrast in the expected direction was found at the root of the tree. A contrast in the opposite direction was between Bethylinidae species, the temperate species range from 1.33–3.50 mm in body length, whereas a tropical species (*Pristocera rufa*) is 6.05 mm long. *Pristocera rufa* attacks a weevil species that is 10–14 mm long (Baker 1976), whereas the other bethylinid species in this contrast have very small hosts, up to 4 mm in length (Mayhew and Hardy 1998). Another unexpected contrast is between the Braconinae species, which vary in clutch size, and this could confound the effect of latitude on body size. Another unexpected result is between two species of *Comperiella* (Chalcidoidea: Encyrtidae: Habrolepidini), the temperate species (*Comperiella bifasciata*) is 0.61 mm and the tropical species (*Comperiella unifasciata*) is 1.30 mm long. Both species are solitary, therefore differences in host size are the likely reason for this contrast.

Because previous work has suggested that the large-bodied Ichneumonidae are more species rich in some temperate than tropical latitudes, the cross-species result could simply be the result of sampling more ichneumonids at low latitudes. To test this possibility, we repeated the analysis without any ichneumonids. Across species there was still a significant association between body size and latitude, with larger bodies associated with temperate as opposed to tropical species (Kruskal–Wallis test, $\chi^2=5.08$, $df=1$, $P=0.024$). A cross-species analysis, with only the Ichneumonidae, was marginally non-significant but in the same direction (Kruskal–Wallis test, $\chi^2=2.97$, $df=1$, $P=0.085$). In PR, this relationship is also not significant (Table 1).

Across all species, clutch size was not significantly associated with latitude (Kruskal–Wallis test, $\chi^2=0.62$, $df=1$, $P=0.300$), although the relationship approached significance when only gregarious species were considered (Kruskal–Wallis test, $\chi^2=3.17$, $df=1$, $P=0.075$; Fig. 5b). PR analyses for all species considered together are non-significant (Table 1). However, when analysing the subset of gregarious species, significance was obtained in the conservative and resolved phylogenies, whilst using the taxonomic levels the result was marginally non-significant (Table 1). In all cases larger clutches are found in tropical species than in temperate species.

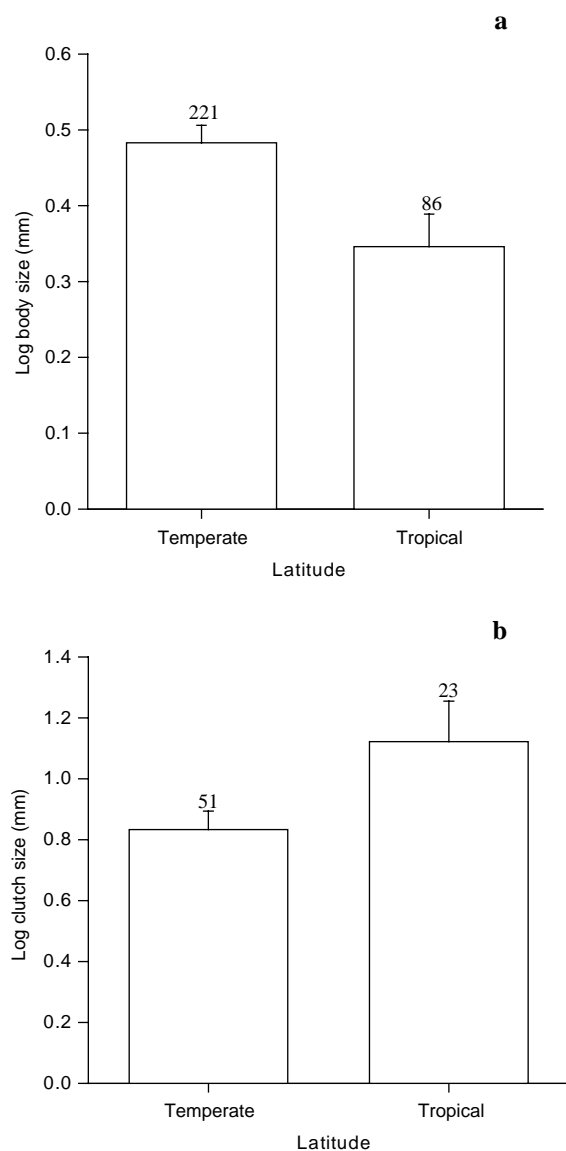


Fig. 5. Log body size across all species (a) and log clutch size in gregarious species (b) against latitudinal distribution (means + SE). Numbers indicate sample sizes.

Discussion

The major finding of this study is that clutch size and body size are strongly associated with host stage attacked both in extant species phenotypes and in terms of evolutionary correlations. The evolutionary association between clutch size and latitude is also significant, for gregarious species. A number of other hypothesized trait associations are found across species, explaining more of the existing phenotypic variation, but without evidence that these are due to consistent evolutionary correlations. Instead, a few evolutionary events may have had disproportionate historical effects on present pheno-

types. Below we discuss the implications of our findings for studies of life history evolution, and for comparative studies in general.

We have found that both body size and clutch size are associated with host stage attacked both across species and in phylogenetic analyses. This is to our knowledge an entirely novel finding. Parasitoid species attack a wide variety of different types of host, both different taxonomic groups, species of different size, different host stages, and in different ecological niches (Gauld and Bolton 1988, Quicke 1997). Theoretical models (Mayhew and Glaizot 2001), predict that the size of the host is a critical influence on both clutch size and body size across species: host size influences body size because it ultimately limits the amount of resource available for developing offspring, and hence also regulates the clutch sizes allocated to hosts by females. Solitary species by definition cannot vary their final brood size and hence their body size should be highly sensitive to host size, increasing with host size across species. Even gregarious populations, experiencing different average host sizes, may be selected to increase both the clutch size and the body size of offspring on larger hosts (Mayhew and Glaizot 2001).

Two previous comparative analyses provide evidence for these trends in the braconid genus *Apanteles* and the family Bethyridae respectively (le Masurier 1987, Mayhew and Hardy 1998). In both taxa body size and clutch size are positively correlated with host size as predicted by theoretical models. In the present study we have a much larger data set covering the taxonomic breadth of the parasitoid Hymenoptera, but we do not have information on the body sizes of the host species involved. However, unlike in the above studies, host stage attacked varies widely in our data and is recorded. Some associations are concordant with expectations based on the relative sizes of the host stages; the largest body and clutch sizes are associated with relatively late host stages (prepupae) and the smallest bodies and clutches are associated with attacking eggs or the nymphs of hemimetabolous insects such as aphids, mealybugs and scale insects, which in general are very small.

Unexpected results are that some egg parasitoids are very large-bodied or lay large broods. These are koinobionts, which allow their hosts to grow considerably after parasitization; for example, *Copidosoma* species (Encyrtidae) lay in host eggs but the offspring emerge from fully developed host larvae, and several hundred can develop polyembryonically from a single host: these are amongst the largest brood sizes in any parasitoid. In addition, parasitoids attacking adult insects are not generally the largest bodied and do not lay the largest clutches. In fact, adult parasitism is rather rare amongst the parasitoid Hymenoptera, and the host species concerned are not large: in our data such parasitoids include braconids of the subfamily Euphorinae such as

Microctonus hyperodae, which parasitizes the adult of the argentine stem weevil, and *Microterys flavus* (Encyrtidae), a parasitoid of scale insects.

Variation in body size is generally much more reflective of the size of the host stage attacked than clutch size (Fig. 1). For example, egg parasitoids are very much smaller than larval, prepupal and pupal parasitoids. In contrast, average clutch sizes are very similar for egg, larval, pupal and adult parasitoids. Theoretical models predict that body size will be much more responsive to variation in host size in species that display contest competition amongst their larvae (solitary species) than species that display scramble competition amongst their larvae (gregarious species; Mayhew and Glaizot 2001). In our data the majority of species are solitary, and this is probably representative of the parasitoid Hymenoptera as a whole (Mayhew 1998), so contest competition between larvae seems a likely reason for this result.

An alternative type of explanation might be that there are selection pressures on body size or clutch size that depend on the stage of host attacked but are independent of its size. For example, clutch size might be modified in response to host immune system, which might be more efficient in larvae than in eggs. Our data should now stimulate studies comparing the fitness consequences of body size and clutch size in parasitoids attacking different host stages.

Interestingly, in a previous comparative study of body size and clutch size (Mayhew and Hardy 1998), evolutionary correlations were non-significant over the entire phylogeny, but were significant over different parts of it, indicating perhaps that clutch size and body size differ in their evolutionary lability (Strand 2000). In the present study both variables are significantly correlated with host stage attacked over the entire phylogeny. One possible reason for this difference is that important changes in both variables occurred at the origin of major groups which were not represented in the previous study. Intuitively, this seems likely, since the major groups of parasitoid Hymenoptera represent contrasting body and clutch sizes (e.g. Ichneumonoidea vs Chalcidoidea). These results therefore cast doubt on the generality of previous results on the lability of these traits, though they remain valid for bethylids.

Previous comparative work across families (Mayhew 1998) suggested that body size and clutch size were negatively evolutionarily associated, as expected if they trade off for a given host size. In this study we find a negative correlation between the traits across species, but fail to find a significant evolutionary correlation, even after controlling for host stage attacked. One possible reason for the difference in our results is that the data of Mayhew (1998) represented all families but did not investigate relationships between close relatives. In contrast, the present study does contain many contrasts

between members of the same genus or closely related genera, but does not represent all major higher taxa. It is likely that differences in clutch size or body size between close relatives are also the result of confounding variables, which we are unable to control for here. This indicates that any association between body size and clutch size is probably dependent on a number of other variables (host stage attacked, host species size, development mode) remaining constant.

We expected, as others have speculated (Godfray 1994), that body size would be related to development mode, because of the ability of koinobionts to allow their hosts to grow after parasitization. Mayhew and Blackburn (1999) failed to detect an evolutionary correlation between the two traits, but one possible confounding variable is the host stage attacked, which is highly variable across both koinobionts and idiobionts. After controlling for this, there is a significant association across species between development mode and body size, with koinobionts being larger than idiobionts attacking a given host stage. There is also a significant interaction, with the largest difference being amongst egg parasitoids. This makes intuitive sense, as the potential for hosts to increase in size is greatest if early host stages are attacked. However, once again there is no evidence that this is the result of a consistent evolutionary correlation. Examination of the contrasts revealed that variation in the size of the host species is likely to be the cause of the unexpected contrasts, like that found within the Ichneumonidae attacking larval host stages. There is no evidence that clutch size is related to development mode. This suggests that any fitness advantage of koinobiosis is not reaped through an increase in clutch size but may be reaped through an increase in body size.

We also speculated that latitude might explain some of the variation in body size and clutch size in parasitoids, as it does in several other organisms. Our data on latitude are very crude, since species are simply coded in a binary fashion as either temperate or tropical. However, in many ways, this crude classification is powerful as it ensures that any contrasts are between species that differ markedly in the latitudinal extent of their ranges (Cardillo 1999, 2002). Although we found a positive relationship between body size and latitude (Bergmann's rule) across species, this was not replicated in the phylogenetic analyses. At least some of the contrast variation is due to differences in clutch size and host size: for example in the Bethyloidea.

Although there was not a significant across-species relationship between clutch size and latitude when all species were considered, the relationship did approach significance in the phylogenetic analyses. In a number of groups, such as the Ichneumonidae, clutch size is evolutionarily conserved and is nearly always one (solitary groups; Mayhew 1998). If solitary species are

removed from the analysis, such that we only consider gregarious species, which are generally regarded as having labile clutch sizes (Godfray 1994), then the significance of all analyses increases, and two of the phylogenetic regressions are now significant. Interestingly, the direction of this relationship (temperate species lay smaller clutches than tropical ones) is the opposite to that found in birds, but is that expected from the body size trends if clutch size and body size are traded off together.

Recently, there has been some interest in explaining latitudinal gradients in species richness by considering latitudinal gradients in life history traits. Specifically, if life history traits vary with latitude, they might in turn affect speciation or extinction rates at different latitudes, and hence species richness (Cardillo 2002). In this respect it is interesting that one group of parasitoids, the Ichneumonidae, which are generally large bodied and solitary, have often been observed to be less species rich in tropical than in some temperate latitudes (Godfray 1994, Gaston and Blackburn 2000). Our studies raise the possibility that selection on life history traits, such as body size and clutch size, might contribute towards this trend. Previous work on explaining the lack of tropical ichneumonid diversity has concentrated on factors, such as host density declining in the tropics, that are rather general to parasitoids. However, there is good evidence that the decline in tropical diversity seen in ichneumonids is not general to all parasitoids (Hanson and Gauld 1997). Explanations focussing on traits particular to ichneumonids should hold much more prospect of success. Large body size and small clutch size are two such traits.

Our analyses have implications for comparative methodology and for research on the phylogenetic relationships of parasitoid wasps. One argument for not carrying out phylogenetic comparative analyses is that they generally give the same answer as cross-species analyses (Ricklefs and Starck 1996). However, several of our analyses here give significant results across species but not when phylogeny is taken into account. In many cases values of ρ are moderately high (although never very high), indicating some phylogenetic dependence. In contrast, where there is agreement between cross-species and PR analyses, the values of ρ are often very small, indicating a lack of phylogenetic dependence. In addition, we even find one case of a significant phylogenetic analysis when the cross species analysis is non-significant! Therefore use of phylogeny is justified. Another reason to abandon phylogenetic analysis is if there is no phylogenetic dependence in the data (Abouheif 1999). Phylogenetic dependence is however not absent from our data.

The earlier studies on the current data have been criticised due to the fact that only a poorly resolved taxonomy was used. This has the effect of lowering

power, and also of possibly introducing bias if the taxonomy is not an accurate reflection of phylogeny. Here we have conducted analyses including the most up-to-date phylogenetic information. The result has been to improve the power of the analyses, since the number of nodes over which we can calculate contrasts has increased. The relationship between body size and host stage attacked is only marginally significant using taxonomy, but is highly significant in both analyses using phylogenetic evidence. In addition, the relationship between clutch size and latitude in gregarious species is significant in both analyses incorporating phylogenetic studies, but is marginally non-significant using taxonomy alone. Thus, only about a decade of phylogenetic work has produced significant benefits for comparative studies (as long as these studies are better representative of phylogeny than the taxonomy alone).

Finally, we put the present results in a wider context. In many organisms adult body size is probably determined by organisms deciding on the optimal time to divert resources away from growth and into reproduction (Stearns 1992, Charnov 1993, Kozłowski and Weiner 1996, Gemmill et al. 1999, Kozłowski and Gawelczyk 2002). In these cases variation in mortality rates is likely the most important factor causing variation in maturation time, and hence body size. In contrast, with some possible exceptions (Harvey et al. 2000), studies to date suggest that the most important biological factors affecting parasitoid body size are the size of the host when it is finally consumed, which ultimately limits how large a parasitoid larva can grow, and the clutch size per host, which affects how many offspring the host must be divided between. Both these are parental optimization decisions. These selection pressures, although different from those in many other organisms, may not be unique to parasitoid Hymenoptera; many other insects develop by consuming small highly depletable resource patches, and the possibility remains that body size and clutch size in many other insects, and hence a large proportion of the planet's species, are determined in much the same way.

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