Deducing how tropical rhyssines (Hymenoptera, Ichneumonidae) mate from body measurements

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Abstract
The biology of many Darwin wasp (Hymenoptera: Ichneumonidae) species is poorly known. Existing museum specimens can potentially be used to get information on e.g. how species live, what they eat, and what their life cycle is. One example of this is a 1991 study by Eggleton in which he measured some rhyssine (Ichneumonidae: Rhyssinae) species, and used the results to deduce how the species likely mate.

We extend this work by measuring five tropical species. We found no evidence that the males of our species scramble for females before the females emerge, which matches what was hypothesised by Eggleton. Further measurements of more species would provide information on how other species mate, and field observations of mating rhyssines would help confirm that Eggleton’s method for deducing rhyssine mating strategies gives true results.

Keywords
Amazon Malaise trapping 2000, Darwin wasps, Ichneumonidae, Rhyssinae, Uganda Malaise trapping 2014–2015

Introduction
The Darwin wasps (Hymenoptera: Ichneumonidae) are an extremely diverse but poorly known family, with possibly over 100000 species (Gauld et al. 2002) of which only about 24000–25000 are known (Yu et al. 2016; Bennet et al. 2019; Roskov et al. 2019). With so many species still undiscovered, it is scarce surprising that our
knowledge of most described species is restricted to the mere fact that they exist. We often know nothing about the biology of species: how they live, what they eat, what their life cycle is.

Existing museum specimens can be used to deduce details of the biology of a species. Eggleton (1991), for example, measured the metasomas of male rhyssine wasps (Ichneumonidae: Rhyssinae) in the Natural History Museum (London) collections, and deduced how the different species mate. The assumption behind the measurements was that rhyssine males often emerge from their pupas before the females and that species whose males scramble for emerging females will tend to invest in long, slender and elongated male metasomas, so as to reach females before other males. Interestingly, according to Gauld (1991) the male metasomas are slender in some temperate species, while most tropical rhyssine species possess shorter and stouter metasomas.

Eggleton (1991) divided rhyssine mating strategies into three categories. These are before-emergence scramble competition (BESC), before-emergence female defence (BEFD) and after-emergence scramble competition (AESC). The first of these (BESC) involves males competing to be the first to insert the metasoma into an emerging female’s burrow and inseminate her. Eggleton showed that in several species known to use this strategy, large-sized males have relatively more slender metasomas than small males. This strategy is only known in *Megarhyssa* Ashmead, 1900 and *Rhyssella* Rohwer, 1920.

Eggleton then suggested using this information to deduce the likely mating strategy of other species. Eggleton’s sample sizes were very small, however, so he was unable to deduce the mating strategy of more than a few (mainly European or North American) species. Five of his species were from the genus *Epirhyssa* Cresson, 1865, a mainly tropical genus found in both the Old and New World, and one that is suspected not to be monophyletic (Hopkins et al. 2019).

After Eggleton’s (1991) work, a large number of rhyssine wasps have been collected and described in Uganda (Hopkins et al. 2019) and Peruvian Amazonia (Gómez et al. 2015). These include 81 males in three Ugandan species, and 26 males in two Peruvian species, all in the genus *Epirhyssa*. This relatively large sample size allows us to use the method proposed by Eggleton to deduce the likely mating strategy of several Afrotropical and Amazonian species.

**Materials and methods**

Our material consisted of 107 male rhyssines in 5 species. Three of these species were collected in Uganda: *Epirhyssa ghesquierei* Seyrig, 1937 (42 males), *E. overlaeti* Seyrig, 1937 (16 males) and *E. quagga* Hopkins et al., 2019 (23 males). Two species were collected in Peru: *E. braconoides* Porter, 1978 (7 males) and *E. diatropis* Porter, 1978 (19 males). We also collected data from two non-tropical species: *Rhysa persuasoria* (Linnaeus, 1758) (3 males), collected in Finland, and *Megarhyssa nortoni* (Cresson, 1864) (4 males) from the USA. Their sample sizes were too low to be analysed. The data on all
To find out which mating strategy these species most likely use, we measured the length of the male mesosoma and the slenderness (length/width) of the third metasomal tergite. The former was used as a proxy for body size, and the latter as a proxy for metasoma slenderness. We measured the mesosoma from the foremost edge of the mesoscutum/pronotum to the insertion of the metasoma (Figure 1). We measured the width of the tergite at its widest point, and the length at its midline (Figure 1). All measurements were done under a microscope, and images saved for future reference.

We plotted metasoma slenderness versus body size, fitted a linear regression line to the plot, and interpreted a significantly increasing line (slenderness increase in large males) as evidence of the BESC mating strategy. For consistency with Eggleton’s (1991) work, we also calculated the significance of the association between body size and tergite slenderness based on Pearson’s correlation coefficient. This is otherwise mathematically equivalent to the linear regression, except that the regression uses a two-tailed test whereas the correlation coefficients are one-tailed.

We analysed the data in the R software v. 3.4.0 (R core team 2017). Digital images of the wasps were taken with a CANON DS126461 digital camera attached to the rhyssine individuals is available online (https://doi.org/10.5281/zenodo.5528589; Keronen et al. 2021).
an OLYMPUS SZX16 stereomicroscope. We used the software Quickphoto Micro 3.1 to take the images and to get measurements from them. The analyses and images are available online (https://doi.org/10.5281/zenodo.5528589 Keronen et al. 2021).

Our analyses differ somewhat from those of Eggleton (1991). He used forewing length as a proxy for body size (mesosoma length) matched the earlier used proxy (forewing length). We found mesosoma length and tergite 3 easier to measure for our material. The forewings of our specimens were sometimes hard to get in focus for a reliable measurement, and were occasionally curled or otherwise unmeasurable. Tergite 5 sometimes telescoped under the previous tergite, making accurate measurements difficult. We expect all tergites to give similar results, with the choice of tergite being a compromise between easy visibility (posterior tergites) and the tergite not telescoping under other tergites (anterior tergites). To validate that forewing length and mesosoma length are sufficiently equivalent, we measured both of them for our Epirhyssa quagga (23 males), and fitted a linear regression between them (Figure 2).

Eggleton also used correlations, instead of linear regression, to analyse his data; again, we expect the results to be similar whichever method is used. The p-values in particular are calculated the same way, since both are based on the t-statistic; the only difference arises from Eggleton’s analyses using a one-tailed test whereas the linear regressions are two-tailed. We felt that a linear regression with its corresponding plots gives a better visualisation of the results, but give the p-values of both approaches.
Results

Mesosoma length matched the previously used measure of body size, wing length (linear regression $p < 0.01$, $R^2 = 0.81$; Figure 2).

The slenderness of tergite 3 did not significantly vary with mesosoma length in any of our study species (linear regression, $p$ range = 0.09–0.44, $R^2$ range = 0.03–0.19, Figures 3, 4). However, the slenderness significantly increased (when analysed with a one-tailed correlation test) with metasoma length for one species, *Epirhyssa overlaeti* ($p = 0.047$, Figure 3). Metasomas were conspicuously similar in slenderness, irrespective of body size or species (Figure 3).

Discussion

We found (almost) no evidence that the males of our tropical study species scramble for females before the females emerge. The one exception was *Epirhyssa overlaeti* which showed a significant increase in tergite slenderness with body size, when analysed with one-tailed correlation tests instead of linear regression. We do not feel this merits too much attention, since this was only marginally significant and we would in any case expect some false positives when testing multiple species.

![Figure 3. Mesosoma length and tergite 3 slenderness (length/width) of 107 male rhyssine wasps. Males of all species had similarly slender tergites 3, irrespective of body size. The only exception was *Epirhyssa overlaeti*, whose tergites got significantly more slender with body size when the significance was estimated by one-tailed correlation (c.f. Figure 4).](image)
These results fit what has been hypothesised. Eggleton (1991) suggested that scrambling for females while they emerge (the BESC mating strategy) only evolved in one rhyssine lineage (*Megarhyssa* plus *Rhyssella*), and that the males of other genera either guard a female emergence point (BEFD), or mate after the female has emerged (AESC). He also suspected that tropical rhyssines do not use the BESC strategy due to population densities being low. Gauld (1991) noted that males of most tropical rhyssine species possess stout metasomas because they are Batesian mimics of stout-bodied vespid wasps. Our results support these hypotheses. We did not find any evidence (except for the *E. overlaeti* mentioned above) of BESC mating in our tropical *Epirhyssa* species despite reasonable sample sizes.

Although our results provide some interesting insights on the biology of some rhyssine species, it remains a fact that there are over 250 rhyssine species (Hopkins et al. 2019), and that Eggleton’s method for detecting BESC mating is still insufficiently tested. We recommend further measurements on more species, especially in the two genera which we measured but for which we did not get sufficient sample sizes. These are *Megarhyssa*, for which Eggleton predicted BESC mating, and *Rhyssa* Gravenhorst, 1829, for which he predicted some other mating strategy. Measurements of some la-
benine and pimpline genera (e.g. *Apechoneura* Kriechbaumer, 1890 and *Dolichomitus* Smith, 1877), which may employ similar mating strategies (Gauld and Wahl 2000: p. 310), would also be useful. Field observations of mating rhyssines would help confirm that the species whose males scramble for emerging females genuinely follow the morphological pattern proposed by Eggleton.

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