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Wasp Fights: Understanding and Utilizing Agonistic Bethylid Behaviour

The Bethylidae is a family of parasitoid hymenopteran wasps comprising three extant subfamilies (Bethylinae, Epyrinae and Pristocerinae) and a total of around 2000 described species. Here we consider their effectiveness as biocontrol agents and especially focus on how studies of agonistic interactions between adult females can be used to both warn of detrimental non-target effects and suggest ways to improve their biocontrol potential.

Bethylids and Pest Control

Bethylids attack almost exclusively the immature stages of coleopterans and lepidopterans, including pests of many important agricultural crops, such as coffee, coconut, sugarcane and almonds, in the field, stored product pests and pests that damage domestic carpets and museum specimens. Aside from a few species that can cause human dermatitis, bethylids can be regarded as beneficial insects and they have been deployed in around 50 classical biocontrol programmes around the world since the mid-1920s. Despite their apparent potential, there have been no biocontrol programmes that have achieved complete pest control (such that no other measures are needed) using bethylid wasps. Overall, establishment of introduced bethylids has been achieved by about a quarter of programmes, with around 5% of the total achieving partial control. The available evidence, however, suggests that the rates of establishment and (partial) success have improved since the 1970s such that about half of the relatively recent programmes have resulted in establishment of released bethylids and around 10% of programmes have achieved partial control of the target pest.

Model Organisms

Despite being underachievers as biocontrol agents, there is a manner in which bethylids have proven undoubtedly beneficial: as study organisms in behavioural and evolutionary research. Parasitoid wasps in general are extraordinarily useful organisms for behavioural and evolutionary ecologists; to some extent this is due to their ease of handling in the laboratory and short and relatively simple life cycles, but the crucial attribute is the direct link between many of their behaviours and their evolutionary fitness. This link is strong because female parasitoids often forage directly for reproductive opportunities rather than for food that will be at some, possibly distant, time in the future be converted into a difficult-to-quantify number of offspring. Bethylids have played an important role in several key areas of parasitoid behavioural ecology research, most notably the evolution of clutch size and sex ratio decisions and also factors determining the outcomes of dyadic contests for indivisible resources. For instance, W.D. Hamilton's seminal work explaining female biased sex ratios in terms of local mate competition (justifiably one of the most famous theoretical advances in evolutionary biology) was bolstered by his knowledge of the sex ratio biology of three bethylid species. Subsequent sex ratio theory was stimulated and tested by empirical and comparative work on numerous further bethylids, leading to a sound understanding of sex ratio precision and the effects of offspring developmental mortality on optimal sex ratio decisions, as well as useful knowledge concerning the relationships between sex ratio and clutch size and the possible influence of some degree of non-local mating ([1] and references therein). Similarly, in clutch size research, bethylids have been used to test theory predicting the 'Lack clutch size', the evolution of gregarious clutches and relationship between offspring size and clutch size[2].

Payback Time

Much of the background knowledge, and indeed some of the data, on which these theoretically-oriented studies were built was gleaned from the taxonomic and biocontrol oriented literature. Reciprocally, an understanding of parasitoid behavioural ecology has great potential to improve biocontrol practice and success. For instance, there are already good examples of the understanding of sex allocation behaviour being used to reduce significantly the cost of parasitoid mass rearing for augmentative biocontrol[3], although it seems that many of the insights generated by behavioural ecology have yet to be fully utilized in biocontrol.

Here we focus on two ways in which initially 'pure' or 'basic' studies of parasitoid contest behaviour have generated results useful, or at least potentially useful, to biocontrol programmes involving bethylid wasps.

Bethylid Contests: Pure

Evolutionary game theoretic models predict that when two animals contest an indivisible resource, the winner will be determined by the interplay between three factors: which contestant is the better (e.g. more able or more powerful) fighter, which is the prior owner, and to which contestant the resource is worth the most. Bethylid wasps, particularly two species of *Goniozus* (subfamily Bethylinae), *G. nephantidis*, a parasitoid of coconut pests, and *G. legneri*, a parasitoid of almond pests, have proven to be extremely useful organisms for testing these theories. On finding a suitable host, a female *Goniozus* first stings and paralyzes it and then stays with it for 24 hours before laying a clutch of eggs onto it. If another foraging female encounters the paralyzed host and its 'owner', violent female–female fights readily ensue, with the loser female being driven away and the winner eventually laying its eggs on the host. These *Goniozus* species also remain with the host after laying eggs until their offspring reach the advanced larval or pupal stage; this is unusual for parasitoids in general but common among the Bethylidae, and owner–intruder contests for host bearing offspring also occur.

By experimentally manipulating differences in size, age, the number of mature eggs stored, and prior owner status between contestant females, and varying the size (quality) of the contested host and the stage of the brood it bears (no eggs, eggs or larvae) the relative importance of the predicted influences on contest outcomes has been established. Differences in female body size are usually of major importance, with bigger females tending to win[4]. This finding led to a theoretical advance connecting size-dependent contest outcomes to optimal clutch size decisions based upon the relationship between body size and parasitoid fitness: even though only one female lays eggs on a given host, her clutch size decision is influenced by the sizes of clutches that other females in the population lay because, on a fixed sized host, smaller clutches give rise to larger offspring which will fare better in subsequent contests for hosts[2]. We have recently found empirical support for this prediction, namely that females exposed to several intruder females before laying eggs reduce the size of the clutches they lay compared to undisturbed females. The above-mentioned empirical studies of contests further showed that outcomes can also be influenced by all of the listed factors additional to body size. These can be interpreted in terms of a difference in the value that the contestant females place on possession of the host[5,6]. Prior ownership status does not appear to be of direct importance but has an effect via the larger number of stored eggs that owners have compared to intruders which enhances their ability to exploit the host thus giving it, to them, a higher value[7]. While there are many other studies of dyadic contests in the animal behaviour and behavioural ecology literature, the particular value of these studies of bethylids is that an unusually large number of many factors contributing effects on contest outcome has been explored, both separately and simultaneously.

Bethylid Contests: Applied

Two issues of ongoing concern to biocontrol practitioners are the question of how many species of natural enemy to deploy to best control a given pest, with the associated danger of detrimental intra-guild interactions, and the dangers of non-target effects of introduced biocontrol agents. Given that multiple species are involved, these are essentially issues at the 'community ecology level' but can be addressed reductively at the 'behavioural' or 'individual' level. Studies of bethylid contests, and associated behaviours, have played a role in evaluating intra-guild interactions in the coffee agroecosystem. Coffee, an originally African crop, is now grown in many tropical regions and is of great economic importance. The principal insect pest of coffee, the coffee berry borer (*Hypothenemus hampei*, a scolytid beetle) also has African origins but has now spread to virtually all coffee growing regions where borer infestations can be intense and coffee production seriously affected. Two species of African bethylids in the subfamily Epyrinae, *Cephalonomia stephanoderis* and *Prorops nasuta*, were released into New World and other coffee growing regions in an attempt to control the borer[8]. Generally, these species established but, typical of bethylids, failed to control the borer sufficiently. Therefore, further candidate agents of biological control were sought. In the late 1980s, *Cephalonomia hyalinipennis*, a bethylid native to North America, was found naturally attacking the borer in southern Mexican coffee plantations. The biology of this 'new' species was evaluated: one encouraging facet of *C. hyalinipennis* was that it is a gregarious species, producing more than one offspring per host attacked (in contrast the two African species released only lay one egg per host) which is predicted to lead to better host population suppression.

In considering whether to encourage, for instance by mass rearing and release, *C. hyalinipennis* in Mexico (augmentative biocontrol) or to introduce it in other coffee producing countries (classical biocontrol) its possible intra-guild interactions with the two African bethylid species were studied. There are many ways in which populations and individuals of different species could interact in an agroecosystem but female–female contests can provide a direct means of assessing the strength and outcomes of inter-specific interactions in the laboratory and thus offer an initial approach to evaluating the possibility of disruptive intra-guild interactions in the field. The three bethylids attacking the coffee berry borer were duly competed against each other, with pairs of females contesting possession of small groups of host inside an artificial coffee berry[9]. It was found that intense contests readily occurred and that the losing female was often killed (*C. stephanoderis* being the most violent and generally successful species). In contrast, when contests were set up between females of the same species, the death of the loser was never observed, even though fighting behaviour appeared to be equally intense. Community ecology theory predicts that species will not be able to coexist when competition is stronger between species than within species: thus oft-fatal inter-species interactions and the non-fatal intra-specific interactions suggest that these bethylids may not be able to coexist ecologically. However, factors other than fighting behaviour may also influence both coexistence and the choice of biocontrol agents to encourage. Investigations of the biology of *C. hyalinipennis* further revealed that it can act as a facultative hyperparasitoid of other bethylids[10], including those deployed against the coffee berry borer (constituting disruptive intra-guild predation) and *G. legneri* and *G. nephantidis* deployed against almond and coconut pests (constituting a potential for detrimental non-target effects if *C. hyalinipennis* were to spread into coconut or almond agroecosystems). A small number of preliminary observations suggest that *C. hyalinipennis* may even be able to win fights against *Goniozus* females, despite being very much smaller, and then hyperparasitize their developing offspring. Studying dyadic contests, normally the preserve of 'pure' behavioural ecology, has thus indicated that the initially promising *C. hyalinipennis* should probably not be encouraged in Mexico nor released as a biocontrol agent of the coffee berry borer elsewhere. More generally, this case history supports the view that when candidate biocontrol agents are screened, consideration may need to be given to potential intra-guild effects and not just host specificity selection criteria.

Bethylid Volatiles: Pure

Until recently, the understanding of bethylid contests was based only on visually observable physical behaviour. However, a few chemically-oriented studies had shown that *Cephalonomia* species can emit a volatile chemical when behaviourally stressed[11]. We recently investigated the occurrence of chemical release by *G. legneri* and *G. nephantidis* (subfamily Bethylinae) and six species in the subfamily Epyrinae (including the natural enemies of the coffee berry borer mentioned above): when artificially stressed the bethylines emit a spiroacetal, called 2-methyl-1,7-dioxaspiro [5.5]undecane (molecular weight 170), while the epyrines emit a methylindole called 3-methylskatole (molecular weight 131). By coupling contest behaviour experiments to chemical analysis apparatus (atmospheric pressure chemical ionization-mass spectrometer, APCI-MS) which is able to monitor continually the chemical composition of the air around interacting wasps we were able to show that, when two *Goniozus* females fight for possession of a host, spiroacetal is usually only released during the most behaviourally aggressive interactions[12]. Once release occurs, the frequency of the most aggressive behaviour is greatly decreased.

Because both contestant females produce and release the same volatile, and thus their natural emissions are chemically indistinguishable, we developed a method of chemical marking by molecular weight manipulation[12]. We reared some females on hosts injected with insect saline made up with deuterated water (heavy water). Deuterium is an isotope of hydrogen, bearing an 'extra' neutron: some deuterium atoms became incorporated into the wasps' bodies during development. We have found no discernable effects on life-history characteristics, such as longevity or fecundity, of the wasps following exposure to deuterium, except that deuterated individuals have reduced fighting abilities but fortunately this effect is much smaller than factors of more biological interest such as differences in body size or ownership status. Importantly, we found that deuterated females emitted volatile profiles that were readily distinguishable from undeuterated females because around 25% of the spiroacetal they emitted had molecular weight raised to 171, i.e. some spiroacetal molecules produced had 17 hydrogen atoms and one deuterium atom rather than the usual 18 hydrogen atoms. This chemical marking revealed that, without exception, it is the losing female that releases the volatile[12]. Although we have currently found no negative effects of exposure to the spiroacetal on *Goniozus*, at high concentration the same chemical has been shown to kill *Drosophila* fruit flies rapidly. Current evidence suggests that *Goniozus* employ spiroacetal released as a weapon of rear-guard action to increase their chance of being able to withdraw from a behaviourally aggressive encounter. Given that these encounters will often take place within the confines of small tunnels and cavities excavated by the host, the concentration of spiroacetal could become locally high and thus the contestant remaining near the host could be temporarily incapacitated while the spiroacetal-releasing loser retreats.

This work advanced the study of chemical interactions between organisms because we were able to monitor simultaneously chemical events and physically-observable behaviours. We consider that the technique will be used to study a wider array of chemically related behaviours, such as the attraction of wasps to plant-released volatiles. Deuterium marking also has wider potential, for instance in mark–recapture studies which could help address the well-known problem of tracking the activities of small parasitoids in the field.

Bethylid Volatiles: Applied?

To date the study of volatile emissions by bethylids has been largely 'pure' in scientific nature but does have a link with biocontrol practice. It has been found that when *C. stephanoderis* is mass reared in laboratory facilities for release against the coffee berry borer in southern Mexico, its rate of establishment (in terms of entering and remaining within target berries) in the agroecosystem is very low, seemingly because adult females disperse away from the release site[13]. Such results are obtained if dozens of wasps are collected into large jars in the laboratory and then transported to the field for release. Establishment rates at the release site can be improved five-fold (but still remain low) if berries containing developing wasps are placed in the field, allowing the wasps to emerge and start to forage naturally[13]. Recently, chemical analysis has shown that there is no volatile release in jars containing 20 female *C. stephanoderis* when the jar is not subjected to disturbance[11]. There are, however, considerable releases of 3-methylskatole within jars that are subjected to one minute of shaking. Given that transport to the field is likely to involve shaking, it is likely that the difference in establishment between naturalistic and *en masse* releases from jars can be explained by the latter method stimulating volatile release which, considering its association with agonistic behaviour, leads to released wasps choosing to disperse rather than remain in the target locality. It seems, therefore, that an understanding of how to reduce chemical release behaviour can improve the efficacy of these wasps in pest control.

Conclusions

Bethylid wasps have proven to be highly useful research organisms in the study of behavioural ecology but only moderately useful as agents of biological pest control. Nonetheless, they are generally beneficial and the greater understanding of their biology gleaned from investigations into female–female contests, volatile release and other areas of behavioural ecology has been, or at least has the clear potential to be, usefully applied to the improvement of biocontrol strategies.

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Further information on the coffee berry borer: www.cabi.org/datapage.asp?iDocID=476

By: Ian C.W. Hardy & Marlène Goubault,
School of Biosciences, University of Nottingham,
Sutton Bonington Campus, Loughborough,
Leicestershire, LE12 5RD, UK.
Email: ian.hardy@nottingham.ac.uk
Fax +44 115 9616060