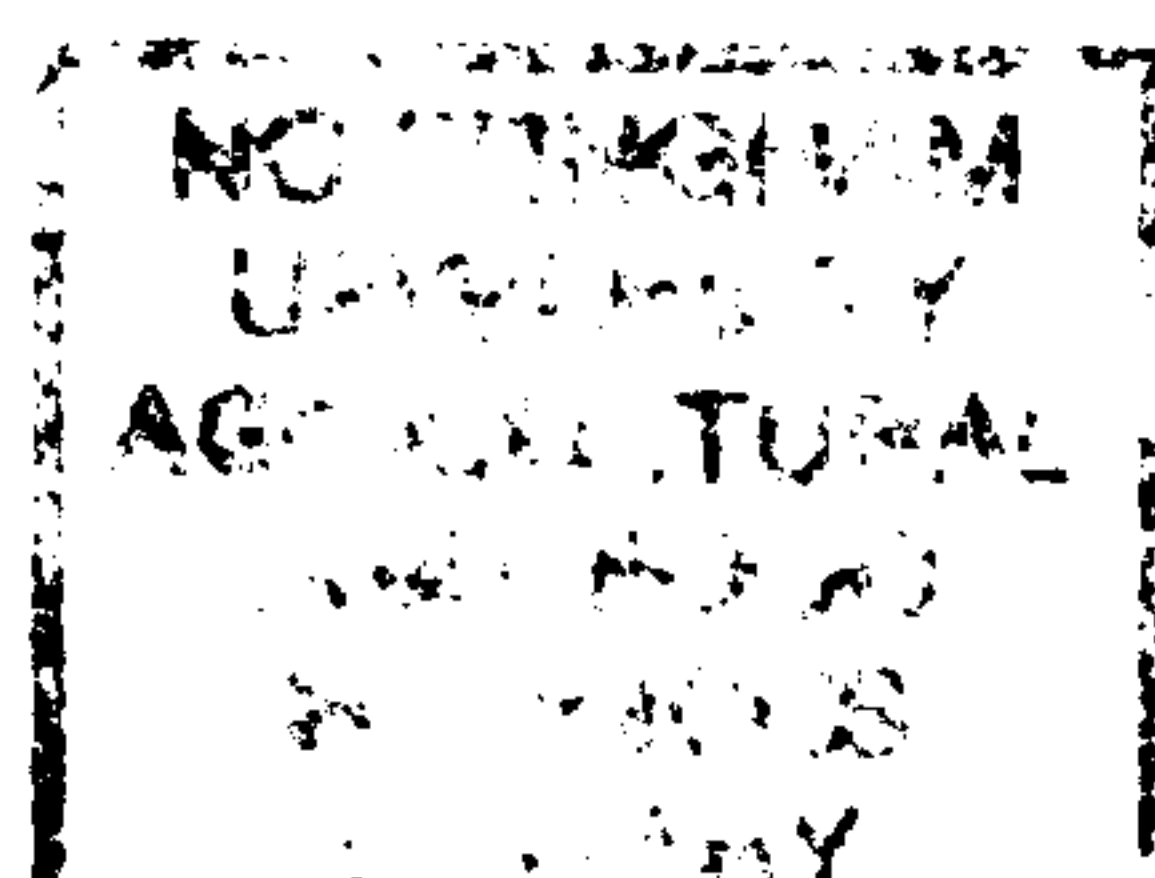


# Parasitoid Interactions in Behavioural Ecology and Biological Control

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## ABSTRACT

This thesis presents laboratory investigations on the competitive interactions which take place within and between bethylid parasitoids. Part one investigates the compatibility of three bethylids (*Cephalonomia hyalinipennis*, *Cephalonomia stephanoderis* and *Prorops nasuta*) for biocontrol releases against the principal pest of coffee, the coffee berry borer (CBB), *Hypothenemus hampei*. *Cephalonomia hyalinipennis* is able to hyperparasitise and consume pupae of *C. stephanoderis* and *P. nasuta*. *Cephalonomia stephanoderis* also engages in intra-guild predation, consuming pupae of *C. hyalinipennis*. In contests for CBB hosts, fatal fighting occurs in 69% of inter-specific replicates but never occurs in intra-specific replicates. This suggests that interspecific competition is stronger than intraspecific competition and that species coexistence may be compromised. *Cephalonomia stephanoderis* is the superior interspecific contestant while *P. nasuta* is the least successful and never kills an opponent. Where CBB infested coffee berries are provided to the three bethylids, coexistence between species is possible, but rare, within a single coffee berry. *Prorops nasuta* is the most successful species in interspecific replicates and replicates containing *C. hyalinipennis* generally have low production, regardless of the species combination added. Part two investigates contest interactions, the variables that influence contest outcome between *Goniozus nephantidis* females and chemical release. Prior ownership and difference in contestant weight have positive influences on contest outcome. Host weight positively influences the outcome of contests between two 'owners' and 'intruder' take-over success increases when intruders are older than owners. Seven bethylid species are found to release volatile chemicals when stressed. A pilot study identifies the volatile chemical in *G. nephantidis* and employs Atmospheric Pressure Chemical Ionisation-Mass Spectrometry for real-time analysis of chemical release during contest interactions. The appendix contains an advanced investigation using this technique. Bethylids are useful model organisms for the study of competitive interactions but appear to be generally ineffective as biological control agents.

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## CHAPTER 1 – GENERAL INTRODUCTION

This thesis is unified by the core theme of intra- and inter-specific interactions between adult female bethylids, which are parasitic wasps considered as potential biological control agents. The first part of the thesis (Chapters 2-4) explores the interactions that take place between three species, *Cephalonomia hyalinipennis* Ashmead, *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (all Hymenoptera: Bethyridae), which have been investigated for biological control attempts against the coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae). The second part of the thesis (Chapters 5-6) investigates intra-specific contests in *Goniozus nephantidis* Muesebeck, a bethylid natural enemy of the coconut pest *Opisina arenosella* Walker (Lepidoptera: Oecophoridae). Each of the chapters can be read in isolation but are all inter-related. In chapter seven I summarise the results of the thesis and suggest possible future investigations.

### 1.1 BIOLOGICAL CONTROL

With the knowledge that chemical control of pest species has numerous disadvantages (Table 1.1), recent research on pest control has focussed on biological control as a constituent of Integrated Pest Management (IPM) (Mackauer, Ehler and Roland, 1990; Baker, 1999; Waage, 2001). These IPM programmes use knowledge of the biology of a pest species to assess which control methods could reduce the economic damage caused by the pest to acceptable levels and may often incorporate the use of chemicals (Baker, 1999). Essential to the functioning of IPM are the conservation of local natural enemies of pests and/or mass releases of screened exotic biological control agents against exotic pests (Waage, 2001). These methods can result in the establishment, and persistence, of a natural enemy population which can suppress pest populations within the release environment. Thus it could maintain the pest population level below an economic threshold and provide the possibility of bringing long-term stability to the system. In addition, when biological control is effective it can be cheap, environmentally clean and pose no health risk to humans.

### 1.1.1 Non-target effects and biological control decisions

Biological control is not without problems. Many researchers consider that release of exotic natural enemies into novel environments to control an exotic pest (classical biological control) may lead to detrimental non-target effects which could reduce biodiversity in the release area (Simberloff and Stiling, 1996; Thomas and Willis, 1998). This may lead to the exclusion, and subsequent extinction, of beneficial or endemic species from the region. Thus a major focus of biological control in recent years has been to incorporate considerations of the ecology, population dynamics, behaviour and host range of pests and their natural enemies into prior screening of biological control agents before their release into a novel environment (Murdoch and Briggs, 1996; Thomas and Willis, 1998; Waage, 2001; Hopper, 2001; Kidd and Jervis, 2005). For this reason, in many programmes quarantine tests are required before the natural enemy is released (Abraham, Moore and Godwin, 1990; Murphy and Moore, 1990; Simberloff and Stiling, 1996; Lopez-Vaamonde and Moore, 1998; Waage, 2001; van Lenteren *et al.*, 2003). This includes screening the agent for any direct non-target effects, for example by assessing its specificity for the target host, and any indirect non-target effects, such as transfer of exotic hyperparasitoids or if it may act as a vector for exotic disease.

**Table 1.1 Advantages and disadvantages of chemical control**

Advantages	Disadvantages
Can have high efficiency	Environmental damage and reduction of biodiversity Low specificity to pest Costly for small farmers Only short term solution – continued spraying required Pest can develop resistance Health risk to sprayer Residues in crop can taint flavour Bad publicity, public require natural control methods



The level of non-target testing required before release of natural enemies remains one of the most debated topics in biological control research. Equally, if not more, hotly contested is the issue of whether it is better to introduce a single or multiple natural enemy species to control a pest (reviewed in Hågvar, 1989; Myers, Higgins and Kovacs, 1989; Denoth, Frid and Myers, 2002). General consensus agrees that multiple natural enemies are generally more successful against weeds because there are numerous niches for specialists to attack, such as the roots, stem and leaves (Denoth *et al.*, 2002). Recommendations for arthropod pest control are far more contentious.

### **1.1.2 Single or multiple species releases against arthropod pests?**

There are three main categories of natural enemies that are released against arthropod pests: entomopathogens, predators and parasitoids. Entomopathogens include fungi, nematodes, protozoa, viruses and bacteria which are sprayed on crops in the form of bioinsecticides (Lacey *et al.*, 2001). The most widely used entomopathogen is *Bacillus thuringiensis* (Lacey *et al.*, 2001), but fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* are also commonly employed (e.g. Bustillo *et al.*, 1999; Lord, 2001). Although entomopathogens are fairly commonly used, most biological control agents of arthropod pests are parasitoids or predators. Predators can be vertebrates or invertebrates, some of which are arachnids, but deployment of insects is most common.

Entomopathogens, parasitoids and predators can be liberated in single or multiple species release programmes. When released against arthropod pests, there appears to be more support for the release of single species to control the pest (e.g. Myers *et al.*, 1989; Wen and Brower, 1995; Denoth *et al.*, 2002; Matsumoto *et al.*, 2003; Pedersen and Mills, 2004) but there is also some support for the use of multiple natural enemies (e.g. Ehler, 1978; Gutiérrez *et al.*, 1988; Kindlmann and Ruzicka, 1992; Heinz and Nelson, 1996; Bogran, Heinz and Ciomperlik, 2002).

When multiple natural enemies are released, there are often two approaches adopted. The first is to release several possible natural enemies until one of them achieves successful biological control of the pest, referred to as the “lottery model” by Denoth

*et al.* (2002). The second, perhaps more informed, approach is to release agents which may act additively or synergistically and thus may achieve greater control together than a single species acting alone, an approach referred to as the “cumulative stress model” by Denoth *et al.* (2002). Reviews of the biological control literature by Myers *et al.* (1989) and Denoth *et al.* (2002) have indicated that release programmes adopt the lottery model more often than the cumulative stress model. Myers *et al.* (1989) reviewed 50 published biological control programme successes in which a mean of three natural enemies were released. They found that in 68% (34) of projects, control could be attributed to a single species from the complex, whereas only 32% (16) of the successes involved control by a complex of natural enemies. Furthermore, Denoth *et al.* (2002) reviewed a random, bias-controlled sample of 108 biological control programmes against insect pests from the BIOCAT database (Greathead and Greathead, 1992) and reported that 64 of these projects resulted in successful biological control. Of the 64 successful projects, 39 involved the release of a single species to control the pest and 25 involved multi-species releases. However, of the programmes that involved release of multiple species, control could be attributed to the actions of a single species in 56% (14) of cases.

These studies indicate that, if the literature reviewed provides a valid sample of biological control programmes, the release of a single species against arthropod pests is generally preferable to multi-species releases. In addition the greater proportion of successes that do occur in multi-species releases are brought about by a single species. If a single natural enemy exerts sufficient control on an arthropod pest, there is no need to introduce further control agents. In fact, adopting a lottery approach and introducing more than one natural enemy to control a pest can actually reduce the success of the agent with the most potential and disrupt control (Myers *et al.*, 1989; Rosenheim, Wilhoit and Armer, 1993; Briggs, 1993; Snyder and Ives, 2001; Denoth *et al.*, 2002). This could happen if a competitively superior natural enemy displaces, or reduces the impact of, a more effective biological control agent in a complex. Such consequences can occur via interspecific interactions such as interference competition, exploitative competition and Intra-Guild Predation (IGP), which includes facultative- and heterospecific hyperparasitism.



### 1.1.3 Exploitation and interference competition

Both interference and exploitative (scramble-type) competition may be frequent between species which attack a common host. The most effective exploitative competitors may reduce the resource available to their competitors (Hågvar, 1989), for instance if they attack earlier host stages or develop faster than their opponents (e.g. Yu, Luck and Murdoch, 1990; Briggs, 1993; Murdoch, Briggs and Nisbet, 1996). On the other hand, interference competitors may consume and parasitize their opponents (IGP) or use aggressive behaviours and resource defence to prevent access to a resource for feeding or parasitism. This may take the form of fatal larval contests (e.g. Chow and Mackauer, 1984; Lawrence, 1988; Mackauer, 1990), or adult contests that can involve lethal (e.g. Collier, Kelly and Hunter, 2002; Pérez-Lachaud, Hardy and Lachaud, 2002) or non-injurious interactions (e.g. Mills, 1991; Murdoch *et al.*, 1996; Ryoo, Yoon and Shin, 1996). In these situations, if the best competitor is not the best control agent, it would be unwise to release the least effective species. This is also of concern if there is little or no resource partitioning between the natural enemy species.

Coexistence of more than one species on the same stages of a host may be unlikely, leading to the persistence of only the superior competitor, whether through interference or exploitation, at the expense of the inferior competitor (Turnbull and Chant, 1961). It may, however, be possible for two natural enemies to coexist on the same host if one of the species is superior in intrinsic competition (e.g. larval and adult contests, development time) and the other is superior in extrinsic competition (adult searching efficiency, dispersal, reproductive capacity and synchronisation with the host) (Hågvar, 1989; Mills, 1991; van Alebeek, Rojas-Rousse and Leveque, 1993; Collier *et al.*, 2002). This is the counter-balanced competition principle proposed by Zwölfer (1971).

#### 1.1.3.1 Intra-guild predation

Another aspect of interference competition is the propensity to take part in IGP. The incidence of IGP in natural communities was first reviewed by Polis, Myers and Holt (1989) and then brought into the context of biological control by Rosenheim *et al.*

(1995), who stated that IGP “occurs when two species that share a host or prey (and therefore may compete) also engage in a trophic interaction with each other (parasitism or predation)”. Intuitively, if a biological control agent feeds on or parasitizes one of its competitors rather than the pest prey there may be some disruption in pest population suppression. This can arise because the more effective natural enemy is the Intra-Guild prey (IG prey), so its population size and effectiveness may be reduced by the Intra-Guild predator (IG predator). Alternatively, if the more effective natural enemy acts as an IG predator, it may divide its attack between the pest and its intra-guild competitor, thus attacking a smaller proportion of the pest population. In the extreme, IGP may lead to the competitive exclusion of the IG predator or IG prey (Rosenheim *et al.*, 1995; Williams, 1996; Holt and Polis, 1997; Müller and Brodeur, 2002).

IGP theory predicts that when the IG predator is competitively superior to the IG prey, the IG prey will be outcompeted and its population will be driven to extinction (Polis *et al.*, 1989; Holt and Polis, 1997). To achieve coexistence between an IG predator and IG prey, the IG predator needs to be competitively inferior to its IG prey (Holt and Polis, 1997; Arim and Marquet, 2004). Theory also predicts that coexistence between an IG predator and its IG prey is only likely when the basal resource is at intermediate productivity (Polis *et al.*, 1989; Holt and Polis, 1997). These conditions are unlikely to be met when natural enemies are released to try to control an outbreak pest population, so we need to consider what may be expected to occur at high basal resource.

At high host production, if the IG predator has a high numerical response it may build up large populations that then consume all of the IG prey, leading to the loss of the IG prey from the system (Holt and Polis, 1997; Polis *et al.*, 1989). Therefore the IG prey may be driven to extinction when released against large pest populations. Conversely, at low pest population levels the IG prey is better at finding hosts than the IG predator, so the IG prey could persist at lower pest densities and thus outcompete the IG predator, leading to the loss of the IG predator from the system (Polis *et al.*, 1989; Holt and Polis, 1997). Therefore, after biocontrol releases against an abundant pest, if the IG prey persists with the IG predator and they bring the pest population down to low levels, it is then the IG predator that may be driven to



extinction, which could feasibly raise pest population levels once more. This is preferable to the establishment of the IG predator in this system alone as the greater suppressive effect of the IG prey would mean the pest population is lower when only the IG prey persists than when only the IG predator persists.

Caution should therefore be applied when releasing multiple species against large pest populations, because if one of the natural enemies acts as an IG predator it may exclude a more effective biological control agent, leading to the possible disruption, or a reduction in the level, of biological control (Rosenheim *et al.*, 1995; Borer *et al.*, 2003).

The species which take part in IGP include predators, parasitoids and pathogens. Entomopathogens are often not very specific to the pest they are sprayed against. They may infect and reduce the beneficial impact of insect predators and parasitoid populations that are released in tandem with them (de la Rosa *et al.*, 2000; Lord, 2001). Predators are also relatively oligo- or poly-phagous and may directly engage in IGP whilst consuming the immature stages of their predator and parasitoid competitors, or indirectly by consuming hosts which harbour developing endoparasitoids.

There are also several ways that parasitoids can engage in IGP. Firstly, they may act as predators and consume the immature stages of their competitors to derive nutrient benefits or to reduce the effect of larval competition for their own offspring (Hardy and Blackburn, 1991; Infante *et al.*, 2001a). Secondly, some species may be facultative hyperparasitoids. These have the capability of acting as primary parasitoids of the arthropod pest, but also have the ability to parasitize the immature stages of their competitors (van Alebeek *et al.*, 1993). Depending on the preference of the facultative hyperparasitoid, they may often hyperparasitise allospecific parasitoids or only choose this option when host resources are limited. Thirdly, some natural enemies are heteronomous hyperparasitoids (also referred to as autoparasitoids). These species act as primary parasitoids when laying female eggs and hyperparasitoids when laying male eggs. Autoparasitoids can be heterospecific (laying males on developing allospecific female parasitoids) or conspecific (laying males on developing conspecific female parasitoids) (Briggs and Collier, 2001;

Hunter, Collier and Kelly, 2002). Facultative- and heteronomous hyperparasitoids are often predicted to be superior competitors than primary parasitoids, which may prevent the persistence of multiple species in these systems and can lead to reduced pest control (Williams, 1996; Briggs and Collier, 2001). However, if the heteronomous hyperparasitoid prefers to oviposit male eggs on or in conspecifics, rather than heterospecifics, this may promote coexistence with a primary parasitoid. The probability of coexistence increases as the preference for conspecific hyperparasitism increases, due to self-limiting density dependence in the autoparasitoid population (Briggs and Collier, 2001). Thus hyperparasitoids should not be disregarded entirely from control programmes; in some cases they may coexist with their primary parasitoid competitors and achieve greater pest suppression together (e.g. Heinz and Nelson, 1996; Bogran *et al.*, 2002; Pedersen and Mills, 2004) and in other cases the hyperparasitoid may exert equal or better suppression of the pest in the absence of a primary parasitoid (e.g. Hunter *et al.*, 2002).

Parasitoids are less likely to cause disruption to biological control than predators and pathogens. This is because both predators and pathogens can engage in IGP without attacking the common pest (Rosenheim *et al.*, 1995; Ehler, 1996; Müller and Brodeur, 2002). In contrast, parasitoids that indulge in IGP consume and parasitize con- and allo-specific eggs and immatures that are already present in or on the pest, which means that the host must have been attacked previously by another parasitoid (Ehler, 1996). Therefore the pest will always be attacked when parasitoids engage in IGP, reducing the likelihood of complete disruption to control, but the overall success of pest suppression may still be reduced and lead to larger pest populations.

One strategy employed by a small proportion of females of a parasitoid species to reduce the incidence of IGP, and increase their own reproductive success, is to practice maternal care and resource defence. Such defence may take the form of direct behavioural contests. These behaviours can be observed in several bethylid parasitoid species which have been considered as biological control agents. The life histories of four of these species, which are investigated in this thesis, are detailed in sections 1.4 and 1.5 below.



## **1.2 CONTEST BEHAVIOUR**

Contest interactions have attracted a great deal of interest from behavioural ecologists, who investigate why contests take place, their dynamics and what decides their outcome. Contest behaviour research has been conducted throughout the animal kingdom, mainly focussing on male-male interactions over food, water, mates and protection from predators (Huntingford and Turner, 1987; Mesterton-Gibbons and Adams, 1998; Riechert, 1998). Females also enter into fights, normally in order to protect their offspring (current reproduction) or their opportunity to reproduce in the future (future reproduction). Although most theoretical models and predictions have been devised to understand male-male contests, many of these can also be applied to female combat.

### **1.2.1 Evolutionary game theory and contest interactions**

Particular emphasis has been placed on how individuals should behave in a contest to maximise their own success. To address this, Maynard Smith (1974) modified economic game theory to devise the term Evolutionary Stable Strategy (ESS). This term refers to the one or more strategies that individuals in a population adopt during a contest in order to maximise their own fitness. Once the majority of individuals within a population assume these ESS's, an individual that adopts an alternative contest strategy cannot 'invade' the population because its strategy will bring fewer benefits, or more costs (Maynard Smith, 1974). These theories have spawned a great deal of theoretical research (e.g. Maynard-Smith, 1982; Grafen, 1987; Mesterton-Gibbons, 1992).

Attention has also been focussed on actual contest interactions and what influences the success of the individuals involved. In a contest between two individuals, if both adopt the same displays and fighting behaviour, the winner may be determined by asymmetries in the ability of each of the contestants in resource defence and aggressive interactions. The combination of these abilities is referred to as the Resource Holding Potential (RHP; also termed Resource Holding Power) (Parker, 1974; Maynard Smith and Parker, 1976). Components of RHP may include contender size and ownership, and the individual with the greater RHP may be

expected to win the contest. However, other factors could also influence which individual wins a contest, for example the value that each of the contestants places on the resource (Resource Value, RV) (Maynard Smith and Parker, 1976; Enquist and Leimar, 1987). If one of the contenders places a higher value on that resource than its opponent, it may be willing to fight more aggressively than the opponent that estimated a lower RV. This may result in an individual with a lower RHP beating an opponent with a higher RHP.

Although contests can involve aggressive interactions, there is a wide range of possible contest dynamics. Contests can involve non-aggressive displays, aggressive non-injurious interactions and full escalation to injurious, and sometimes fatal, fighting. Contests are often settled by convention before aggressive interactions occur. Convention could rely on a diverse range of cues, for example estimation by a competitor of its own and an opponent's body size, which could allow contestants to infer RHP and minimise the chance of personal injury. Escalation to high levels of aggression is only expected where there is little chance of injury from such aggressive displays or where both contestants estimate a high RV, relative to future expectations, and are thus more willing to risk injury than they are over a low RV (Maynard Smith and Parker, 1976; Grafen, 1987; Enquist and Leimar, 1990). This can occur regardless of how large the asymmetry in the RHPs of the two contestants is.

There is ongoing debate as to whether contestants fight solely on the basis of what they perceive their own RHP to be or if they also assess their opponents RHP in relation to their own (Gammell and Hardy, 2003; Taylor and Elwood, 2003). As contests escalate, individuals may gain more information on their opponent's RHP and so, if they can assess their opponent's RHP, this assessment is likely to change as the contest escalates to more aggressive interactions. Likewise, the competitor's assessment of its own RHP may change as the contest progresses, perhaps through injuries sustained during escalation (Payne, 1998). Thus, as the contest escalates, the likelihood of an individual winning or retreating increases, through improved assessment of RHPs. When there is little asymmetry in RHPs between the two contestants, the duration of contests is expected to be longer and involve higher



levels of escalation than when there is a large asymmetry between competitors (Parker, 1974; Maynard Smith and Parker, 1976; Wells, 1988).

There are several ways assessment may occur within a contest, and theory has concentrated on disputes where competitors repeat the same signal but can vary the strength of this signal. These theories are the 'sequential assessment rule', the 'best-so-far rule' and the 'cumulative assessment rule' (Enquist and Leimar, 1983; Payne and Pagel, 1997; Payne, 1998). In the sequential assessment rule, both competitors repeat the same signal multiple times, but with no change in the signal intensity (Enquist and Leimar, 1983). This is adopted in a situation where there is large error in information transfer, so, as the signal is repeated, an individual gathers an increasingly more accurate estimate of its opponent's relative fighting ability.

The best-so-far rule relies on the two contestants aiming to minimise the energy they expend on contests (Payne and Pagel, 1996b). They both begin by producing a small, low cost display. As the contest progresses they repeat this display, and the intensity of the display becomes successively larger until one of the individuals concedes. In this way, both contestants ensure that they invest the minimum energy needed to resolve the contest.

The final rule involves the cumulative assessment model (Payne and Pagel, 1996a; Payne, 1998). Again, the contest starts with a particular signal, which is then repeated. The intensity of each signal repetition can stay the same or become stronger or weaker as the contest proceeds. Assessment in this model takes account of all previous actions, so the overall signal intensity increases with each display even if the strength of the current repetition of the signal is weaker than the previous one. In conducting these signal repetitions, the contestants perform a war of attrition or endurance. Payne (1998) takes the cumulative assessment model a step further, incorporating physical contact into the model. Decisions as to when to concede a contest are then reliant on the amount of physical damage each contestant is willing to sustain and competitors are unable to assess the damage they have done to their opponent.

In some situations, individuals may continue to accumulate damage until one of the individuals is killed, termed fatal fighting. This is only predicted to occur where there is little risk of damage to the winner when escalating or when the loser will have very few future reproductive opportunities if it concedes (Grafen, 1987; Enquist and Leimar, 1990).

### **1.2.2 Contest interactions and insects**

Contest theory has been applied to a wide variety of organisms, including insects. Much study in the class of insects has inevitably focussed on male-male contests for mating opportunities, which can range from non-contact wars of attrition between butterflies competing for territories in which females enter to be mated, such as sunspots (Stutt and Willmer, 1998), through to direct, injurious combat in the vicinity of females, such as in fig wasps (Cook, Bean and Power, 1999).

Less research has been conducted on adult female-female fights, which generally take place for reproductive opportunities, most commonly in parasitoid hymenoptera searching for hosts to parasitize (Hardy and Blackburn, 1991; Field, 1992; Field and Calbert, 1999; Pérez-Lachaud *et al.*, 2002). Several of these parasitoid species are bethylids.

## **1.3 BETHYLID PARASITOIDS**

The Aculeate family Bethylidae is distributed globally and contains about 1900-2200 described species (Evans, 1978; Hawkins and Gordh, 1986; Gordh and Móczár, 1990; Polaszek and Krombein, 1994) and probably at least as many undescribed species (Polaszek and Krombein, 1994). Most bethylids develop as gregarious external parasitoids of lepidopteran and coleopteran larvae (Hawkins and Gordh, 1986; Greathead, 1986; Gordh and Móczár, 1990; Polaszek and Krombein, 1994). Many of these hosts have cryptic life histories, and attack agricultural crops and stored products. Their cryptic lifestyle makes them difficult to control with pesticide applications so there has been interest in the use of their natural enemies as biological control agents. Bethylids are one family of parasitoids that are investigated for control of these pests, and they seem fairly promising when considering their life



history traits; bethylids tend to have high search efficiency, female biased sex ratios, are relatively species-specific, and have low juvenile mortality due, in part, to host guarding by foundress females (Griffiths and Godfray, 1988; Gordh and Móczár, 1990; Hardy and Blackburn, 1991; Hardy and Mayhew, 1998; Hardy *et al.*, 1998; Pérez-Lachaud and Hardy, 2001).

This thesis investigates female competitive behaviours of four species of bethylid parasitoids. Three of these species belong to the sub-family Epyrinae (*Cephalonomia hyalinipennis*, *Cephalonomia stephanoderis* and *Prorops nasuta*) and attack *Hypothenemus hampei*, a scolytid beetle that is a major pest of coffee worldwide. The other species, *Goniozus nephantidis*, belongs in the sub-family Bethylinae and is a natural enemy of the coconut pest *Opisina arenosella*.

## **1.4 STUDY SPECIES – COFFEE AGRO-ECOSYSTEMS**

I begin with a summary of the life history of the coffee berry borer and its parasitoid natural enemies and then focus on the brief life histories and biological control histories of the three study species, *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta*.

Both coffee and the coffee berry borer (CBB), *Hypothenemus hampei*, are indigenous to central Africa (Davidson, 1968; Le Pelley, 1968). From here, coffee has been introduced into tropical climates throughout the world and has become a major economic product. The CBB was first noticed in South America, in Brazil, in 1924 (Moreira, 1928 in Le Pelley, 1968) then in Central America in 1971 (Baker *et al.*, 1992), reaching Mexico in 1978 (Baker, 1984). It is suspected that the spread of the CBB occurred through infestations of traded berries (Le Pelley, 1968; Damon, 2000). The CBB has subsequently reached virtually all coffee producing regions around the world, and it is now considered the most serious pest of coffee worldwide (Le Pelley, 1968; Baker, 1999; Damon, 2000; Jaramillo *et al.*, 2005).

### **1.4.1 The coffee berry borer**

The CBB is a small scolytid beetle pest, the females of which primarily attack coffee species, particularly *Coffea arabica* and *C. canephora* (robusta variety) (Le Pelley,



1968; Damon, 2000). Adult females are approximately 2.2mm in size and males are considerably smaller (Hargreaves, 1926). The female locates ripe red or yellow coffee berries and bores a tunnel into the fruit until she reaches the endosperm (bean), where she excavates galleries and tunnels. Within these galleries she oviposits 2-3 eggs per day, laying a batch of up to 70 eggs in her lifetime (Le Pelley, 1968; Baker, 1984). The larvae that eclose from the spanandrous broods consume the endosperm and emerge as adults. Sibling mating is normal and the sex determination mechanism is pseudoarrhenotoky (Brun *et al.*, 1995; Borsa and Kjellberg, 1996a, 1996b). The life cycle lasts 28-34 days in field conditions (Le Pelley, 1968; Damon, 2000) and longevity varies from 35-112 days (Hargreaves, 1926).

Damage is not only caused by the developing immatures consuming the endosperm; the tunnel that the female has bored can act as a source for secondary infection by fungi or further pest attack. When there are few suitable mature berries to attack, such as early in the season or where frequent harvesting is practiced, females may bore into green, unripe berries which are unsuitable for breeding (Le Pelley, 1968; Baker, 1999; Damon, 2000). This may stop the development of these berries and can cause their decay and premature fall, all of which reduce the coffee yield to farmers.

Levels of infestation by the CBB are often high, with reports of 80% of berries infested in Uganda (Hargreaves, 1926), 90% in Tanzania (Le Pelley, 1968) and 60-100% in Mexico (Baker, 1984). This can lead to large economic losses, such as the US \$58 million per year estimate that has been quoted for Colombia (Ruiz, Uribe and Riley, 2000).

#### **1.4.2 Natural enemies of the coffee berry borer**

When considering control options for the CBB, its cryptic within-berry lifestyle not only provides it with a refuge from pesticide and bio-insecticide applications, but also limits the range of natural enemies that can attack it; concealed hosts like the CBB tend to have parasitoid complexes that are relatively species poor compared to more exposed hosts (Hawkins, 1988; Hawkins, Thomas and Hochberg, 1993). Pesticides such as Endosulfan have been reasonably effective in controlling CBB when applications have been made when females were beginning to bore into the

berry, but there are many reasons why the use of chemical control has become less favoured (Table 1), including the development of resistance to Endosulfan and Lindane in some CBB populations (Brun *et al.*, 1990, 1995; Parkin, Brun and Suckling, 1992). For these reasons, attention turned to the use of classical biological control.

The first instance of a classical biological control attempt against the CBB was the introduction of *P. nasuta* into Brazil in 1929 (Hempel, 1934). Although *P. nasuta* established, it had little impact on CBB populations. Once the CBB had reached Central and North America (Mexico) biological control practitioners began further explorations within the African native range of the CBB to look for more natural enemies of potential use in classical biological control. Several parasitoid species were identified and investigated in the laboratory, including the braconid *Heterospilus coffeicola* Schmiedeknecht (Hymenoptera: Braconidae) and three bethylid species *Cephalonomia stephanoderis*, *Sclerodermus cadavericus* Benoit and the previously released *Prorops nasuta* (Moore and Prior, 1988; Murphy and Moore, 1990). Of these species, *S. cadavericus* causes dermatitis in humans so could not be introduced outside of its native range (Murphy and Moore, 1990) and rearing attempts of *H. coffeicola* in the laboratory repeatedly failed (Hargreaves, 1935; Moore and Prior, 1988; Murphy and Moore, 1990; Baker, 1999). Quarantine and screening trials for *H. coffeicola* are necessary before it can be released as an exotic parasitoid against the CBB as it may act as a vector for the fungus *Colletotrichum coffeanum*, which causes coffee berry disease within Africa and has been found to be carried by the congener, *H. prosopidis* (Nemeye, Moore and Prior, 1990).

Both *C. stephanoderis* and *P. nasuta* were investigated further and imported into Mexico in 1985 and Ecuador in 1987 from Togo, via quarantine in the UK, for a CABI Bioscience biological control programme against the CBB (Barrera *et al.*, 1990b; Baker, 1999). I summarise the life histories of *C. stephanoderis* in section 1.4.2.1 and *P. nasuta* in section 1.4.2.2, focussing on Mexico, where releases began in 1988 (Moore and Prior, 1988; Barrera *et al.*, 1990a).

Following the release of *C. stephanoderis* and *P. nasuta*, a further parasitoid natural enemy of the CBB, *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae), was



discovered in the Ivory Coast and Togo (LaSalle, 1990). The life cycle of *P. coffea* was investigated in the laboratory (Feldhege, 1992; Infante *et al.*, 1994a; Lopez-Vaamonde and Moore, 1998) and it has subsequently been released as a classical biological control agent around the world (Vergara-Olaya *et al.*, 2001a, 2001b; Baker, Jackson and Murphy, 2002; Jaramillo *et al.*, 2005).

Briefly, *P. coffea* is a synovigenic gregarious endoparasitoid which attacks adult female CBBs as they are beginning to bore into the coffee berry (Feldhege, 1992; Infante *et al.*, 1994a; Baker, 1999). Adult female *P. coffea* lay one or two eggs inside the adult CBB and then disperse to search for further CBB adults in different berries (Feldhege, 1992). The adult females are approximately 1mm in length and live for 2-4 days, in which time they can parasitize adult CBBs in more than 10 coffee berries (LaSalle, 1990; Feldhege, 1992; Infante *et al.*, 1994a; Lopez-Vaamonde and Moore, 1998; Baker, 1999). They are relatively specific to scolytid beetles (Lopez-Vaamonde and Moore, 1998) and have now been released, and are believed to have established, in Guatemala, Colombia, Mexico, Ecuador and Honduras (Vergara-Olaya *et al.*, 2001a, 2001b; Baker *et al.*, 2002; Jaramillo *et al.*, 2005).

#### ***1.4.2.1 Cephalonomia stephanoderis***

The bethylid parasitoid *Cephalonomia stephanoderis* was identified by Betrem (1961). Adults are black and females, which are approximately 2mm in length, are larger than males (Abraham *et al.*, 1990; Infante *et al.*, 1994b). It is a solitary, primary, idiobiont ectoparasitoid that can feed on all stages of the CBB, but preferentially consumes eggs and adults and parasitizes large larvae, prepupae and pupae of the CBB (Abraham *et al.*, 1990; Barrera, Gómez and Alauzet, 1994; Lauzière, Brodeur and Pérez-Lachaud, 2001a; Lauzière, Pérez-Lachaud and Brodeur, 2001b). It is indigenous to Africa and is reported in the Ivory Coast (Le Pelley, 1968; Vega *et al.*, 1999) and Togo (Moore and Prior, 1988; Vega *et al.*, 1999), where it has only been found attacking the CBB.

An adult female locates a coffee berry containing a suitable range of CBB stages, enters and paralyzes all developmental stages. Females are synovigenic and anautogenous; in order to mature eggs they must host feed on CBB stages which are



suitable for oviposition (Lauzière *et al.*, 2001b). They normally display non-concurrent destructive host feeding, except at very low host densities when concurrent host feeding is possible (Lauzière, Pérez-Lachaud and Brodeur, 1999).

After a pre-oviposition period of 2-9 days, a single egg is deposited on each pupa, prepupa or large larva of the CBB (Abraham *et al.*, 1990; Infante and Luis, 1993; Lauzière *et al.*, 2001a) at an oviposition rate of 1-2 eggs per day (Lauzière *et al.*, 1999). The life cycle lasts from 20-22 days at a mean temperature of 25°C, but is inversely related to temperature (Abraham *et al.*, 1990; Infante *et al.*, 1992). Adult females can feed on 70 or more hosts, lay up to 70 eggs and longevity can be up to 49 days under field temperatures (25-35°C) in the laboratory (Abraham *et al.*, 1990; Infante *et al.*, 1992; Infante and Luis, 1993; Lauzière, Pérez-Lachaud and Brodeur, 2000a). A foundress female *C. stephanoderis* remains within a single berry guarding the paralysed hosts and her developing brood (Abraham *et al.*, 1990; Pérez-Lachaud *et al.*, 2002). On emergence of the brood, sibling mating occurs and brood sex ratio is female biased, with up to seven females for each male (Infante and Luis, 1993; Cabrera and Barrera, 1998; Lauzière *et al.*, 1999). Mated females then disperse to find new CBB infested berries.

*Cephalonomia stephanoderis* was previously thought to be monophagous but Pérez-Lachaud and Hardy (2001) demonstrated that it is able to host feed and develop on alternative curculionids in the laboratory. Further, Damon (1999) suggested that the CBB may actually be the secondary host of *C. stephanoderis* in Togo, because large populations of *C. stephanoderis* only build up in coffee plantations close to the inter-harvest period. Parasitism levels by *C. stephanoderis* in the Ivory Coast can reach 50% of CBB infested berries (Le Pelley, 1968) but are generally a lot lower (Barrera *et al.*, 1990a) and Damon (1999) found a maximum of only 6% parasitism of CBB-infested berries by *C. stephanoderis* in Togo.

Where it has been released in classical biological control programmes, *C. stephanoderis* has normally established but has contributed little to CBB population suppression (Bustillo-Pardey *et al.*, 1996; Baker, 1999; Damon, 1999). Within Mexico, the first releases of *C. stephanoderis* lead to parasitism rates of up to 80% of CBB infested berries within a month of release, but this quickly fell to low levels

(Barrera *et al.*, 1990b). Despite continued releases and its establishment, parasitism levels in Mexican agro-ecosystems remain below 1% of CBB infested berries (Damon, 1999).

#### ***1.4.2.2 Prorops nasuta***

*Prorops nasuta* was first reported attacking the CBB by Hargreaves (1926). Females are 1.5 - 2.5mm in length (Evans, 1978), but normally 2 - 2.3mm (Hargreaves, 1926; Abraham *et al.*, 1990), and are larger than males. They are solitary primary ectoparasitoids that consume all stages of the CBB, feeding preferentially on eggs and adults and parasitizing large larvae, prepupae and pupae of the CBB (Abraham *et al.*, 1990; Infante, Mumford and Baker, 2005). It is indigenous to Africa and is reported attacking the CBB in Uganda (Hargreaves, 1926), the Congo (Le Pelley, 1968), Kenya (Le Pelley, 1968; Murphy and Rangi, 1991), Tanzania (Le Pelley, 1968), Zaire (Le Pelley, 1968), Togo (Moore and Prior, 1988), Cameroon (Barrera *et al.*, 1990a) and the Ivory Coast (Barrera *et al.*, 1990a).

The life-history of the primary, idiobiont parasitoid *P. nasuta* is very similar to that of *C. stephanoderis*. Females are synovigenic and anautogenous: in order to mature eggs they must destructively host feed on CBB stages which are suitable for oviposition (Infante *et al.*, 2005). Adults will feed and develop on a small number of alternative coleopterans in the laboratory if access to CBB stages is prevented, but juvenile mortality is high on these hosts (Pérez-Lachaud and Hardy, 2001). They paralyse all CBB stages in a single berry and, after a pre-oviposition period of 3-16 days (Infante, 2000; Infante *et al.*, 2005), they lay one egg on pupae, prepupae or large larvae at a rate of 1-2 eggs per day (Murphy and Rangi, 1991). The life cycle lasts 22-30 days at 25-26°C (Abraham *et al.*, 1990; Murphy and Rangi, 1991). Adult females can lay up to 28 eggs and live for up to 65 days under field temperatures (25-35°C) in the laboratory (Le Pelley, 1968; Abraham *et al.*, 1990; Infante, 2000). A foundress female remains within a single berry guarding the paralysed hosts and her developing brood (Murphy and Rangi, 1991; Pérez-Lachaud *et al.*, 2002). On emergence, sibling mating occurs within the female biased brood, which can have up to seven females to each male (Barrera, 1991; Infante *et al.*, 2005). Mated females disperse to find new CBB infested berries.



Parasitism levels in Kenya have never been more than 20% of CBB infested berries (Barrera *et al.*, 1990a; Murphy and Rangi, 1991) and population levels of *P. nasuta* are low when found (Vega *et al.*, 1999). When released in classical biological control programmes, *P. nasuta* has had little impact on CBB populations (Infante *et al.*, 2005), but has established in several release countries, such as Brazil (Hempel, 1934) and Colombia (Bustillo-Pardey *et al.*, 1996). *Prorops nasuta* has failed to persist in Mexican coffee agro-ecosystems, despite repeated releases of cultures originating from Togo and Brazil (Infante, Mumford and Mendez, 2001b; Infante, Mumford and Garcia-Ballinas, 2003).

#### **1.4.3 New association parasitoids of the coffee berry borer**

In addition to efforts to use classical biological control against the CBB, some practitioners have noticed new associations forming involving the CBB. In Mexico, field nematodes have been discovered naturally attacking the CBB (Castillo *et al.*, 2002) and Pérez-Lachaud (1998) found the bethylid *C. hyalinipennis* parasitizing the CBB in Mexican coffee agro-ecosystems. This species was then investigated further to assess its potential as a biological control agent against the CBB.

##### ***1.4.3.1 Cephalonomia hyalinipennis***

*Cephalonomia hyalinipennis* is a small black bethylid native to Europe, North and South America which has been reared from a range of coleopteran species and galls of cynipids (Evans, 1978; Gordh and Móczár, 1990; Pérez-Lachaud and Hardy, 2001). According to Evans (1978) females vary in length from 1.5 - 2.2mm, but they tend to be smaller than this (1.2 - 1.7mm) when reared on the CBB (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 1999). Males vary from 1 - 1.5mm (Evans, 1978).

Pérez-Lachaud and Hardy (1999, 2001) have investigated the biology of *C. hyalinipennis* to assess its suitability for mass release against the CBB. Females are



primary ectoparasitoids<sup>1</sup> that consume all stages of the CBB except the adult, feeding preferentially on eggs and small larvae and parasitizing large larvae, prepupae and pupae of the CBB (Pérez-Lachaud and Hardy, 1999). Females are synovigenic, anautogenous and practice both concurrent and non-concurrent host feeding (Pérez-Lachaud and Hardy, 1999, 2001). They are semi-gregarious, laying 1-4 eggs on pupae, prepupae or large larvae of the CBB after a pre-oviposition period of 2-5 days (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 1999). Development lasts 19.6 - 20.9 days at 28°C (Pérez-Lachaud and Hardy, 1999).

Adult females lay a mean of 88 eggs and live for up to 95 days at 28°C in the laboratory (Pérez-Lachaud and Hardy, 1999). On locating a batch of CBB hosts, a foundress female will paralyse them before guarding these hosts and her developing brood (Pérez-Lachaud *et al.*, 2002). Similarly to *C. stephanoderis* and *P. nasuta*, sex ratios are female biased, male sex ratio being 0.212, and sibling mating occurs (Pérez-Lachaud and Hardy, 1999).

*Cephalonomia hyalinipennis* has not been identified attacking the CBB in other regions of North and South America. It has not been released against the CBB and parasitism levels in the field appear to be low (Pérez-Lachaud, 1998).

#### **1.4.4 Interactions between species in coffee agro-ecosystems**

The release of *C. stephanoderis* and *P. nasuta* into coffee agro-ecosystems where *C. hyalinipennis* is indigenous prompts the question of whether these species are able to coexist, or if it may be better to release a single species to attempt CBB control. The obvious overlap in life history of the species suggests that there may be competition between them for access to CBB infested berries.

Previous experimental research has provided conflicting evidence as to whether coexistence is possible between the three bethylid species. Infante *et al.* (2001a) concluded that *P. nasuta* and *C. stephanoderis* can co-exist in laboratory 'coffee jar

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<sup>1</sup> But Pérez-Lachaud and Hardy (1999) suggested that it may, however, act as a facultative hyperparasitoid, and chapter 2 of this thesis addresses this issue.

experiments' with numerous berries. However, Pérez-Lachaud *et al.* (2002) and Batchelor *et al.* (2005; Chapter 3) have demonstrated that, on a one-to-one basis, *P. nasuta*, *C. stephanoderis* and *C. hyalinipennis* females will fight violently for possession of *H. hampei* hosts in an artificial arena, often resulting in the death of the loser.

The only theoretical study into the population dynamics involved in coffee biocontrol was conducted by Gutierrez *et al.* (1998). They used population parameters collected from Colombia to model the population dynamics of *H. hampei*, *C. stephanoderis*, *P. nasuta* and the eulophid *P. coffea*. The model suggested that, if releasing only one parasitoid, *P. coffea* was the only species that could feasibly reduce *H. hampei* populations to significant levels. *Prorops nasuta* and *C. stephanoderis* were effectively treated as having the same life-histories, with competition between the two species assumed to be symmetrical. This assumption was subsequently investigated and contest work suggested that *C. stephanoderis* was the superior competitor (Pérez-Lachaud *et al.*, 2002). Within the models, both bethylid species had little impact on *H. hampei* populations and were predicted to have even lower success when released together due to interference. Of note, however, is that simulations that included *P. coffea* and one bethylid species (either *P. nasuta* or *C. stephanoderis*) had the capacity to reduce *H. hampei* populations to slightly lower levels than *P. coffea* alone. The addition of *C. hyalinipennis* into the simulations and experimental studies could feasibly change the predictions for whether coexistence and control are possible.

With these gaps in our knowledge, the aim of Part one of this thesis is to assess the competitive interactions that take place between the three bethylid species *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* in order to infer the release strategy that could have the most impact on CBB populations. Despite the debate on the pros and cons of multiple and single species releases, few studies have actually attempted to establish the compatibility of multiple natural enemies to be introduced into the field to control a pest.



## 1.5 CONTEST BEHAVIOUR IN *GONIOZUS NEPHANTIDIS*

Part two of this thesis concentrates on intraspecific contest interactions between females of *G. nephantidis*. I start with a brief description of the life history of *G. nephantidis* and then summarise its known contest interactions.

### 1.5.1 *Goniozus nephantidis*

*Goniozus nephantidis* is an idiobiont gregarious ectoparasitoid that is a natural enemy of *Opisina arenosella* in coconut plantations in India and Sri Lanka. Adult females are 3 – 4.5 mm in length and males are 3 - 3.5mm (Remadevi, Mohamed and Abdurahiman, 1981; Kapadia and Mittal, 1986). *Goniozus nephantidis* has been investigated as a biological control agent against *O. arenosella*, but investigation into alternative hosts for mass rearing of *G. nephantidis* demonstrated that it could successfully parasitize *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in the laboratory (Kapadia and Mittal, 1986). The life history of *G. nephantidis* reared on *C. cephalonica* is similar to on *O. arenosella* and the details reported below are from both species. Because of the ease in culturing as a factitious host, *C. cephalonica* has been used to rear *G. nephantidis* for many subsequent experiments (e.g. Hardy and Blackburn, 1991; Petersen and Hardy, 1996).

Adult female *G. nephantidis* paralyse their host and exhibit host guarding behaviour during a 3 - 8 day pre-oviposition period (Antony and Kurian, 1960; Kapadia and Mittal, 1986). They then lay a clutch of 3 - 18 eggs on larval hosts (Antony and Kurian, 1960; Hardy, Griffiths and Godfray, 1992) and aggressively defend their developing brood. Adult females can lay between 20 – 110 eggs over their lifespan (Antony and Kurian, 1960; Kapadia and Mittal, 1986; Cock and Perera, 1987; Hardy *et al.*, 1992), which generally lasts for 3 - 23 days (Antony and Kurian, 1960; Remadevi *et al.*, 1981; Kapadia and Mittal, 1986). On emergence, the offspring have a female biased sex ratio (proportion male 0.093 – 0.16) and sibling mating occurs (Cock and Perera, 1987; Hardy and Cook, 1995; Hardy *et al.*, 1998; Hardy *et al.*, 1999).

Under natural conditions, *G. nephantidis* parasitism rates are generally below 5% (Kapadia and Mittal, 1986), but can reach 28% where there are outbreaks of *O. arenosella* (Kapadia and Mittal, 1986; Cock and Perera, 1987). *Goniozus nephantidis* is attacked by at least five species of hyperparasitoid in India and at least one species in Sri Lanka (Jayaratnam, 1941). Hyperparasitism rates of *G. nephantidis* by one hyperparasitoid, *Pediobius imbrues*, can reach 90.14% in India (Kapadia and Mittal, 1986). The high prevalence of hyperparasitism in the field could partly explain why brood guarding behaviour is important in this species (Hardy and Blackburn, 1991). Despite the low parasitism rates of *G. nephantidis*, it continues to be investigated, mass reared and released against *O. arenosella* (e.g. Venkatesan *et al.*, 2003).

### 1.5.2 Prior contest research on *Goniozus nephantidis*

The host and brood guarding behaviour of female *G. nephantidis* has been investigated in the laboratory (Hardy and Blackburn, 1991; Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). These studies used the factitious host *C. cephalonica*. Hardy and Blackburn (1991) demonstrated that guarding behaviour increases the probability of brood survivorship because it prevents superparasitism by conspecifics and reduces multiparasitism by the allospecific parasitoid *Bracon hebetor*. Egg broods that were unguarded were vulnerable to ovicide by conspecifics, which can subsequently lay their own clutch onto the host (Hardy and Blackburn, 1991; Hardy *et al.*, 1999). In contrast, larval broods were rarely attacked by conspecifics. This could occur because the larvae have already consumed much of the host and left little resources for development of superparasitoids or practicing larvicide could be too time-consuming (Hardy and Blackburn, 1991). When the allospecific *B. hebetor* intruded on unguarded hosts, larvicide, followed by multiparasitism, always occurred.

Further research established that adult female *G. nephantidis* engage in aggressive intraspecific contests for paralysed hosts. These contests rapidly escalate to chasing, biting, grappling and (usually) non-injurious fighting and were generally resolved within the 90 minute experimental period (the length of videotapes available) used by Stokkebo and Hardy (2000), although they suggest that natural contests may occur over a longer period. The probability of a female winning a contest has been



found to be positively influenced by its weight relative to its competitor (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). The importance of body size (wasp weight) asymmetries in female-female contests has implications for the 'Lack' clutch size that should be laid on hosts; the Lack clutch size is the number of eggs a female should lay to maximise clutch fitness (Godfray, 1994; Mesterton-Gibbons and Hardy, 2004). When host size increases, there is evidence that females of many bethylid species, including *G. nephantidis*, increase their clutch sizes (Gordh, Woolley and Medved, 1983; Hardy *et al.*, 1992; Mayhew, 1998; Mayhew and Hardy, 1998). In addition, female *G. nephantidis* that emerge from larger hosts tend to be larger (Hardy *et al.*, 1992). Because larger females are advantaged in contests, foundresses that lay fewer eggs than their competitors on a similarly sized host provision more resource to each of their offspring, thus producing larger progeny that subsequently have a competitive advantage during contests. It is argued that natural selection would lead to clutch sizes of one if contests always occurred, and larger individuals always won, because this is the only non-invasible clutch size strategy (Petersen and Hardy, 1996; Mesterton-Gibbons and Hardy, 2004). Hardy *et al.* (1992) report that, for standard sized hosts, the calculated Lack clutch size for *G. nephantidis* is 18, whereas the average observed clutch size is 9. The Lack clutch size prediction of 18 eggs was based on a static optimality model that considers fitness components that are related to absolute body size (longevity and fecundity). The observed clutches of 9 may be produced because the fitness effects of body size were underestimated (Hardy *et al.*, 1992) and/or because it is body size relative to competitors that is important for success in contest interactions (Petersen and Hardy, 1996).

The discrepancy between observed and predicted clutch size in *G. nephantidis* lead to the development of a game-theoretic model, which incorporated the effect of body size on contest outcome (Mesterton-Gibbons and Hardy, 2004). In this model, the clutch size optima of individual mothers depend on the clutch size decisions made by other mothers in the population. Many of the model's parameter combinations predict the 'true Lack clutch size' (based on game theory) to be around half of the 'traditional Lack clutch size' (based on static optimality theory). Thus the observed clutch size of 9 in *G. nephantidis* may be an optimal strategy.

Further variables that positively influence contest outcome include prior ownership (Petersen and Hardy, 1996) and the number of mature eggs in competing females' abdomens (egg load) (Stokkebo and Hardy, 2000). In laboratory investigations published to date, prior owners always win contests against smaller intruders, and larger intruders generally need a size advantage of more than 0.25mg in order to displace the owner from the host (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). Stokkebo and Hardy (2000) suggested that ownership advantage results from resource value asymmetries between the owner and intruder. They predicted that, when owners have oviposited prior to the contest, they win because they place a higher RV on the host than intruders do. When contests occur for unparasitized hosts, Stokkebo and Hardy (2000) hypothesised that the ownership advantage is due to egg load asymmetries. They showed that, once owners paralyse the host, they increase their egg maturation rates compared to intruders. This results in larger egg loads in owners, who might place a larger value on the resource because they can oviposit on (exploit) the host sooner than intruders. However, they also found a positive relationship between wasp weight and egg load, which introduces a confounding variable. Further, in these experiments egg load asymmetries did not influence contest outcome in owner-intruder contests; owners won 23 of 24 contests regardless of egg load or body size asymmetries. In contrast, contests between two owners, in which asymmetries in ownership and body size were absent, found a strong positive advantage from larger egg load (Stokkebo and Hardy, 2000). Whilst these results are consistent with ownership advantage being a result of RV asymmetries (instead of, or as well as RHP), they do not prove that egg load determines resource value. Additional attributes that influence contest outcome remain to be investigated.

Part two of this thesis investigates some of the candidate further explanatory variables that may influence contest outcome between *G. nephantidis* females. Emphasis is placed on host size and wasp age, which might be expected to correlate with RV, and the release of chemicals by females is explored during contest interactions.



## **1.6 SUMMARY OF THESIS STRUCTURE**

In summary, the structure of the thesis is as follows:

### **1.6.1 Part One: Competitive interactions between bethylid parasitoids of the coffee berry borer**

Chapter 2 examines the laboratory hyperparasitic and predatory capacity of adult female *C. hyalinipennis* on con- and allo-specific bethylid parasitoid immatures, and the predatory capacity of adult female *C. stephanoderis* on *C. hyalinipennis* immatures. The aim is to assess whether intra-guild predation is possible between the bethylids that attack the CBB.

Chapter 3 investigates inter- and intraspecific contest interactions within and between *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta*. The aim is to compare the strength of intra- and inter-specific competition via contests, and to identify any competitive superiority of these species.

Chapter 4 evaluates the production in terms of female recruitment of *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* when provided with CBB-infested coffee berries under varying intra- and inter-specific competition intensities. The aim is to assess whether the bethylid species are likely to coexist under limited CBB availability.

The overall aim of chapters 2-4 is thus to evaluate the ‘compatibility’ of the three bethylids and to investigate whether particular combinations of species are likely to provide enhanced, or reduced, control of the CBB.

### **1.6.2 Part Two: *Goniozus nephantidis* contests and chemical release**

Chapter 5 explores the influences of asymmetries in host weight and wasp age on contest outcome between *G. nephantidis* females. The aim is to understand more fully the various influences on contest outcome.

Chapter 6 investigates the release of volatile chemicals by female *G. nephantidis* and seven other bethylid species. The aim is to provide a pilot study using a new technique to be used for assessing the influence of volatile release within contests.

The overall aim of chapters 5 and 6 is to assess the influence of further variables on contest interactions between bethylid females.

### **1.6.3 Format of the thesis**

All five experimental chapters, and the manuscript in the Appendix, are written in paper format. Those that have been published already are chapter 2 (Pérez-Lachaud, Batchelor and Hardy, 2004), chapter 3 (Batchelor *et al.*, 2005) and chapter 4 (Batchelor *et al.*, 2006). Chapter 5 has been submitted to a behavioural journal. The appendix contains a manuscript that I am a co-author for, which follows from the work in Chapter 6. Although I was involved in the discussion and development of the work, it is not included as a chapter in the thesis because I did not play a major role in collecting and analysing the data and writing the manuscript. The references and acknowledgements for each chapter are collected together into the final reference list and acknowledgments sections, respectively.



**Part One: Competitive interactions between bethylid parasitoids of  
the coffee berry borer**

## CHAPTER 2 – WASP EAT WASP: FACULTATIVE HYPERPARASITISM AND INTRA-GUILD PREDATION BY BETHYLID WASPS<sup>1</sup>

### 2.1 ABSTRACT

Bethylid wasps are primary parasitoids of coleopteran and lepidopteran pests of economic importance and have thus been deployed as biological control agents. We show that *Cephalonomia hyalinipennis* (Hymenoptera: Bethylidae) is also a facultative hyperparasitoid of four other bethylid species: *Cephalonomia stephanoderis* and *Prorops nasuta*, natural enemies of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), and of *Goniozus nephantidis* and *G. legneri*, which have been released against lepidopteran pests of coconuts and almonds respectively. Conspecific and allospecific ovicide and larvicide are also observed, constituting intra-guild predation. Such trophic interactions have the potential to disrupt biological control in coffee, coconut and almond agro-ecosystems.

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<sup>1</sup> Published as: PÉREZ-LACHAUD, G., BATCHELOR, T.P. and HARDY, I.C.W. (2004) Wasp eat wasp: facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biological Control* **30**, 149-155



## 2.2 INTRODUCTION

The Bethylidae (Hymenoptera: Aculeata) is a cosmopolitan family of ectoparasitoid wasps consisting of more than 100 genera and approximately 2000 described species (Gordh and Móczár, 1990). The hosts of bethylids are nearly always larval or pupal Coleoptera or Lepidoptera, often those occurring in cryptic locations such as plant stems, seeds, wood or soil (Clausen, 1940; Evans, 1964). Many of these host species are also economically important pests of crops including almonds, coconuts, coffee, cotton, dates, rice and sugarcane. Bethylids also attack pests of coniferous trees, stored grain, wool and museum specimens. There have consequently been attempts to deploy bethylids as biological control agents, with variable results (e.g. Legner and Gordh, 1992; Baker, 1999).

Host range is often an important criterion in the selection of a potential agent of biological control (e.g. Waage, 2001), as host specificity minimizes the risk of unwanted non-target effects and/or potentially disruptive intra-guild predation (Rosenheim *et al.*, 1995; Holt and Hochberg, 2001; Müller and Brodeur, 2002). A few bethylid species seem to be truly monophagous (e.g. *Cephalonomia waterstoni*; Howard and Flinn, 1990), but most studied species are oligophagous (e.g. Finlayson, 1950; Gordh, 1976; Evans, 1978) and even species that have been reported as monophagous may readily be reared on alternative factitious hosts in the laboratory (e.g. *Cephalonomia stephanoderis*; Pérez-Lachaud and Hardy, 2001). It has, however, been generally accepted that bethylids do not develop hyperparasitically. For instance, Brodeur's (2000) survey of the taxonomic affiliation of hyperparasitoids did not list any Aculeate species as being hyperparasitic.

The bethylid *Cephalonomia hyalinipennis* Ashmead (sub-family Epyrinae) is a widely distributed (Evans, 1978), gregarious ectoparasitoid, that attacks a broad range of coleopteran hosts both in nature (Gordh and Móczár, 1990), and in the laboratory (Pérez-Lachaud and Hardy, 2001)<sup>2</sup>. In the Soconusco region of Chiapas,

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<sup>2</sup> Note that at the time of publishing this paper, we were unaware that *C. hyalinipennis* has been reported to have been reared from galls of *Amphibolips* and *Dischalcaspis* spp. (Hymenoptera: Cynipidae) (Evans, 1978). This indicates that our report was not the first documented case of hyperparasitism by *C. hyalinipennis* on another hymenopteran.

Mexico, *C. hyalinipennis* naturally attacks prepupae and pupae of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) (Pérez-Lachaud, 1998), which has spread from its African origin to become the most important pest of coffee worldwide (e.g. Baker, 1999; Damon, 2000). The borer remains a major agricultural problem for Mexican coffee growers despite the introduction, in the late 1980s, of two of its African natural enemies, the bethylid wasps *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (Barrera *et al.*, 1990a; Infante *et al.*, 2001b), both also belonging to the sub-family Epyrinae. Improved biological control practices, including the possible use of additional or alternative agents, are thus desirable.

We have previously examined various aspects of the reproductive biology and behaviour of *C. hyalinipennis* in order to assess the desirability of its augmentative release within its natural range and possible introduction into other coffee growing areas (Pérez-Lachaud and Hardy, 1999, 2001; Pérez-Lachaud *et al.*, 2002). During studies of interactions between *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta*, adult *C. hyalinipennis* females were serendipitously observed to parasitize the larvae and prepupae of the two other species. In this paper we confirm this finding and report it for the first time, and additionally document inter- and intra-specific predation (ovicide and larvicide) between these species. We further report that *C. hyalinipennis* can develop hyperparasitically on two further bethylid species, *Goniozus nephantidis* Muesebeck and *G. legneri* Gordh; these belong to the sub-family Bethylinae and are natural enemies of lepidopteran pests of coconut (Cock and Perera, 1987) and almonds (Legner and Gordh, 1992) respectively. We discuss the implications of these trophic interactions for biological control.

## 2.3 MATERIALS AND METHODS

### 2.3.1 Hyperparasitism of allospecific Epyrinae by *C. hyalinipennis*

Adult female *C. hyalinipennis* were placed singly into experimental arenas consisting of chambers in plastic blocks with a clear plexiglass lid (as described by Pérez-Lachaud and Hardy, 1999; see also Petersen and Hardy, 1996). In 'naive female' replicates (n = 10), females had emerged as adults within the previous 24h. had



mated but had not, as adults, had previous contact with hosts. In 'experienced female' replicates ( $n = 5$ ), females had been allowed to attack healthy *H. hampei* hosts for 3 days prior to transfer to the arenas.

Each arena also contained 5 healthy hosts (prepupae and/or pupae of *H. hampei*) and 5 *H. hampei* prepupae or pupae previously parasitized by *C. stephanoderis* females. *Cephalonomia stephanoderis* females lay one egg per host. *Cephalonomia stephanoderis* immatures were either at the egg stage (5 naïve female replicates) or in late larval stages of development (still attached to the host, or detached and beginning to spin a cocoon) (5 naïve and 5 experienced replicates). Arenas were examined at least twice a day, and often more frequently, for four days after introducing the *C. hyalinipennis* female. The behaviour of some of the females was also videotaped. We measured the head width of *C. hyalinipennis* females that eventually matured from eggs laid during the observation period. Experiments were carried out at  $28 \pm 2^\circ\text{C}$ .

Following the above protocol, we set up an additional replicate in which a naïve *C. hyalinipennis* female was provided with *H. hampei* hosts bearing single *P. nasuta* (rather than *C. stephanoderis*) larvae. Due to lack of biological material, we were unable to further assess hyperparasitism of *P. nasuta*.

### **2.3.2 Conspecific hyperparasitism by *C. hyalinipennis***

Using a similar protocol, 4 naïve and 5 experienced *C. hyalinipennis* females were each provided with 5 healthy hosts and 5 late-instar conspecific larvae (produced by another *C. hyalinipennis* female).

### **2.3.3 Hyperparasitism of *C. hyalinipennis***

A similar protocol was used to assess whether *C. stephanoderis* hyperparasitise *C. hyalinipennis*: 5 naïve *C. stephanoderis* were each provided with 5 healthy hosts plus 5 hosts bearing larval *C. hyalinipennis*. The capacity of *P. nasuta* females to attack other bethylids could not be investigated due to a shortage of biological material.

#### 2.3.4 Hyperparasitism of Bethylinae by *C. hyalinipennis*

Naïve adult female *C. hyalinipennis*, originating from mixed sex broods, were placed singly into experimental arenas formed by taping together two excavated microscope slides (Pérez-Lachaud and Hardy, 1999). Each arena contained 3 late-stage larvae of *G. nephantidis* or *G. legneri* that had ceased to feed and detached from the host larva in order to pupate. Both *G. nephantidis* and *G. legneri* were reared on the factitious host *Corcyra cephalonica* (Lep.: Oecophoridae). Experiments using *G. nephantidis* were carried out at 26-29°C and those using *G. legneri* at 24-28°C. We measured the head width of *C. hyalinipennis* females that eventually matured.

#### 2.3.5 *Cephalonomia hyalinipennis* reared on *H. hampei*

For comparison of adult body size when developing on different hosts, we measured the head widths of 130 *C. hyalinipennis* reared on *H. hampei* in naturally infested berries collected from the field in Mexico.

### 2.4 RESULTS

#### 2.4.1 Hyperparasitism of allospecific Epyrinae by *C. hyalinipennis*

All *C. hyalinipennis* females used *H. hampei* hosts bearing *C. stephanoderis* eggs, either by feeding on the eggs followed by oviposition on the *H. hampei* (2/5), adding eggs (multiparasitism) without ovicide (2/5) or feeding on *H. hampei* without oviposition (1/5). The also present healthy *H. hampei* were oviposited on, fed upon (destructive host feeding) or both (concurrent host feeding). When females were provided with healthy hosts and late-stage *C. stephanoderis* larvae (still on their host's remains or already spinning a cocoon), they similarly fed upon both healthy *H. hampei* and *C. stephanoderis* larvae, and oviposited on healthy *H. hampei*. Continuous videotape recording allowed us to observe that all *C. hyalinipennis* females chewed holes into, and entered, newly constructed silk cocoons of *C. stephanoderis* larvae (Table 2.1, Fig. 2.1). At the end of the 4-day observation period 43/47 (93.5%) *C. stephanoderis* cocoons had been entered by *C. hyalinipennis*.



**Table 2.1** Actions of *C. hyalinipennis* females provided with conspecific, *C. stephanoderis* or *P. nasuta* larvae

Species of larvae provided	<i>C. hyalinipennis</i>		<i>C. stephanoderis</i>		<i>P. nasuta</i>
<i>C. hyalinipennis</i> prior experience	naïve	experienced	naïve	experienced	naïve
Replicates	4	5	5	5	1
Females opening cocoons	0	0	5	5	?
Females that fed on developing parasitoids <sup>1</sup>	1	3	3	2	1
Females that hyperparasitised	0	1	5	4	1
Replicates in which <i>C. hyalinipennis</i> offspring eclosed	-	0	4	3	1

<sup>1</sup>Values are minima as definitive observations of host feeding are difficult and because some *C. hyalinipennis* eggs were accidentally killed during experimental inspection



**Figure 2.1** A *C. hyalinipennis* female feeding on a *C. stephanoderis* larva inside its cocoon.



Cocoons that were later dissected under a stereomicroscope were found to contain prepupae and pupae of *C. stephanoderis* that had been parasitized by *C. hyalinipennis*. From 1 to 3 eggs were laid on individual *C. stephanoderis* prepupae/pupae (Figs. 2.2 and 2.3). However, not all *C. stephanoderis* prepupae/pupae inside cocoons entered by *C. hyalinipennis* were parasitized or fed upon: 6 *C. stephanoderis* adults emerged from such cocoons. No effect of prior experience of *H. hampei* hosts on *C. hyalinipennis* behaviour was discernable and the proportion of naïve and experienced females that hyperparasitised at least some *C. stephanoderis* larvae was similar (log-likelihood ratio test,  $G_1 = 1.49$ ,  $P > 0.05$ ). Most individuals developing as hyperparasitoids died as late-instar larvae or as a

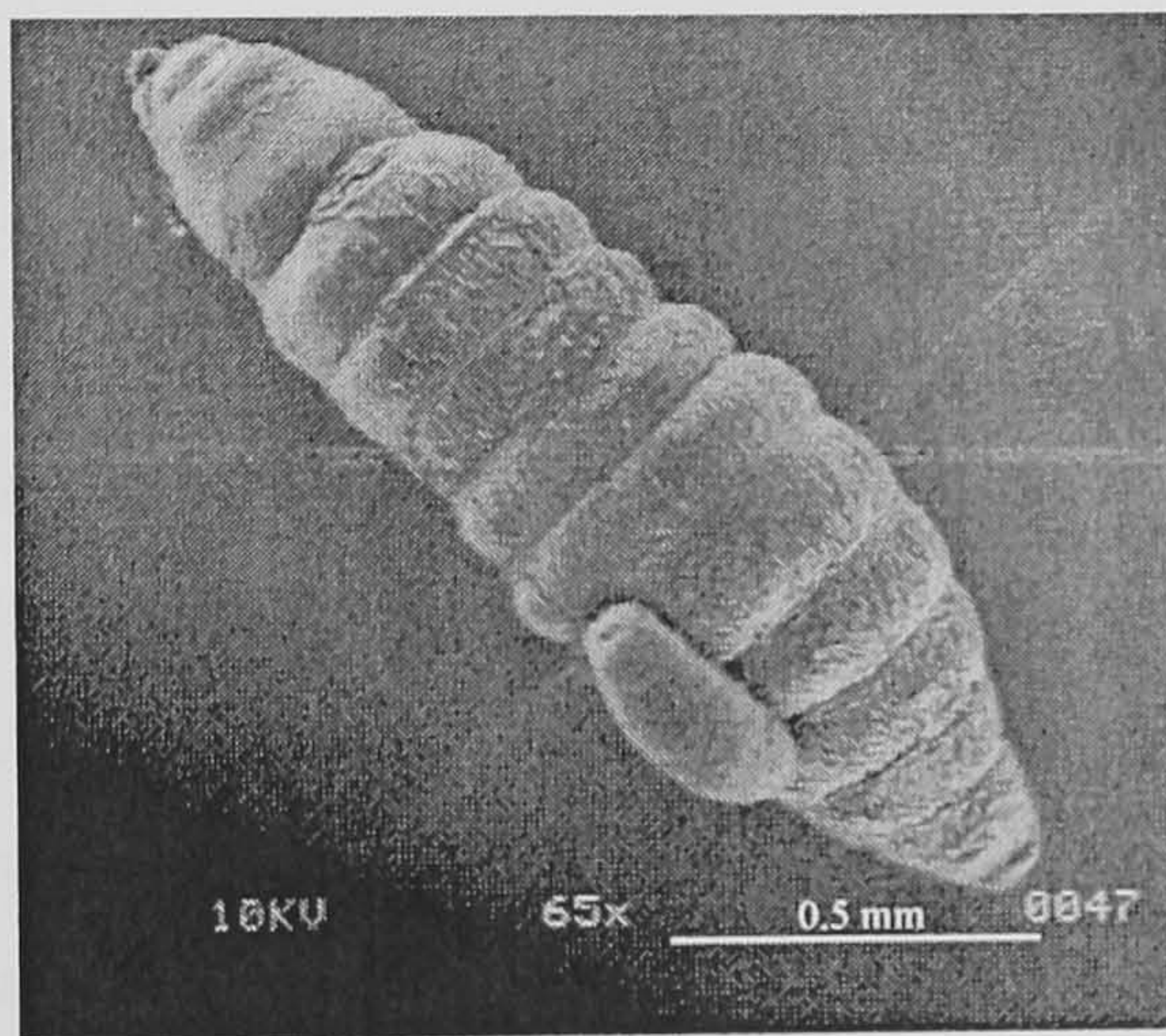


Figure 2.2 A *C. stephanoderis* larva bearing an egg of *C. hyalinipennis*.

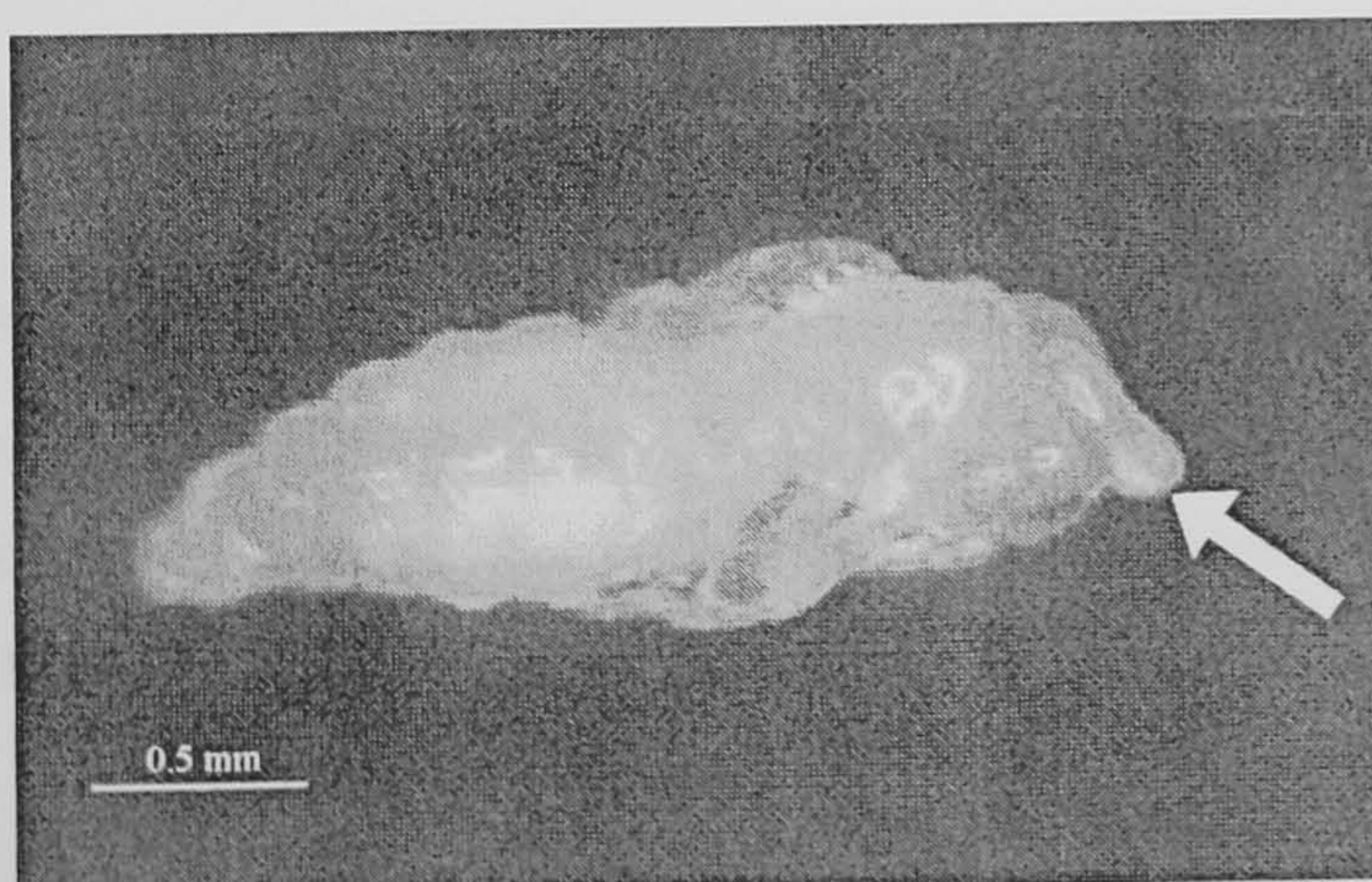


Figure 2.3 A *C. stephanoderis* pupa parasitized by *C. hyalinipennis*.



consequence of experimental manipulation during the egg stage. Successful reproduction via hyperparasitism was, however, achieved by 7/10 *C. hyalinipennis* females (Table 2.1), with 14 adults obtained from 24 hyperparasitised *C. stephanoderis*. *Cephalonomia hyalinipennis* females developing as hyperparasitoids of *C. stephanoderis* (head width, mean 0.156mm  $\pm$  SD 0.009) were smaller than individuals from stock cultures that developed as primary parasitoids of *H. hampei* (mean = 0.263 mm, SD  $\pm$  0.020, n = 130) (Fig. 2.4).

In the single replicate in which a naïve *C. hyalinipennis* was provided with 5 *P. nasuta* larvae, one larva was destroyed by host feeding and 4 were parasitized after they had left the *H. hampei* host and spun cocoons. One adult *C. hyalinipennis* female developed to adulthood as a hyperparasitoid of these *P. nasuta* (Table 2.1).

#### 2.4.2 Conspecific hyperparasitism by *C. hyalinipennis*

No *C. hyalinipennis* female was observed to open cocoons spun by conspecific larvae (Table 2.1), but some were observed to feed upon these larvae before cocoon spinning (Table 2.1). One (experienced) female both fed on and laid an egg on a conspecific larva when there were no suitable *H. hampei* available, but the egg died on hatching. The proportion of females that acted as conspecific hyperparasitoids was uninfluenced by experience ( $G_I = 1.27$ ,  $P > 0.05$ ) and, overall, the proportion of females that hyperparasitised conspecifics was lower than the proportion that parasitized allospecifics ( $G_I = 14.54$ ,  $P < 0.001$ ).

#### 2.4.3 Hyperparasitism of *C. hyalinipennis*

All 5 *C. stephanoderis* were observed feeding upon *C. hyalinipennis* larvae before cocoons were spun, but these females did not behave as hyperparasitoids. The difference between the proportions of *C. stephanoderis* and *C. hyalinipennis* females that acted as allospecific hyperparasitoids is significant ( $G_I = 5.58$ ,  $P < 0.05$ ).

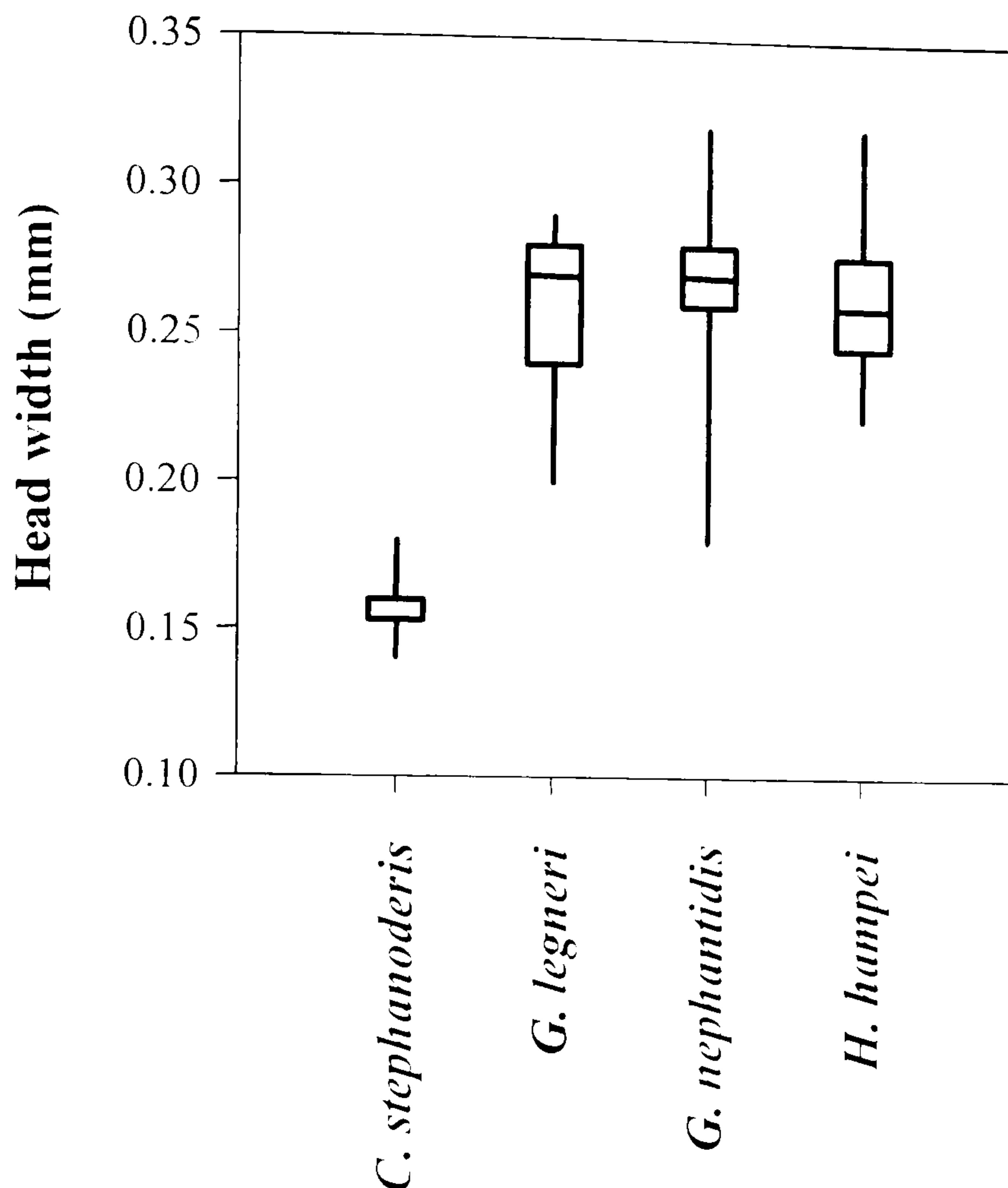


Figure 2.4 Size of adult *C. hyalinipennis* females developing from different host species, expressed as head width medians with 25% quartiles and 95% CI. Median head width differed significantly according to host species (Kruskal-Wallis 1-way ANOVA,  $H = 56.09$ , d.f. = 3,  $P < 0.001$ ).

#### 2.4.4 Hyperparasitism of Bethylinae by *C. hyalinipennis*

*Cephalonomia hyalinipennis* females readily oviposit on both *G. nephantidis* and *G. legneri* larvae, with up to 7 adults developing from each of the three hosts provided. We have reared more than 11 generations of *C. hyalinipennis* on *Goniozus* hosts, providing a further alternative culturing method (Pérez-Lachaud and Hardy, 2001). The mean head widths of adult female *C. hyalinipennis* reared on *Goniozus* (*G. legneri* =  $0.261 \pm 0.027$ mm,  $n = 14$ ; *G. nephantidis* =  $0.270 \pm 0.017$ ,  $n = 134$ ) are similar to those of conspecifics developing as primary parasitoids of *H. hampei* and larger than those reared hyperparasitically on *C. stephanoderis* (Fig. 2.4).



## 2.5 DISCUSSION

We have shown that *C. hyalinipennis* can develop as a hyperparasite on *C. stephanoderis* and *P. nasuta*, currently the two main biological control agents used against the coffee berry borer, and also on *G. nephantidis* and *G. legneri*, which have respectively been released against coconut and almond pests. This is atypical as bethylid species usually parasitize a limited range of coleopteran or lepidopteran hosts (however, under laboratory conditions, some will develop on a wider range of insect species, sometimes spanning several orders, e.g. Zhang, Song and Fan, 1984; Chen, Lu and Lin, 1989). Although *C. hyalinipennis* is able to develop on a wide range of species, their suitability for its development varies (Pérez-Lachaud and Hardy, 2001). We were unable to evaluate formally the developmental mortality of hyperparasitic individuals, but mortality appeared similar to primary parasitoid mortality (Pérez-Lachaud and Hardy, 2001). Further, adults that develop on *C. stephanoderis* are smaller than those from *H. hampei* or *Goniozus*, and thus may not be as long-lived, fecund or as effective in competition for hosts (Hardy *et al.*, 1992; Petersen and Hardy, 1996).

We have also shown that adult *C. hyalinipennis* females readily feed upon eggs and larvae of *C. stephanoderis*, *P. nasuta* and conspecific larvae. Similarly, adult female *C. stephanoderis* feed on larval *C. hyalinipennis*. *Cephalonomia stephanoderis* has been previously reported to feed on fully developed *P. nasuta* larvae but not on larvae that are still attached to the host or on eggs, while *P. nasuta* feeds on *C. stephanoderis* eggs, feeding larvae and fully developed larvae (Infante *et al.*, 2001a). Ovicide, and later use of previously parasitized hosts, is reported in a number of other bethylid species, and larvicide has been documented in some, but not all (Goertzen and Douth, 1975; Hardy and Blackburn, 1991; Mayhew, 1997).

The fact that *C. hyalinipennis* acts as a facultative hyperparasitoid and an intra-guild predator may present a challenge for the biological control of *H. hampei* and of pests of almonds and coconut. Such actions are generally thought to be detrimental to the 'top-down' pest regulation aimed at by biological control practitioners (e.g. Rosenheim, 1998; Hassell, 2000) and there have been many concerns about the introduction of facultative hyperparasitoids (e.g. Weseloh, Wallner and Hoy, 1979;

Rosenheim *et al.*, 1995; Sullivan and Völkl, 1999). On current evidence, we thus do not advise the introduction of *C. hyalinipennis* into coffee growing regions outside its natural range (e.g. Africa, India, South East Asia). If introduced into the Indian sub-continent, any detrimental effects of *C. hyalinipennis* could extend beyond the coffee agro-ecosystem, as it has the potential to attack beneficial *G. nephantidis* in coconut growing areas.

Turning to areas in which *C. hyalinipennis* is naturally present, its widespread occurrence in the USA (Evans, 1978; Gordh and Móczár, 1990) leads us to caution that it could begin to attack *G. legneri* in, for instance, Californian almond growing regions, in much the same way as it naturally became present in the coffee agro-ecosystem (Pérez-Lachaud, 1998). *Cephalonomia hyalinipennis* could thus disrupt what has possibly been the most successful biological control exerted by an introduced bethylid (Legner and Gordh, 1992). It is however, more difficult to evaluate whether the presence of *C. hyalinipennis* in the coffee agro-ecosystem in parts of the New World (Pérez-Lachaud, 1998) is desirable or undesirable for the control of *H. hampei*, and thus whether augmentative releases should be made. The risk that the (increased) presence of *C. hyalinipennis* will disrupt successful biological control by *C. stephanoderis* or *P. nasuta* seems low, since the introduction of these species has to date not lead to acceptable levels of pest suppression (e.g. Baker, 1999; Damon, 2000; Infante *et al.*, 2001b). However, population dynamic studies and biological control case history reviews each generally caution against the deployment of multiple natural enemies, and recommend that only a single ‘best species’ should be used to avoid negative interactions (e.g. Myers *et al.*, 1989; Murdoch and Briggs, 1996; Denoth *et al.*, 2002). It is unclear which of these three bethylid species is the superior, in population dynamic terms, competitor under field conditions. Current laboratory evidence suggests that *C. hyalinipennis* will compete effectively for resources via facultative hyperparasitism, and has a higher intrinsic rate of increase than *C. stephanoderis* or *P. nasuta* due to the larger number of eggs laid per host (Pérez-Lachaud and Hardy, 1999) and will also win behavioural contests against *P. nasuta*, but not *C. stephanoderis* (see below). There are further relevant aspects of the biologies of these species, such as foraging ability, that have not been assessed. Anecdotal evidence, from the Soconusco region of Mexico, suggests that *C. hyalinipennis* is currently rare where it has been found, and that *C.*



*stephanoderis* is recovered more frequently (T.P. Batchelor, pers. obs.). It is possible, though we suspect unlikely, that hyperparasitism, predation and lethal behavioural attacks by *C. hyalinipennis* are responsible for the non-establishment of *P. nasuta* in the region (Infante *et al.*, 2001b). Possible interactions between *C. hyalinipennis* and further candidate agents, such as *Phymastichus coffea* (Hym.: Eulophidae) (Baker, 1999) remain unevaluated.

The success of *C. hyalinipennis* as a hyperparasitoid and intra-guild predator, and thus the influence of these behaviours on biological control, may be moderated by the brood guarding behaviour exhibited by *C. stephanoderis*, *P. nasuta*, *G. nephantidis* and *G. legneri*. Females of these species may stay with their brood until pupation and most have been shown to engage in direct, in some cases lethal, contests for the possession of hosts (Hardy and Blackburn, 1991; Petersen and Hardy, 1996; Pérez-Lachaud *et al.*, 2002; Chapter 3). *Cephalonomia hyalinipennis* females are usually unsuccessful when intruding on guarded *C. stephanoderis* broods (Pérez-Lachaud *et al.*, 2002; Chapter 3), which would reduce the likelihood of predation and hyperparasitism. The success of *C. hyalinipennis* in contests with *P. nasuta*, however, may be higher (Chapter 3) and its success against *Goniozus* species is unevaluated. The time that *C. stephanoderis* females spend brood guarding seems to depend on the size of the brood and, ultimately, the number of available hosts: small broods of 1-3 individuals, produced when hosts are scarce, are abandoned earlier than larger broods (G. Pérez-Lachaud and I.C.W. Hardy, unpublished). The occurrence of unguarded broods is expected to increase the likelihood of hyperparasitism by *C. hyalinipennis*.

In summary, trophic and competitive interactions between *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* in the field seem likely to occur and to be complex. Such interactions could have a negative impact on *H. hampei* control, but neither the importance of the impact nor the best biological control strategy is currently clear. Similarly, hyperparasitism by *C. hyalinipennis* appears to have the potential to disrupt natural enemy action in almond and coconut agro-ecosystems.

# CHAPTER 3 - INSECT GLADIATORS II: COMPETITIVE INTERACTIONS WITHIN AND BETWEEN BETHYLID PARASITOID SPECIES OF THE COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE)<sup>1</sup>

## 3.1 ABSTRACT

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) causes substantial reductions in coffee production. It originates from Africa but is now present in almost all of the major coffee producing countries. Classical biological control attempts around the world, including South and Central America, the Caribbean, Indian sub-continent, Indonesia and Polynesia, including introductions of the African bethylid wasps *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem, have not yet proved sufficiently successful. In Mexico, a bethylid wasp indigenous to North America and Europe, *C. hyalinipennis* Ashmead, has naturally started to attack the borer. The presence of multiple species of natural enemies has the potential to disrupt biological control via negative interspecific interactions. We evaluate both inter- and intraspecific competition among these three bethylids in the laboratory, focussing on pairs of adult females competing directly for hosts. In interspecific contests, the loser is frequently killed. *Cephalonomia stephanoderis* is the most successful species, while *P. nasuta* females never killed their opponents. Intraspecific interactions often involved fighting behaviour but were non-fatal. We discuss the implications of the differing strengths of such inter- and intraspecific interactions for the coexistence of these natural enemies of the coffee berry borer and for biological control.

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### 3.2 INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), continues to cause substantial reductions in coffee production throughout the world (Baker, 1999; Baker *et al.*, 2002). Native to Africa, it is now present in almost all of the major coffee producing countries, including Mexico. *Hypothenemus hampei* was first observed in the Soconusco region (Chiapas) in 1978 and has since become a major pest throughout Mexico (Baker, 1984; Infante *et al.*, 2001b). As part of a biological control program, releases of two parasitoid natural enemies from Africa, *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyridae) commenced in Chiapas in 1988 (Barrera *et al.*, 1990a). *Cephalonomia stephanoderis* has established but is rare (Damon, 1999; Damon and Valle, 2002) and *P. nasuta* has never persisted longer than 15 months (Infante *et al.*, 2001b); neither species has achieved economically significant pest control. A new association has also developed in Mexico where *Cephalonomia hyalinipennis* Ashmead, a bethylid wasp indigenous to North America and Europe (Gordh and Móczár, 1990), has been found naturally attacking *H. hampei* in the Soconusco region of Chiapas (Pérez-Lachaud, 1998).

These three bethylid species share many similar life-history traits. All three species are synovigenic and need to host-feed before they can mature eggs. Adult females feed on all developmental stages of the borer, preferentially consuming eggs, small larvae and adults (Barrera *et al.*, 1990b; Lauzière *et al.*, 2001a), with the possible exception of *C. hyalinipennis*, which does not appear to attack adult borers. Females preferentially parasitize prepupae, pupae and occasionally large larvae of the CBB (Barrera *et al.*, 1989; Abraham *et al.*, 1990; Infante and Luis, 1993; Pérez-Lachaud and Hardy, 1999, 2001; Lauzière *et al.*, 2001b). Individual females usually oviposit within only one berry.

Negative interactions between these bethylids are known to occur. Intra-guild predation occurs between *P. nasuta* and *C. stephanoderis* in the laboratory via consumption of allospecific eggs and larvae (Infante *et al.*, 2001a). *Cephalonomia hyalinipennis* has recently been identified as a facultative hyperparasitoid of several bethylid parasitoid species, including *C. stephanoderis* and *P. nasuta*, in the

laboratory (Pérez-Lachaud *et al.*, 2004: Chapter 2). In addition dyadic contests for hosts occur between adult females. After entering a berry containing CBB, females exhibit host guarding (Pérez-Lachaud *et al.*, 2002), a behaviour observed in many bethylid species (Griffiths and Godfray, 1988; Hardy and Blackburn, 1991; Petersen and Hardy, 1996). Guarding females attempt to repel con- and allo-specific intruders entering the berry. The contests that result can involve a series of chases, biting and, at full escalation, attempts to attack and paralyze each other with their stingers (modified ovipositors), which house venom to induce paralysis in hosts prior to oviposition. Direct interspecific contests frequently result in the paralysis and death of the defeated female (Pérez-Lachaud *et al.*, 2002), demonstrating the potency of the venom when used on allospecifics. The outcome of dyadic contests is, in general, expected to be related to the costs incurred (Enquist and Leimar, 1990; Payne, 1998), differences in competitive ability between individuals (Maynard-Smith and Parker, 1976; Hammerstein, 1981; Grafen, 1987), prior ownership (Petersen and Hardy, 1996; Papaj and Messing, 1998) and to the resource value (Wells, 1988; Dugatkin and Biederman, 1991; Field and Calbert, 1998) to the competitors.

Pérez-Lachaud *et al.* (2002) documented dyadic contests between the abovementioned bethylid species that are parasitoids of the CBB. They placed emphasis on contests involving *C. stephanoderis* and investigated 7 of the 12 possible combinations of owner versus naïve or experienced intruder for these three species in the laboratory. Here we present data on the remaining 5 combinations of interspecific contests. Further, we extend these investigations to intra-specific contests for CBB hosts. We discuss these findings in the contexts of the evolution of fatal fighting and the relative importance of intra- and inter-specific interactions in relation to species coexistence and their implications for biological control of the CBB.

### 3.3 MATERIALS AND METHODS

Parasitoids were reared at El Colegio de la Frontera Sur (ECOSUR, Tapachula, Chiapas, Mexico). The *C. stephanoderis* stock was derived from insects collected in Togo in 1988, and the *P. nasuta* stock from insects reared in Brazil in 1992. The *C. hyalinipennis* culture was initiated in 1997 from material collected in the Soconusco



region (Mexico). Pupae and prepupae of the coffee berry borer were removed directly from naturally attacked coffee berries collected from various localities around Tapachula.

The experimental procedure followed that of Pérez-Lachaud *et al.* (2002) in which full methodological details are given. Briefly, we observed behavioural interactions during encounters between pairs of adult female bethylids. In all interspecific, and most intraspecific, experiments, one female was a prior 'owner' of a batch of 10 hosts, which had been paralyzed and fed upon by that female. Owners had already oviposited in the majority of these contests. We refer to the non-owner females as the 'intruder'. Intruders were either 'naïve' (had not previously encountered hosts) or 'experienced' (had host fed and oviposited prior to the experiment). We studied those combinations of owner versus naïve or experienced intruder not addressed by Pérez-Lachaud *et al.* (2002), such that, by combining these studies all twelve possible combinations of interspecific contests were observed (Table 3.1). We also enhanced the sample size of two of the combinations previously studied by Pérez-Lachaud *et al.* (2002). Intraspecific contest experiments involved either an owner and an intruder (Table 3.2) or two naïve non-owners (Table 3.3), of known body size (head width), with methodology otherwise identical to that used for interspecific contests.

Interactions between females were observed within the apparatus used by Pérez-Lachaud *et al.* (2002) consisting of an opaque plastic block with three chambers linked by a slot (through which the parasitoids studied can pass) and covered with clear Plexiglas. This apparatus was modified from that originally developed by Petersen and Hardy (1996), who provide a detailed description and illustration. Due to the relatively small size of bethylids attacking the coffee berry borer, the apparatus used in this study, and by Pérez-Lachaud *et al.* (2002), was made to a smaller scale than the original, such that the dimensions of the chambers were 10 mm diameter and 5 mm depth, with 10 mm between each chamber and a slot of 1 mm in width. Barriers can be pushed into the slot to isolate one or more chambers, and be withdrawn to connect the chambers. This apparatus allows interactions between females to occur within a confined space, similar to in a coffee berry, with each female free to enter and leave the immediate vicinity of the hosts. Observations were made from above, through a binocular dissection microscope. Environmental

conditions were  $28 \pm 2^{\circ}\text{C}$  and  $75 \pm 5\%$  RH under natural lighting with an approximately 12:12 (L: D) h photoperiod.

Interactions between prior owners and intruders were set up as follows: a newly emerged mated female (>2 d old) was placed in a glass tube (dimensions 1.5 cm x 7.3 cm, sealed with gauze) and presented with a batch of 10 host pupae and prepupae for 3-6 days prior to the experiment and moved, with any resulting progeny, into an observation chamber in the apparatus one day before the experiment. On transfer, the owner was presented with a further five fresh hosts. Thus owner females were aged from 4 to 7 days old. An 'intruder' female was placed into an adjoining chamber the day before the observations, with the chambers isolated by barriers. Naïve intruders were aged 4-7 days and had been fed on concentrated honey solution. Experienced intruders were of a similar age as owners and had been presented with hosts during the first 4-5 days of adult life, but neither their resulting offspring nor the hosts' remains were present in the apparatus.

Intraspecific interactions between two naïve non-owners were set up and observed in a similar manner to owner-intruder experiments, except that a female aged 2-5 days, that had been fed on honey was placed into each of the two extreme chambers in the apparatus, and 10 hosts were placed in the central chamber.

In all experiments, the barriers were withdrawn during the day following set-up, allowing both females access to all three chambers and the hosts. Behaviours of both females were observed for 3 h following barrier withdrawal and the locations of the females within the apparatus were recorded every 15 min (scan sampling). Individual females were used in experiments only once to avoid confounding variables arising from prior experience of contests (Mesterton-Gibbons, 1999). A total of 130 interspecific owner-intruder interactions were observed (Table 3.1): 57 of these observations were made in this study and 73 derived from the prior work of Pérez-Lachaud *et al.* (2002). The current study also provided a total of 55 intraspecific interactions: 25 owner-intruder interactions (Table 3.2) and 30 naïve non-owner interactions (Table 3.3).



When direct interactions (pursuit and attempts to sting or bite) occurred during the 3 h observation period, the outcome of interactions was scored on the basis of the number of encounters won by each female. The female that won the majority of encounters (and possessed the hosts) or eventually paralyzed and/or killed the other female, was scored as the winner. However, direct aggressive interactions frequently did not occur during the observation period. In these cases, we continued the experiment overnight scoring the position and/or behaviour at 24 h (or for every subsequent 24 hours until an outcome was evident) after the beginning of the experiment.

As in Pérez-Lachaud *et al.* (2002), the influences of experience and species were explored using log-likelihood ratio tests (*G*-tests) of data in 2 x 2 contingency tables for comparing between rows in Table 3.1 or Table 3.2 and influence of ownership status was explored using two-tailed binomial tests for small samples with the null hypothesis that owners and intruders win equal numbers of contests (comparisons within rows of Table 3.1 or Table 3.2). The influence of body size in intraspecific non-owner interactions was studied using two-tailed binomial tests. The incidence of fatal fighting in inter- and intra-specific contests was compared using *G*-tests.

### **3.4 RESULTS**

#### **3.4.1 Interspecific interactions**

Interactions between previously unobserved species combinations comprised similar behaviours to those reported by Pérez-Lachaud *et al.* (2002). Owners generally remained near their hosts prior to barrier removal. About half of the intruders that entered following barrier withdrawal fed upon the hosts they encountered (30/57). Owner-intruder encounters generally involved no immediate agonistic response, but these often occurred during the observation period. Experienced intruders engaged more readily in direct contests with owners than did naïve intruders. Agonistic interactions consisted of pursuit, biting and stinging. Stinging often resulted in paralysis and death.

*Cephalonomia stephanoderis* was the most aggressive species, and *P. nasuta* the least. When *C. stephanoderis* had prior experience of hosts (i.e. when females were owners or experienced intruders), after the initial encounter, females immediately became more active and commonly attacked and attempted to paralyze their opponents within 10 minutes (12/17 interspecific contests, 5/10 intraspecific contests). *Cephalonomia hyalinipennis* females with prior experience of hosts generally inspected allospecifics several times before chasing, biting and attempting to sting them. Although initially less aggressive, once agonistic interactions commenced, *C. hyalinipennis* appeared to be as aggressive as *C. stephanoderis*. Both *C. stephanoderis* and *C. hyalinipennis* owners usually chased intruders throughout the apparatus, while *P. nasuta* owners usually did not (5/7, 14/20 and 6/30 replicates respectively, based only on results collected in this study). Experienced *C. stephanoderis* and *C. hyalinipennis* had usually laid eggs prior to the experiment, but experienced *P. nasuta* (owners and intruders) had generally paralyzed some hosts but not oviposited (43/57 replicates). We noticed that the least aggressive species, *P. nasuta*, has less flexibility of abdominal movement than the two *Cephalonomia* species and that it often adopted a static threatening/defensive posture, standing with its abdomen curved under its thorax with the stinger pointing forwards, but appeared unable to mount an active attack.

#### **3.4.1.1 Outcome of contests**

The outcomes of interspecific contests are summarized in Table 3.1. Our analysis combines data collected during this study with those reported by Pérez-Lachaud *et al.* (2002). In half of the twelve experimental scenarios, owners won significantly more contests than did intruders (Table 3.1, rows 1-5 & 7) and in two (rows 6 & 12) intruders won significantly more than owners.

In scenarios in which the owner was *C. stephanoderis*, owners won all, or nearly all, of the contests (Table 3.1, rows 1-4). When the owner was *C. hyalinipennis*, owners were significantly advantaged against naïve but not experienced *C. stephanoderis* or *P. nasuta* intruders (Table 3.1, rows 5-8), and lost significantly against experienced *C. stephanoderis* intruders (Table 3.1, row 6). In contrast, *P. nasuta* owners had no significant ownership advantage in individual contests (Table 3.1, row 9-12), and lost



Table 3.1 Outcomes of dyadic interspecific interactions

Row	Owner	Intruder	Intruder state	Contests won by prior owners					Contests won by intruders					Mean time to contest resolution (h)
				n	Total	P <sup>c</sup>	Intruder		Intruder not killed	Total	Owner		Owner not killed	
							killed				killed			
1 <sup>a</sup>	<i>C. stephanoderis</i>	<i>C. hyalinipennis</i>	naïve	12	11	0.006	6		5	1	0	1	20.7	
2 <sup>b</sup>			experienced	10	9	0.021	8		1	1	0	1	24.0	
3 <sup>a</sup>		<i>P. nasuta</i>	naïve	10	10	0.002	10		0	0	0	0	16.8	
4 <sup>b</sup>			experienced	10	10	0.002	10		0	0	0	0	12.9	
5 <sup>a</sup>	<i>C. hyalinipennis</i>	<i>C. stephanoderis</i>	naïve	14	12	0.013	10		2	2	1	1	40.2	
6 <sup>a</sup>			experienced	11	1	0.012	0		1	10	8	2	8.6	
7		<i>P. nasuta</i>	naïve	10	9	0.021	6		3	1	0	1	20.7	
8			experienced	10	8	0.109	5		3	2	0	2	79.1	
9 <sup>a</sup>	<i>P. nasuta</i>	<i>C. stephanoderis</i>	naïve	13	4	0.267	0		4	9	8	1	28.1	
10			experienced	10	3	0.344	0		3	7	6	1	41.7	
11		<i>C. hyalinipennis</i>	naïve	10	3	0.344	0		3	7	5	2	81.8	
12			experienced	10	1	0.021	0		1	9	6	3	83.6	

<sup>a</sup> Data collected by Pérez-Lachaud *et al.* (2002).

<sup>b</sup> Data are a combination of results obtained by Pérez-Lachaud *et al.* (2002) and the current study.

<sup>c</sup> Probabilities of  $\geq$  observed number of wins by owner (binomial test).

significantly against experienced *C. hyalinipennis* (Table 3.1, row 12).

When all contests involving a particular owner species were combined, *C. stephanoderis* and *C. hyalinipennis* owners won significantly more contests than did the intruders (*C. stephanoderis* won 40: lost 2, Binomial test,  $P < 0.001$ ; *C. hyalinipennis* won 30: lost 15, Binomial test,  $P = 0.036$ ) but *P. nasuta* owners lost significantly more contests than they won (won 11: lost 32, Binomial test,  $P = 0.002$ ).

Overall, when acting as intruders, both *C. stephanoderis* and *C. hyalinipennis* won and lost similar numbers of contests (*C. stephanoderis* won 28: lost 20, Binomial test,  $P = 0.312$ ; *C. hyalinipennis* won 18: lost 24, Binomial test,  $P = 0.441$ ) but *P. nasuta* intruders lost significantly more contests than they won (won 3: lost 37, Binomial test,  $P < 0.001$ ).

We explored the influence of intruder species on contest outcome by comparing across rows of Table 3.1. Experienced *C. stephanoderis* intruders were significantly more successful against *C. hyalinipennis* owners than were experienced *P. nasuta* intruders (comparison of rows 6 & 8:  $G_1 = 11.97$ ,  $P < 0.001$ ). Comparisons of other scenarios indicated that the species of the intruder had no significant influence on contest outcome, whether intruders were naïve (rows 1 & 3:  $G_1 = 1.25$ ,  $P > 0.05$ ; rows 5 & 7:  $G_1 = 0.10$ ,  $P > 0.05$ ; rows 9 & 11:  $G_1 < 0.01$ ,  $P > 0.05$ ) or experienced (rows 2 & 4:  $G_1 = 1.44$ ,  $P > 0.05$ ; rows 10 & 12:  $G_1 = 1.30$ ,  $P > 0.05$ ): in these cases *C. stephanoderis* and *C. hyalinipennis* owners tended to win, and *P. nasuta* owners tended to lose.

The influence of intruder experience was explored by comparing across rows of Table 3.1. One comparison indicated an effect of intruder experience on contest outcome: experienced *C. stephanoderis* won significantly more often than naïve females when intruding on *C. hyalinipennis* owners (rows 5 & 6:  $G_1 = 16.432$ ,  $P < 0.001$ ). In all five other comparisons, no effects of intruder experience were detected (rows 1 & 2:  $G_1 < 0.01$ ,  $P > 0.05$ ; rows 3 & 4:  $G_1 < 0.01$ ,  $P > 0.05$ ; rows 7 & 8:  $G_1 = 0.40$ ,  $P > 0.05$ ; rows 9 & 10:  $G_1 < 0.01$ ,  $P > 0.05$ ; rows 11 & 12:  $G_1 = 1.29$ ,  $P > 0.05$ ).



When both *C. stephanoderis* and *C. hyalinipennis* had prior experience of hosts, *C. stephanoderis* won more often, as both owner and intruder and hence ownership did not influence contest outcome (comparison of rows 2 & 6:  $G_1 < 0.01$ ,  $P > 0.05$ ). Similarly, contests between experienced individuals of *C. hyalinipennis* and *P. nasuta* showed no influence of ownership as *C. hyalinipennis* generally won (rows 8 & 12:  $G_1 = 0.40$ ,  $P > 0.05$ ). In contrast, experienced *C. stephanoderis* achieved greater success against experienced *P. nasuta* when in the role of owner (rows 4 & 10:  $G_1 = 4.69$ ,  $P < 0.05$ ).

*Cephalonomia stephanoderis* thus appears to be the most successful competitor in interspecific interactions: this species nearly always won contests when in the role of owner, defeated *C. hyalinipennis* owners when experienced and generally defeated *P. nasuta* owners irrespective of experience. *Prorops nasuta* is the least successful competitor as it usually lost contests, whether in the role of owner or intruder.

### 3.4.2 Intraspecific interactions

The suite of behaviours observed in intraspecific interactions involving at least one experienced female were the same as in interspecific interactions, with the exception that attempted stinging did not result in paralysis and death. In accord with observations of interspecific interactions, contests between *C. stephanoderis* dyads were generally the most aggressive and those between pairs of *P. nasuta* the least. Both *C. stephanoderis* and *C. hyalinipennis* owners usually chased intruders throughout the apparatus, while *P. nasuta* owners did not (8/10, 9/10 & 0/5 replicates respectively). Experienced *C. stephanoderis* and *C. hyalinipennis* (owners and intruders) had usually laid eggs prior to the experiment (15/15 & 14/15 replicates respectively), but while experienced *P. nasuta* (owners) had generally paralyzed some hosts, they had only oviposited in 1/5 replicates. Experienced intruders of *C. hyalinipennis* chased owners throughout the apparatus in 3/5 replicates; on 2/3 of these occasions the contest began with the owner chasing the intruder.

#### 3.4.2.1 Outcome of intruder-owner contests

In *C. stephanoderis*, owners won all contests against conspecific intruders (Table 3.2), constituting a significant advantage associated with ownership (rows 1 & 2 combined: Binomial test:  $P = 0.002$ ); hence there was no significant effect of size or intruder experience on contest outcome (rows 1 & 2:  $G_1 < 0.01$ ,  $P > 0.05$ ).

*Cephalonomia hyalinipennis* owners won all intraspecific contests against naïve intruders, but lost 3/5 contests against experienced intruders (Table 3.2), indicating that experience is a significant advantage to intruders (comparison of rows 3 & 4:  $G_1 = 5.49$ ,  $P < 0.05$ ). On each of the three occasions that an experienced intruder defeated a conspecific owner, the intruder was the larger individual (head width). *Cephalonomia hyalinipennis* owners won two contests against experienced conspecific intruders: on one occasion the owner was larger and on the other the competitors were the same size. When intruder experience was ignored, the size difference between contestants had a (marginally) significant effect on contest outcome ( $G_1 = 3.85$ ,  $P \approx 0.05$ ), larger owners tending to win against smaller intruders. In *C. hyalinipennis* there was no overall advantage to ownership (rows 3 & 4 combined: Binomial test:  $P = 0.344$ ), due to the loss of three contests against experienced intruders.

*Prorops nasuta* owners won one and lost one contest of five against a naïve conspecific intruder (Table 3.2). The other three contests had no discernable winner, neither female excluding its competitor from the hosts after 168 hours: in these cases agonistic interactions were not observed. No results on contests involving experienced *P. nasuta* intruders were collected.

#### 3.4.2.2 Outcome of contests between naïve females

Agonistic behaviour was relatively rare (*C. stephanoderis* 5/10 contests, *C. hyalinipennis* 3/10 contests, *P. nasuta* 0/10 contests) during the 3 h observation period. Individuals tended to explore the apparatus, inspect each other with their antennae without aggression, and inspect, paralyze and feed on hosts. There was not always a discernable winner in intraspecific contests (Table 3.3), but most contests



Table 3.2 Outcomes of dyadic intraspecific interactions

Row <sup>a</sup>	Owner	Intruder state	n	Contests won by prior owners			Contests won by intruders			Mean time to contest resolution (h)
				Total	Intruder killed	Intruder not killed	No winner	Owner killed	Owner not killed	
1	<i>C. stephanoderis</i>	naïve	5	5	0	5	0	0	0	7.2
2		experienced	5	5	0	5	0	0	0	7.2
3	<i>C. hyalinipennis</i>	naïve	5	5	0	5	0	0	0	3
4		experienced	5	2	0	2	0	0	3	3
5	<i>P. nasuta</i>	naïve	5	1	0	1	3	0	1	120

<sup>a</sup> Experiments were performed on 5 of the 6 possible combinations of owner versus naïve or experienced intruder for three species.

Table 3.3 Outcomes of dyadic intraspecific non-owner contests

Row	Species	n	Contests won by		Contests involving same sized individuals	Contests with no winner	Mean time to contest resolution (h)
			Larger female	Smaller female			
1	<i>C. stephanoderis</i>	10	4	2	2	2	37.1
2	<i>C. hyalinipennis</i>	10	6	1	2	1	70.3
3	<i>P. nasuta</i>	10	2	4	1	3	82.9



were ultimately resolved (Table 3.3). In contests that were resolved, there was no significant advantage associated with being the larger contestant in any of the three species (Table 3.3: *C. stephanoderis*,  $n = 7$ , Binomial test:  $P = 0.688$ ; *C. hyalinipennis*  $n = 8$ , Binomial test:  $P = 0.125$ ; *P. nasuta*  $n = 7$ , Binomial test:  $P = 0.687$ ).

### 3.4.3 Comparison of inter- and intraspecific interactions

Fatal fighting was not observed in any of the 55 intraspecific contests but did occur in 89/130 (68.5%) interspecific contests: this overall difference is highly significant ( $G_1 = 94.13$ ,  $P < 0.001$ ). There were no fatalities in any of the contests, whether intra- or interspecific, won by *P. nasuta*. The significance of the overall difference is due to the high prevalence of fatalities in contests won by *C. stephanoderis* and *C. hyalinipennis* (comparisons of fatalities in intra- and interspecific contests won by *C. stephanoderis*,  $G_1 = 28.87$ ,  $P < 0.001$ , and *C. hyalinipennis*,  $G_1 = 13.96$ ,  $P < 0.001$ ).

Since survival during conspecific and allospecific contests differed within *C. stephanoderis* and *C. hyalinipennis*, we compared the number of attacks made by owners in each type of contest, but were unable to analyze these differences statistically due to small and unequal sample sizes. Using this criterion, *C. hyalinipennis* was about ten times more aggressive in intra- than interspecific contests (mean and range: intra- 15.1, 0 - 40,  $n = 10$ ; inter-specific 1.7, 0 - 16,  $n = 20$ ). In *C. stephanoderis* the difference was much smaller (mean and range: intra- 11.9, 0 - 42,  $n = 10$ ; inter-specific 8.42, 0 - 26  $n = 7$ ). There was also little difference for *P. nasuta* (mean and range: intra- 0, 0,  $n = 5$ ; inter-specific 1.0, 0 - 8,  $n = 30$ ).

## 3.5 DISCUSSION

Extending investigations to all combinations of owner-intruder interspecific interactions between *C. stephanoderis*, *C. hyalinipennis* and *P. nasuta* dyads has confirmed that these species actively and directly compete for hosts, with the death of the loser a common event. Also as found by Pérez-Lachaud *et al.* (2002), *C. stephanoderis* is generally the most successful competitor. Examination of intraspecific interactions revealed that although agonistic contests often occur,

fatalities do not. We discuss these results first in terms of contest behaviour and then in terms of the consequences of such agonistic interactions for the coexistence of natural enemy species and biological control.

Direct aggressive interactions between females have now been documented in several bethylid species (Hardy and Blackburn, 1991; Petersen and Hardy, 1996; Stokkebo and Hardy, 2000; Pérez-Lachaud *et al.*, 2002) and in a few other hymenopterans (e.g. Field and Calbert, 1998, 1999; Moore and Greeff, 2003; Tarpy and Fletcher, 2003). While behavioural defence against allospecifics occurs (Hardy and Blackburn, 1991; Mills, 1991; Pérez-Lachaud *et al.*, 2002), most reports concern intraspecific interactions. Within species belonging to the Parasitica, female-female contests generally do not result in escalation involving attack and retaliation. For example, *Pachycrepoideus vindemmiae* Rondani (Hym.: Pteromalidae) females generally have very brief interactions with minimal physical contact and only occasional biting (Goubault *et al.*, 2005) and Moore and Greeff (2003) report that contests in the fig wasp *Platyscapa awekei* Wiebes (Hym.: Agaonidae) do not result in injurious fighting. Contests are generally decided by size difference, the larger individuals prevailing by lifting their competitors off the surface using their mandibles whilst ovipositing. In contrast, in aculeate species the literature suggests that contests are generally more aggressive. Full escalation, e.g. fights involving grappling, is common between conspecific females of *Anoplius viaticus* (L.) (Hym.: Pompilidae; Field, 1992), *Goniozus nephantidis* (Muesebeck) (Hym.: Bethyridae; Petersen and Hardy, 1996), *C. stephanoderis* and *C. hyalinipennis* (this study). In *C. stephanoderis* and *C. hyalinipennis* attempts to sting opponents are common yet there were no fatalities from intraspecific fighting. In *G. nephantidis*, fatal fighting was long thought to be absent, but one instance has recently been observed (Chapter 5), while in contests between honey bee queens (*Apis mellifera* L., Hym.: Apidae) fatalities are common (Tarpy and Fletcher, 2003). It may be that venom injection does not generally occur when conspecifics attack each other with their stingers or that, in some species, conspecifics are immune to each other's venom.

In contrast to intraspecific interactions, fatalities are common in interspecific interactions between *C. stephanoderis*, *C. hyalinipennis* and *P. nasuta*. Evolutionary game theory predicts that fatal fighting will occur only when resources are severely



limiting (Grafen, 1987; Enquist and Leimar, 1990; Mesterton-Gibbons, 1992; Pérez-Lachaud *et al.*, 2002). under other conditions the risk of injury leads individuals to avoid direct contests or settle them without aggression. If, however, some individuals are able to engage in aggressive contests with little risk of injury while fighting with and killing their competitors, fatal fighting may commonly occur even when resource limitation is not severe (Pérez-Lachaud *et al.*, 2002). The lack of fatal fighting between conspecifics suggests that resources are not an important limiting factor (e.g. due to low parasitoid population densities relative to the CBB: Damon, 1999). This is in contrast to the suggestions of Pérez-Lachaud *et al.* (2002), who only studied interspecific contests. It now seems likely that fatal fighting between *C. stephanoderis* and *P. nasuta* females is due to *P. nasuta* females being unable to injure or kill *C. stephanoderis*, while *C. stephanoderis* can kill *P. nasuta* relatively easily (see below). The interpretation of fatal fighting between *C. stephanoderis* and *C. hyalinipennis* is hampered by the fact that these species have been geographically separated for much of their evolutionary history.

The success of *C. stephanoderis* in interspecific contests could result from several factors, such as temperature (Infante *et al.*, 2001a; Pérez-Lachaud *et al.*, 2002), egg maturation state and morphology. In our experiments, *P. nasuta* often did not lay eggs on hosts. *Prorops nasuta* tends to have a longer pre-oviposition period than the two *Cephalonomia* species (Abraham *et al.*, 1990). We observed that *P. nasuta* exhibits distinctly different paralysis and oviposition behaviour from the two *Cephalonomia* species. These *Cephalonomia* have abdomens that can be flexed in several places while *P. nasuta* appears only able to bend the tip of the abdomen near the stinger and the narrowest part of the abdomen (the 'waist'). When paralyzing a host, both *Cephalonomia* species mount it whilst bending their abdomen around to sting it (*C. stephanoderis*: Lauzière, Pérez-Lachaud and Brodeur, 2000b; *C. hyalinipennis*: T.P. Batchelor, pers. obs.). In contrast, *P. nasuta* does not mount its hosts prior to stinging: it stands near to the host and then bends its abdomen forwards beneath its thorax before advancing towards the host and attempting to insert its stinger (T.P. Batchelor, pers. obs.). Within a coffee berry, stinging may be facilitated by the surrounding bean tissue keeping the host in position. In the apparatus used in our experiments, where hosts are not fixed in position, this method often resulted in unsuccessful attempts at paralysis during which *P. nasuta* females pushed the host

around the arena. These differences in morphology and behaviour are likely to lead to a weaker distinction between experienced and inexperienced females in *P. nasuta* than in *Cephalonomia* in our experiments, and thus provide a candidate explanation for the lack of an influence of 'ownership' in *P. nasuta*. Differences in abdominal morphology are also likely to affect the ability to sting opponents during agonistic interactions, which likely accounts for the lack of fatalities inflicted by *P. nasuta* females, the low levels of aggression exhibited by this species and its overall low level of success in contests.

The occurrence of fatal fighting between allospecifics but not conspecifics implies that interspecific competition is stronger than intraspecific competition, and thus that coexistence of these species may be unlikely (Hassell, 2000, p120). If contest behaviour were the primary determinant of the strength of competitive interactions, we would expect *C. stephanoderis* eventually to exclude *P. nasuta* and *C. hyalinipennis* from the coffee agro-ecosystem. Indeed, in our laboratory, *C. stephanoderis* commonly invades *P. nasuta* cultures, but not vice versa (T.P. Batchelor and J.F. Barrera, pers. obs.). However, a range of factors may act to diminish the importance of interspecific contest behaviour, and thus promote coexistence. In Africa, the natural ranges of *C. stephanoderis* and *P. nasuta* may not greatly overlap (Murphy and Moore, 1990). Where they have been found together, *C. stephanoderis* is more prevalent (Moore and Prior, 1988; Borbon-Martinez, 1989; Barrera, 1991; Vega *et al.*, 1999; Komlan, 2004). Even when multiple species co-occur and exploit the same resource, such as a population of CBB, species-independent aggregation of individuals over resource units (Shorrocks and Sevenster, 1995) or a metapopulation structure (Lei and Hanski, 1998; Hassell, 2000) may allow competitively inferior species to persist.

Persistence of *P. nasuta* in nature or agro-ecosystems may also be promoted by contest avoidance. Once inside a berry, *P. nasuta* females have been observed to block the entrance with the bodies of dead adult borers (Infante *et al.*, 2001a). While such blockages may prevent competitors from entering berries and thus reduce the incidence of contests, they may also prevent the successful emergence of maturing parasitoids. During investigations involving dissections of coffee berries, we have on several occasions found large numbers of dead adult *P. nasuta* inside their natal



berry; emergence may not have been possible due to such a blockage (T.P. Batchelor, I.C.W. Hardy and J.F. Barrera, unpublished data). These companion studies, conducted in parallel and in exactly the same environmental conditions as this contest work, suggest that *P. nasuta* cultures have the highest net population growth rates of the three species.

Persistence of *C. hyalinipennis*, despite being inferior to *C. stephanoderis* in behavioural contests, is likely to be promoted by the fact that it has the ability to develop as a hyperparasitoid of pupal *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud *et al.*, 2004: Chapter 2), and by producing larger clutch sizes than *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud and Hardy, 2001). In New World coffee agro-ecosystems falling within its native range, *C. hyalinipennis* populations may also be able to persist via the exploitation of alternative hosts (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 2001).

To date, only one theoretical study has modelled the population dynamics of the CBB and its parasitoids (Gutierrez *et al.*, 1998). This model considered the regulation of CBB populations afforded by *C. stephanoderis*, *P. nasuta* and the eulophid endoparasitoid *Phymastichus coffea* LaSalle, but not *C. hyalinipennis*. Only *P. coffea* was predicted to exert significant control on CBB. Due to poor numerical responses, *C. stephanoderis* and *P. nasuta* were each predicted to exert poor control when released alone. According to this model, releasing these two species together would lead to even less control due to interspecific competition (although releasing either or both in conjunction with *P. coffea* may improve the control exerted by this Eulophid alone). Competition between *C. stephanoderis* and *P. nasuta* was, however, assumed to be symmetrical: our results indicate that asymmetrical competition is more likely, *C. stephanoderis* being the stronger competitor. Incorporating such observations, and the consideration of *C. hyalinipennis*, into this modelling framework would likely affect the predicted dynamics and perhaps generate different strategic recommendations.

In summary, this study indicates that coexistence of *C. stephanoderis*, *C. hyalinipennis* and *P. nasuta* in coffee agro-ecosystems may be compromised by aggressive interspecific interactions, particularly because negative interspecific

interactions are stronger than intraspecific interactions. The occurrence of interference competition via contest behaviour is likely to lead to the long-term establishment of at most one of these species as a biological control agent, unless the relative importance of interspecific interactions is reduced by factors such as lack of host limitation, alternative host use, contest avoidance or differences in parasitoid reproductive strategy. This conclusion contrasts with that of Infante *et al.* (2001a), which was based on medium-term population trends in laboratory microcosms, without observation of female-female interactions in the immediate vicinity of hosts. Although the species we studied showed the full behavioural repertoire associated with agonistic contests, and fatalities were common, the importance of interspecific interactions in the field remains unevaluated and population-dynamic models that incorporate asymmetric interspecific competition between these species have yet to be developed.



# CHAPTER 4 - INTERACTIONS AMONG BETHYLID PARASITOID SPECIES ATTACKING THE COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE)<sup>1</sup>

## 4.1 ABSTRACT

The question of whether biological control is most likely achieved by deploying single or multiple species of biological control agents is much debated. While utilizing several natural enemies may enhance control, there is also the potential for disruptive inter-specific interactions. Such interactions may be studied in the laboratory by focusing on the details of the interactions themselves and attempting to infer population level consequences from their sum, or by focusing more directly on the overall effects on natural enemy populations: we term these approaches ‘reductionist’ and ‘holistic’. Here we conduct a holistic laboratory study on interactions between three species of parasitoid wasps that are parasitoids of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae): *Cephalonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead and *Prorops nasuta* Waterston (all Hymenoptera: Bethylinidae). We find evidence for both intra- and inter-specific resource competition. Interactions between *C. stephanoderis* and *P. nasuta*, both indigenous to Africa, appear to be approximately symmetrical, while *C. hyalinipennis*, naturally found in the coffee plantations of Chiapas, Mexico, may exert a disruptive influence. *Cephalonomia hyalinipennis* also has a low population growth rate. We now consider it to be a detrimental invader of the Mexican coffee agro-ecosystem that should not be encouraged by augmentative release or introduced into other regions. Overall, the most successful species, in terms of both emergence and female production, was *P. nasuta*. We compare these results with those from prior reductionist and holistic studies, and with observations on patterns of establishment of these bethylid species in the field. Given that it is increasingly clear that disruptive inter-specific interactions are generally common when multiple

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species are deployed in biological control, screening of potential agents should consider such interactions alongside the more 'traditional' focus on host specificity.



## 4.2 INTRODUCTION

There has been much debate as to whether better biological control is achieved by releasing single or multiple species of natural enemies (Watt, 1965; Hågvar, 1989; Myers *et al.*, 1989; Ehler, 1990; Murdoch and Briggs, 1996; Collier *et al.*, 2002; Denoth *et al.*, 2002). Denoth *et al.* (2002) recommend restraint in the introduction of multiple species due to detrimental interspecific interactions, such as intra-guild predation (Rosenheim *et al.*, 1993; Rosenheim *et al.*, 1995; Ferguson and Stiling, 1996; Holt and Polis, 1997; Snyder and Ives, 2001; Collier *et al.*, 2002; Hunter *et al.*, 2002; Pérez-Lachaud *et al.*, 2004: Chapter 2) or that a more effective species, in terms of host suppression, may be competitively inferior to less effective natural enemies and thus fail to establish (Turnbull and Chant, 1961; Force, 1974; Leveque *et al.*, 1993; Hunter *et al.*, 2002). However, some theoretical and field investigations have indicated that multiple natural enemies can, under certain conditions, coexist and may be desirable in that they can additively suppress pest populations, for example by attacking different life-history stages of the host (Huffaker, Messenger and Debach, 1971; Ehler, 1978; Hassell, 1978; Gutierrez *et al.*, 1988; Kindlmann and Ruzicka, 1992; Gutierrez *et al.*, 1998).

The above concerns apply to virtually all biological pest control programs because virtually all pests have more than one species of natural enemy (e.g. Mason and Huber, 2002) but, because different pest species have different biologies and different arrays of natural enemies, pest control solutions need largely to be developed on a case-by-case basis. Here we address the issue of how many, and which, of three species of natural enemy, all bethylid wasps, are most likely to provide biological control of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Our study is an attempt to resolve an interpretational disparity between several prior studies that drew opposing conclusions from different types of experiments designed to evaluate inter-specific competition. We begin with a summary of the CBB control problem and of the prior studies concerning the coexistence of multiple natural enemies of the CBB.

#### 4.2.1 Biological control of the CBB and coexistence of natural enemy species

The coffee berry borer is indigenous to Africa but has now spread throughout the coffee growing world and is considered the most serious pest of coffee worldwide (Baker, 1999; Damon, 2000; Baker *et al.*, 2002). The CBB arrived in southern Mexico (Chiapas) in 1978 and now causes severe reductions in Mexican coffee production (Baker, 1984; Barrera *et al.*, 1990b; Infante *et al.*, 2001b). Control programs in Chiapas, incorporating Integrated Pest Management (IPM), have included the release of two African natural enemies of the CBB, the parasitoid wasps *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (both Hymenoptera: Bethyridae). Neither species has achieved economic control following a classical biological control strategy; *C. stephanoderis* has widely established in the Soconusco region of Chiapas but remains in low numbers (Barrera, 1994; Damon, 1999; Damon and Valle, 2002) and *P. nasuta* has not persisted for more than 15 months (Infante *et al.*, 2001b; Infante *et al.*, 2003). A further bethyrid, *Cephalonomia hyalinipennis* Ashmead, which is indigenous to South and North America and Europe (Gordh and Móczár, 1990), has been discovered naturally attacking CBB in coffee plantations in the Soconusco region (Pérez-Lachaud, 1998). The biologies of each of these three species have been investigated in the context of their potential as agents of biological control (e.g. Barrera *et al.*, 1989; Abraham *et al.*, 1990; Barrera *et al.*, 1990a; Barrera *et al.*, 1993; Infante and Luis, 1993; Damon, 1999; Pérez-Lachaud and Hardy, 1999, 2001; Infante, 2000; Lauzière *et al.*, 2001a). Recent studies have focused on interactions between these species to evaluate whether they are likely to coexist in agro-ecosystems and the influence of interspecific interactions on host population suppression (Gutierrez *et al.*, 1998; Infante *et al.*, 2001a; Pérez-Lachaud *et al.*, 2002, 2004: Chapter 2; Batchelor *et al.*, 2005: Chapter 3).

The first laboratory evaluation of interspecific interactions was carried out by Infante *et al.* (2001a) using medium-term population trends in laboratory microcosms using coffee berries naturally infested by the CBB. Competition experiments were conducted between *C. stephanoderis* and *P. nasuta* (but not *C. hyalinipennis*) released at a range of densities (5 berries per parasitoid or 1, 2, 3 or 5 parasitoids per berry) and temperatures (18°C, 29°C or an alternation between these temperatures) into 1 L jars containing 10-50 infested berries. Allospecific competitors were



released either simultaneously or one species was released 10 days after the other. One month after the initial release, any adult parasitoids observed outside of the berries were collected, identified and counted. Such collections ceased after a further 2.5 months. The outcome of any resource competition was thus determined according to progeny production, over a period that would allow between 2 and 5 parasitoid generations. When competitor species were released simultaneously at  $>18^{\circ}\text{C}$  *C. stephanoderis* generally produced more progeny than *P. nasuta*, but at  $18^{\circ}\text{C}$  *P. nasuta* was more successful. When release was not simultaneous, the species that was released first generally produced more progeny, with the exception that at  $18^{\circ}\text{C}$  *P. nasuta* out-produced *C. stephanoderis* irrespective of temporal priority. Infante *et al.* (2001a) found no evidence of interference competition from this experiment (chases and fights between adult females outside of the berries were never observed). They concluded that interspecific competition between *C. stephanoderis* and *P. nasuta* is mainly of the indirect, exploitative kind, and that the deployment of both species against the coffee berry borer is sound. For the purposes of the present study, we refer to this experimental approach as ‘holistic’ because the focus is on overall ‘output’ rather than on the mechanisms that lead to particular patterns on progeny production and, ultimately, coexistence.

A different, more mechanistic, approach to evaluating competition has been taken by several other studies that have explored factors influencing the outcomes of interspecific interactions. We refer to this as the ‘reductionist’ approach because the focus is on components of the competitive process: it is implicit that patterns of progeny production and coexistence can be inferred from their sum. Dyadic contests for host resources between adult females within a confined space representing an individual berry were studied by Pérez-Lachaud *et al.* (2002) and Batchelor *et al.* (2005; Chapter 3). Interactions between and within *C. stephanoderis*, *C. hyalinipennis* and *P. nasuta*, with or without one individual having temporal priority (‘ownership’) or ovipositional experience, were observed at  $28 \pm 2^{\circ}\text{C}$  for 3 hours (with subsequent daily inspection). Outcome of competition was determined according to behaviour and mortality, rather than progeny production. These experiments showed that, in all three species, prior owners exhibit active resource defence against ‘intruders’ that attempt to take possession of the resource. In intraspecific contests, aggressive behaviours but no fatalities were observed. In

interspecific interactions, losing females were killed in 69% of contests. *Cephalonomia stephanoderis* was the most successful competitor in interspecific interactions as it nearly always won contests when given prior access to resources. usually defeated *C. hyalinipennis* owners when having ovipositional experience and generally defeated *P. nasuta* owners irrespective of experience. *P. nasuta* was the least successful competitor, usually losing contests, whether in the role of owner or intruder. The occurrence of fatal fighting between allospecifics but not conspecifics implies that interspecific competition is stronger than intraspecific competition, and thus that ecological coexistence of these species may be unlikely (e.g. Hassell [2000], p120).

Further to these direct and competitive interspecific contest interactions, aspects of intra-guild predation have been reported between these bethylid species. Infante *et al.* (2001a) provided adult female *C. stephanoderis* and *P. nasuta* with developing allospecifics in the laboratory and observed limited interspecific ovicide and larvicide. *Cephalonomia stephanoderis* has also been reported to feed on *C. hyalinipennis* larvae and *C. hyalinipennis* feeds on immature *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud *et al.*, 2004: Chapter 2). Furthermore, *C. hyalinipennis* can also develop as a hyperparasitoid of *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud *et al.*, 2004: Chapter 2).

While fighting, predation and facultative hyperparasitism constitute interspecific interference competition (species directly reduce each other's survival [Griffith and Poulson, 1993]), suggesting coexistence is unlikely, the importance of these interactions in the field remains unevaluated. Furthermore, there may be additional important yet unidentified aspects of interactions between these species. The reductionist approach by itself may thus not readily lead to robust recommendations concerning multi-species interactions in agro-ecosystems. In contrast, the holistic approach may point to likely outcomes but not to causality. In the case of CBB biocontrol strategy, the holistic approach has lead to the recommendation of multi-species release while reductionist studies have cautioned against this.

Here we conduct laboratory experiments that combine elements of both approaches. Inter- and intra-specific interactions are studied using naturally infested berries with



outcomes evaluated in terms of progeny production (holistic). These interactions, however, occur between small numbers of females within a restricted number of berries, for a single generation, such that the outcomes may more readily be interpreted in terms of trophic and contest interactions (reductionist) than when larger numbers of berries and parasitoids are used in each experimental replicate.

#### 4.3 MATERIALS AND METHODS

Our experiments used coffee berries naturally infested with the CBB. All berries used were *Coffea canephora* (robusta variety) and were collected from various localities around Tapachula (Chiapas, Mexico) between early January and early March in 2003 and 2004. Berries were selected for experiments on the basis of evidence of infestation by CBB and the presence of suitable host stages. Berries that contained suitable host stages were dark brown or black and had dark dust around holes originally bored by CBB adults on entry. To assess the likely content of berries thus selected, we dissected five samples of 20-50 berries, from throughout the experimental period, and recorded the presence and life-history stages of any CBB inside. Of the 130 berries dissected, all contained at least one adult CBB and 128 contained hosts at a suitable stage (eggs, larvae, prepupae and pupae) for oviposition by bethylid parasitoids. There was, however, significant between-sample variation in the number of suitable CBB per berry (log-linear ANOVA corrected for overdispersion,  $F_{4,125} = 12.64$ ,  $P < 0.001$ ): the average number per sample ranged from 15.34 (SD  $\pm 6.1$ ,  $n = 50$ ) to 38.2 (SD  $\pm 19.3$ ,  $n = 20$ ). Even the lower of these host availabilities should provide abundant reproductive opportunities for the bethylid species studied. None of the dissected berries contained parasitoids: we thus assumed that there were no parasitoids already present in the berries used in experiments (prior data on field collections also support this assumption: Damon [1999] found parasitoids in only 1 in every 1136 CBB infested berries dissected).

Parasitoids were cultured at El Colegio de la Frontera Sur (Tapachula). The *P. nasuta* stock was derived from insects reared in Brazil in 1992 and the *C. stephanoderis* stock from insects collected in Togo in 1988. The *C. hyalinipennis* culture was initiated in 1997 from material collected in the Soconusco region (Mexico) (Pérez-

Lachaud, 1998). Experiments were conducted during two periods: late January to mid-April 2003 and mid-January to mid-May 2004.

Each experimental replicate was carried out using a glass tube (1.5 cm diameter · 7.3 cm height) closed with nylon gauze held in place with a flexible plastic ring. The number of berries and the number and species of wasps placed into each tube was varied. A tube contained one, two or three berries and one, two or three ‘foundress’ female parasitoids (no male wasps were introduced into any tubes). Some tubes with multiple foundresses contained only one species of parasitoid; we refer to these as intra-specific competition replicates. The remainder of the multiple foundress replicates evaluated inter-specific competition, and contained wasps belonging to either two or three of the bethylid species studied. In multiple-female replicates, parasitoids were introduced simultaneously to avoid priority effects (Petersen and Hardy, 1996; Infante *et al.*, 2001a; Batchelor *et al.*, 2005: Chapter 3).

The initial combinations of berries and wasps used in intra-specific competition replicates are shown in Table 4.1, comprising nine possible combinations of parasitoids and berries for each species. There were at least 10 replicates of each combination (500 overall, Table 4.1); a high degree of replication was chosen due to the significant variation in the number of suitable CBB per berry in pilot samples (see above). The initial 12 combinations and numbers of replicates (195 overall) used to evaluate inter-specific competition are shown in Table 4.2. Taken together these combinations allowed us to control for effects of density on parasitoid production and to compare between intra- and inter-specific competition replicates.

The prepared tubes were then kept at ambient temperature ( $27.5 \pm 3.5^{\circ}\text{C}$ ) and  $62.5 \pm 32.5\%$  relative humidity. They were kept in darkness for the first 18 days and then transferred to natural daylight timings (approx. 11:13 light: dark photoperiod). Each replicate was inspected daily from the time of parasitoid introduction until parasitoid emergence was thought to be complete. All emerging parasitoids were collected and identified to species and gender. There were usually several CBB adults found dead outside of the berries within a tube, but we did not record their numbers. Berries were dissected and inspected for unemerged parasitoids 14 days after the first parasitoid emerged from a given tube, except when emergence continued beyond 14



days in which case we dissected berries after the first two days with no parasitoid emergence. The production of female parasitoids of each species from each tube was calculated as the total unemerged plus emerged females minus the number of foundress females introduced. We focus exclusively on the production of females, rather than that of both sexes, since it is the number of female progeny that most influences host-parasitoid population biology (parameter  $c$  in classical host-parasitoid population dynamic models, e.g. Hassell, 2000, p10; see also Pérez-Lachaud and Hardy, 2001).

#### 4.3.1 Statistical analysis

Data were analyzed using generalized linear modelling (in Genstat version 7.2.0.208 and GLIM version 4.0), which allows parametric analysis of data with non-normally distributed error variances without prior transformation. Proportional data (replicates with emergences) were explored using logistic analyses assuming binomially distributed residuals (Crawley, 1993; Wilson and Hardy, 2002) and significance was assessed by change in deviance,  $G$  (which approximates  $\chi^2$ ). Count data (number of emerging females per replicate) were explored using log-linear analyses in which the dispersion parameter was estimated empirically to take overdispersion (variances greater than the mean leading to non-Poisson distributed residuals) into account, with significance assessed using  $F$ -ratio tests (Crawley, 1993).  $G$ -tests (log-likelihood ratio tests) were used to analyze data in contingency tables. Data on number of emerging females per foundress were square-root transformed prior to standard parametric analysis (that assume normally distributed residuals) to stabilize residual variances. Our general approach was thus to use, where possible, parametric analyses in which the assumed distribution of residuals was matched to the data rather than transforming data to fit standard assumptions. Assumed error distributions and variances were checked using residual and normality plots (e.g. Wilson and Hardy, 2002). Further, we adopted the top-down approach (i.e., stepwise backward analysis) in which significance is assessed when explanatory variables are removed from an initially complex model (containing all main effects and interaction terms of interest) to obtain a parsimonious statistical description.

Possible Type I errors due to multiple comparisons (e.g. Quinn and Keough, 2002, p48-50) were controlled for using the false discovery rate (FDR) procedure of Benjamini and Hochberg (1995), which retains more statistical power than Bonferroni-based methods. All statistical comparisons were treated as one 'family' of tests and the family-wide  $\alpha$ -value adopted was 0.05. After using this procedure, all P-values  $> 0.011$  were no longer considered to represent genuine underlying relationships: possible Type I errors were thus exposed in 9 cases. However, because multiple comparisons methods, including the FDR, are inherently conservative (possibly generating Type II errors) we also indicate the initial interpretations in these cases.

Analysis of proportion of replicates with emergence included all replicates prepared for each experiment, but subsequent analyses of species emergence, number of emerging females per replicate and number of emerging females per foundress were undertaken on the subset of replicates from which parasitoids emerged.

## 4.4 RESULTS

### 4.4.1 Emergence from berries

Parasitoid progeny emerged in 63.4% of intra-specific competition replicates and 70.4% of inter-specific competition replicates (Tables 4.1 & 4.2). Among intra-specific competition replicates, there was significant inter-specific variation in the proportion of replicates from which progeny emerged ( $G_2 = 25.11$ ,  $P < 0.001$ ,  $n = 500$ ). This was due to the relatively frequent emergence of *P. nasuta* (Table 4.1): the proportion of emergence of the two *Cephalonomia* species did not differ significantly ( $G_1 = 0.61$ ,  $P = 0.79$ ). The proportion of emergence was unaffected by the number of foundresses ( $G_2 = 1.54$ ,  $P = 0.215$ ) and the number of berries provided ( $G_2 = 0.53$ ,  $P = 0.588$ ).

Similar patterns were found for inter-specific competition replicates: emergence from replicates with two allospecific foundresses was unaffected by the number of berries ( $G_2 = 1.24$ ,  $P = 0.29$ ) but varied according to species composition ( $G_2 = 5.26$ ,  $P = 0.005$ ,  $n = 159$ ), again due to the relatively frequent emergence from replicates



**Table 4.1 Initial conditions of, and outcomes from, replicates focussing on intra-specific interactions**

Initial conditions			Replicates	Outcomes	
Species	Number of foundresses	Number of berries		Replicates with emergence	
				Number	%
<i>C. stephanoderis</i>	1	1	19	11	57.9
	2	1	34	13	38.2
	3	1	21	10	47.6
	1	2	23	10	43.5
	2	2	21	10	47.6
	3	2	17	11	64.7
	1	3	21	10	47.6
	2	3	23	12	52.2
	3	3	26	11	42.3
	<i>C. stephanoderis</i> totals		205	98	47.8
<i>C. hyalinipennis</i>	1	1	20	10	50.0
	2	1	23	10	43.5
	3	1	17	12	70.6
	1	2	23	10	43.5
	2	2	19	11	57.9
	3	2	19	11	57.9
	1	3	25	12	48.0
	2	3	17	13	76.5
	3	3	19	10	52.6
	<i>C. hyalinipennis</i> totals		182	99	54.4
<i>P. nasuta</i>	1	1	15	10	66.7
	2	1	13	11	84.6
	3	1	15	12	80.0
	1	2	14	10	71.4
	2	2	10	10	100.0
	3	2	13	12	92.3
	1	3	10	9	90.0
	2	3	13	11	84.6
	3	3	10	10	100.0
	<i>P. nasuta</i> totals		113	95	84.1
Overall totals		500	292	63.4	

**Table 4.2 Initial conditions of, and outcomes from, replicates focussing on inter-specific interactions**

Initial conditions				Replicates	Outcomes				
<i>C. stephanoderis</i>	<i>C. hyalinipennis</i>	<i>P. nasuta</i>	No. Berries		Replicates with emergence	Any species	<i>C. stephanoderis</i>	<i>C. hyalinipennis</i>	<i>P. nasuta</i>
1	1	-	1	22	9	40.9	9.1	36.4	-
1	1	-	2	18	9	50.0	27.8	38.9	-
1	1	-	3	24	13	54.2	20.9	45.8	-
1	-	1	1	21	12	57.1	23.8	-	42.9
1	-	1	2	13	12	92.3	53.8	-	76.9
1	-	1	3	15	11	73.3	33.3	-	60.0
-	1	1	1	13	11	84.6	-	30.8	53.8
-	1	1	2	14	10	71.4	-	42.9	71.4
-	1	1	3	19	12	63.2	-	15.8	57.9
1	1	1	1	12	11	91.7	25.0	41.7	33.3
1	1	1	2	12	10	83.3	8.3	33.3	75.0
1	1	1	3	12	10	83.3	16.7	41.7	66.7
Totals				195	130	70.4	24.3	36.4	59.8

containing *P. nasuta* (comparison of replicates with and without *P. nasuta*:  $G_1 = 10.39$ ,  $P = 0.001$ ). Emergence of each species was not affected by the identity of the other species present (*P. nasuta*:  $G_1 = 0.02$ ,  $P = 0.884$ ,  $n = 95$ ; *C. hyalinipennis*:  $G_1 = 1.29$ ,  $P = 0.256$ ,  $n = 110$ ; *C. stephanoderis*:  $G_1 = 4.23$ ,  $P = 0.04$ ,  $n = 113$ , note that while *C. stephanoderis* females emerged less often from replicates that also contained *C. hyalinipennis* than from those containing *P. nasuta*, this result is interpreted as non-significant following correction for FDR). Emergence rates of the three species were unaffected by the number of berries in a replicate.



## 4.4.2 Progeny production from berries with emergence

### 4.4.2.1 Intra-specific competition

For *C. stephanoderis*, production of female progeny per replicate increased with increasing number of berries ( $F_{2,89} = 8.91$ ,  $P < 0.001$ ,  $n = 98$ , Fig. 4.1a) but there was no significant influence of foundress number ( $F_{2,89} = 0.19$ ,  $P = 0.830$ ) or a berry number  $\times$  foundress number interaction ( $F_{4,89} = 0.21$ ,  $P = 0.934$ ). In terms of female production per foundress, there was an increase with increasing number of berries ( $F_{2,89} = 8.43$ ,  $P < 0.001$ , Fig. 4.1b), but a decrease with increasing number of foundresses ( $F_{2,89} = 25.34$ ,  $P < 0.001$ ). There was no significant berry number  $\times$  foundress number interaction ( $F_{4,89} = 0.59$ ,  $P = 0.672$ ). The effect of foundress number was due to a qualitative distinction between single and multiple foundress cases: the resultant reduction in fit of the statistical model when the 2- and 3-foundress data were combined (i.e. aggregation of factor levels, Crawley [1993], p190) was not significant after correction for FDR ( $F_{1,92} = 4.35$ ,  $P = 0.04$ ).

For *C. hyalinipennis*, female production per replicate (Fig. 4.1c) was not influenced by number of berries ( $F_{2,90} = 0.98$ ,  $P = 0.379$ ,  $n = 99$ ) or foundress number ( $F_{2,90} = 0.92$ ,  $P = 0.402$ ) and there was no significant berry  $\times$  foundress interaction ( $F_{4,90} = 1.27$ ,  $P = 0.288$ ). Production per foundress decreased as foundress number was increased but not significantly after correction for FDR ( $F_{2,90} = 3.91$ ,  $P = 0.024$ , Fig. 4.1d). No significant effect of number of berries on female production per foundress was detected ( $F_{2,90} = 0.86$ ,  $P = 0.426$ ) and there was no berry  $\times$  foundress interaction ( $F_{4,90} = 0.51$ ,  $P = 0.730$ ).

Female *P. nasuta* production per replicate increased with an increase in both the number of berries and the number of foundresses, but neither result was significant after correction for FDR (respectively,  $F_{2,86} = 3.66$ ,  $P = 0.030$ ,  $n = 95$ , Fig. 4.1e:  $F_{2,86} = 4.07$ ,  $P = 0.021$ , Fig. 4.1e). There was no significant berry  $\times$  foundress interaction ( $F_{4,86} = 1.85$ ,  $P = 0.127$ ). Female production per foundress decreased as the number of foundresses was increased ( $F_{2,86} = 10.19$ ,  $P < 0.001$ , Fig. 4.1f): single-foundress replicates had significantly higher female production per foundress than those with

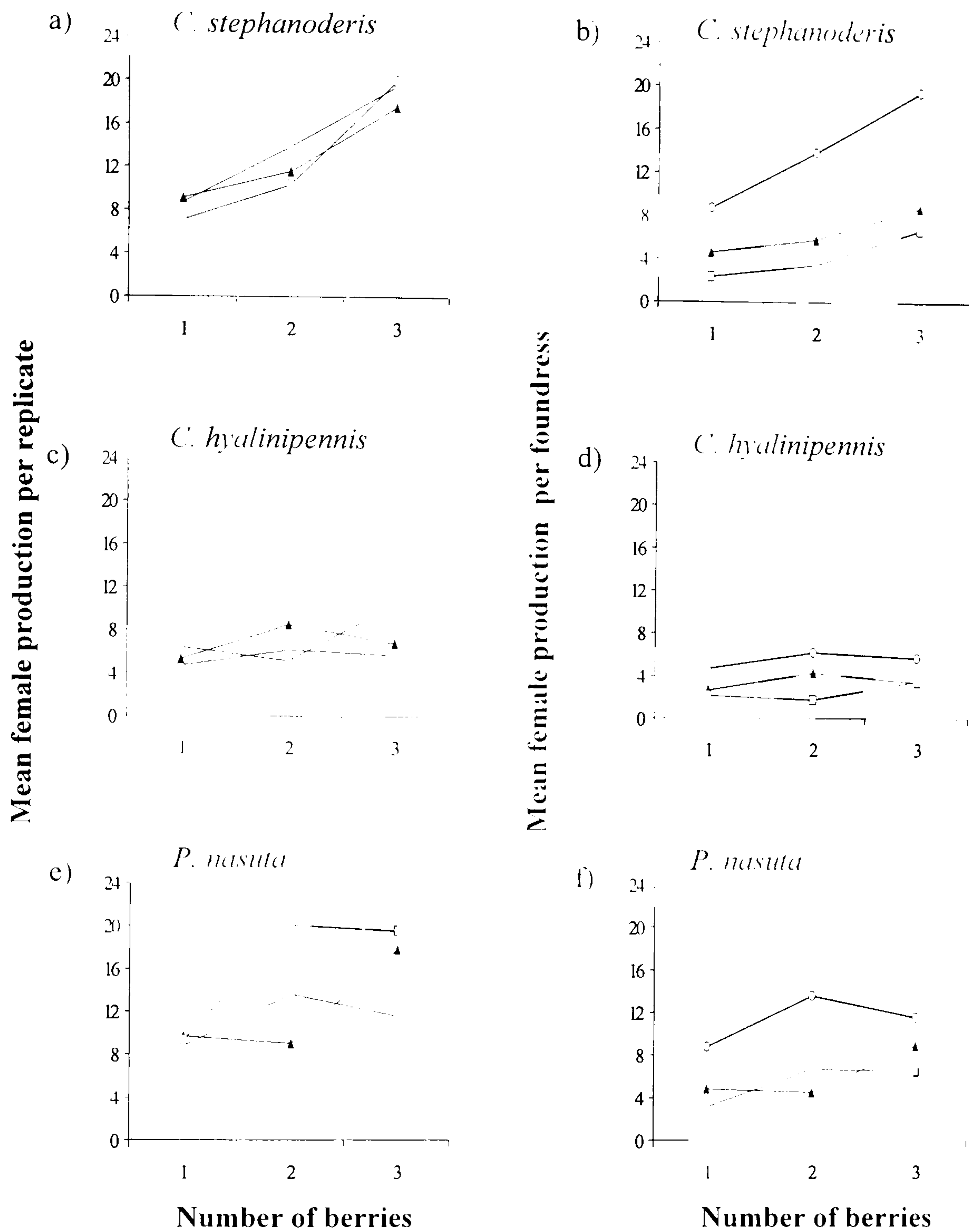


Figure 4.1 Intraspecific competition. Mean female production per replicate a), c) & e). Mean female production per foundress b), d) & f). 1 foundress ○, 2 foundresses ▲, 3 foundresses □.



two or three foundresses (aggregating factor levels, as above, lead to no significant reduction in fit,  $F_{1,89} = 0.01$ ,  $P = 0.905$ ). The number of berries had no significant effect on female production per foundress ( $F_{2,86} = 2.50$ ,  $P = 0.088$ ) and there was no significant berry  $\times$  foundress interaction ( $F_{4,86} = 1.65$ ,  $P = 0.168$ ).

#### Comparisons of intraspecific competition

A comparison of female progeny production among species revealed that, as the number of berries increased, the number of females emerging per replicate also increased ( $F_{2,265} = 13.09$ ,  $P < 0.001$ ,  $n = 292$ ). There were significant differences in female production per replicate between the three species ( $F_{2,265} = 23.58$ ,  $P < 0.001$ ), which could be attributed to lower female production in *C. hyalinipennis* replicates than those which contained *P. nasuta* or *C. stephanoderis* (aggregating factor levels lead to no significant reduction in fit,  $F_{1,274} = 1.64$ ,  $P = 0.105$ ).

Female production per foundress also increased significantly as the number of berries was increased ( $F_{2,265} = 8.11$ ,  $P < 0.001$ ,  $n = 292$ ). Conversely, as the number of foundresses per replicate increased, the number of emerging females per foundress decreased ( $F_{2,265} = 33.82$ ,  $P < 0.001$ ). The three species differed significantly in female production per foundress ( $F_{2,265} = 24.26$ ,  $P < 0.001$ ); *C. hyalinipennis* had significantly lower female production per foundress than *C. stephanoderis* or *P. nasuta* (aggregation of factor levels lead to no significant reduction in fit  $F_{1,284} = 2.49$ ,  $P = 0.060$ ).

#### **4.4.2.2 Inter-specific competition**

##### Species emergence

In this section we analyze results in terms of whether or not species were represented among the progeny emerging from replicates (patterns in the numbers of individuals emerging are explored in the following section). Since laboratory-observed contests commonly lead to the loser's death (Pérez-Lachaud *et al.*, 2002; Batchelor *et al.*, 2005: Chapter 3), we first examined the frequency with which more than one species of progeny emerged from inter-specific competition replicates (Table 4.3).

**Table 4.3 Species composition of female progeny emerging from inter-specific competition replicates**

Foundress species combination	Number of berries	Female progeny emergence						
		<i>C. stephanoderis</i> only	<i>C. hyalinipennis</i> only	<i>P. nasuta</i> only	<i>C. stephanoderis</i> & <i>C. hyalinipennis</i>	<i>C. stephanoderis</i> & <i>P. nasuta</i>	<i>C. hyalinipennis</i> & <i>P. nasuta</i>	<i>C. stephanoderis</i> , <i>C. hyalinipennis</i> & <i>P. nasuta</i>
<i>C. stephanoderis</i> & <i>C. hyalinipennis</i>	1	1	7	-	1	-	-	-
	2	2	4	-	3	-	-	-
	3	2	8	-	3	-	-	-
<i>C. stephanoderis</i> & <i>P. nasuta</i>	1	3	-	7	-	2	-	-
	2	2	-	5	-	5	-	-
	3	2	-	6	-	3	-	-
<i>C. hyalinipennis</i> & <i>P. nasuta</i>	1	-	4	7	-	-	0	-
	2	-	0	4	-	-	6	-
	3	-	1	9	-	-	2	-
<i>C. stephanoderis</i> , <i>C. hyalinipennis</i> & <i>P. nasuta</i>	1	2	5	3	0	1	0	0
	2	0	1	5	0	1	3	0
	3	0	2	4	0	1	2	1

Multi-species emergence was observed in 34/130 replicates: the frequency of multi-species emergence from single berry replicates (4/43) was significantly lower than from replicates containing two or more berries (30/87) ( $G_1 = 10.7$ ,  $P < 0.01$ ), suggesting that contest interactions could be occurring in single berry replicates when individuals are forced to share or compete for resources. We further explored whether being forced to interact within a berry affected the frequency of emergence of each species by comparing emergence from single berry replicates with emergence from replicates with multiple berries (where contests could potentially be avoided) among the replicates with two foundress species (i.e. dyadic replicates). The emergence rates of each of the species when competing with allospecifics were not significantly different when a single berry or multiple berries were provided (*C. stephanoderis*,  $G_1 = 1.43$ ,  $P > 0.1$ ; *C. hyalinipennis*,  $G_1 = 0.01$ ,  $P > 0.1$ ; *P. nasuta*,  $G_1 = 3.71$ ,  $P > 0.05$ ).



Turning to the combinations of species that emerged, *P. nasuta* and *C. hyalinipennis* never emerged together from single-berry replicates ( $n = 22$ , Table 4.3) but did so significantly more often from replicates that contained multiple berries (13/42 occasions, Table 4.3,  $G_1 = 12.63$ ,  $P < 0.001$ ). *Cephalonomia stephanoderis* and *P. nasuta* emerged together from 10/43 replicates containing multiple berries and from 3/23 single berry replicates (Table 4.3,  $G_1 = 1.04$ ,  $P > 0.1$ ). Both *Cephalonomia* species emerged from 1/20 single-berry replicates and from 6/42 multiple-berry replicates (Table 4.3,  $G_1 = 1.32$ ,  $P > 0.1$ ). From the 32 replicates with all three species of foundresses, all three species of progeny emerged only once, from a three-berry replicate (Table 4.3).

Considering the frequency of emergence of each species from interspecific replicates, *P. nasuta* emerged most often (77/99 replicates), whether competing against either or both of the *Cephalonomia* species (Table 4.3). *Cephalonomia stephanoderis* emerged least often from interspecific replicates (35/97 replicates), and had particularly low emergence from replicates initially containing all three species (6/31 replicates, Table 4.3). *Cephalonomia hyalinipennis* emerged from 53/95 interspecific replicates, achieving highest success in dyadic replicates containing *C. stephanoderis* ( $n = 31$ , *C. hyalinipennis* emerged from 26 replicates and *C. stephanoderis* from 12, Table 4.3).

Taken together, the above data on patterns of species emergence suggest that *P. nasuta* was the most and *C. stephanoderis* the least successful, and that interspecific competition occurs.

#### Number of emerging females per replicate

We first examine the influence of interspecific competition on female production per species by comparing between 2-foundress (dyadic) replicates in which foundress density is constant. We then re-analyze these data with the addition of replicates containing all three species of foundresses (triadic interactions).

### Dyadic interactions

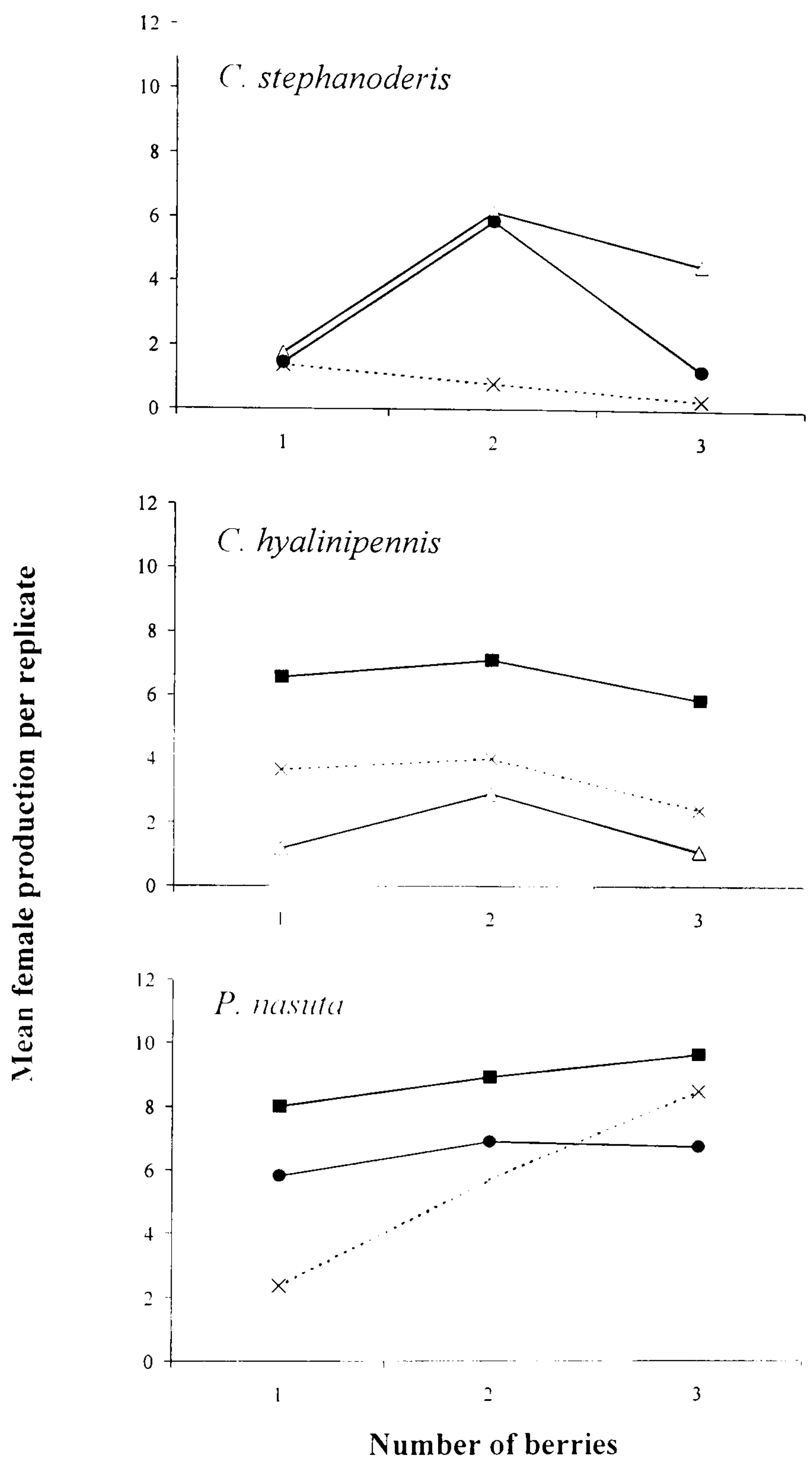
While more female *C. stephanoderis* emerged in two-berry than one-berry replicates the difference was not significant after correction for FDR ( $F_{2,60} = 3.95$ ,  $P = 0.024$ ,  $n = 66$ , Fig. 4.2). Female production was not significantly influenced by the identity of the competing species ( $F_{1,60} = 1.19$ ,  $P = 0.279$ ,  $n = 66$ ) and there was no significant berry number  $\times$  foundress species combination interaction ( $F_{2,60} = 0.98$ ,  $P = 0.383$ ,  $n = 66$ ). For *C. hyalinipennis*, female production was uninfluenced by the number of berries provided ( $F_{2,58} = 0.46$ ,  $P = 0.635$ ,  $n = 64$ ) but was significantly higher in replicates containing *C. stephanoderis* than in those containing *P. nasuta* ( $F_{1,58} = 15.81$ ,  $P < 0.001$ ,  $n = 64$ , Fig. 4.2). There was no berry  $\times$  species interaction ( $F_{2,58} = 0.55$ ,  $P = 0.582$ ,  $n = 64$ ). Production of *P. nasuta* females was not significantly influenced by the identity of the competing species ( $F_{1,62} = 1.88$ ,  $P = 0.175$ ,  $n = 68$ ) or the number of berries provided ( $F_{2,62} = 0.19$ ,  $P = 0.828$ ,  $n = 68$ ), nor was there a berry  $\times$  species interaction ( $F_{2,62} = 0.02$ ,  $P = 0.984$ ,  $n = 68$ ) (Fig. 4.2).

Comparison of overall female production per replicate, irrespective of species identities, indicated that production was higher in replicates containing *C. stephanoderis* and *P. nasuta* rather than *C. hyalinipennis*, but this difference was not significant after correction for FDR ( $F_{2,90} = 3.68$ ,  $P = 0.029$ ,  $n = 99$ , Fig. 4.3). Increasing the number of berries available had no significant effect on overall female production per replicate ( $F_{2,90} = 2.91$ ,  $P = 0.059$ ) and there was no significant berry  $\times$  species interaction ( $F_{4,90} = 0.51$ ,  $P = 0.727$ ).

### Dyadic and triadic interactions

Analysis of female production per replicate using both dyadic and triadic replicates generated the same qualitative conclusions as above, with the exception that for *C. stephanoderis* there was an effect of competing species identity: production was significantly higher in dyadic replicates containing *P. nasuta* than in triadic replicates ( $F_{2,88} = 5.88$ ,  $P = 0.004$ ,  $n = 97$ , Fig. 4.2).





**Figure 4.2 Interspecific competition. Mean female production per foundress for each species when present with *C. stephanoderis* ■, *C. hyalinipennis* ●, *P. nasuta* Δ, or all 3 species present ×.**

#### 4.4.2.3 Comparison between intra- and inter-specific replicates

We compared overall female production, irrespective of species identities, per replicate under intra- and inter-specific competition. Production was influenced by the combination of foundress species present ( $F_{6,209} = 2.99$ ,  $P = 0.008$ , Fig. 4.3) and positively influenced by the number of berries provided but the latter trend was not significant after correction for FDR (log-linear analysis,  $F_{2,209} = 4.09$ ,  $P = 0.018$ , Fig. 4.3). There was no significant berry  $\times$  species combination interaction ( $F_{12,209} = 0.80$ ,  $P = 0.655$ ). We then attempted to combine, in a stepwise manner, with order determined by inspection of parameter estimates (Crawley, 1993), the seven different

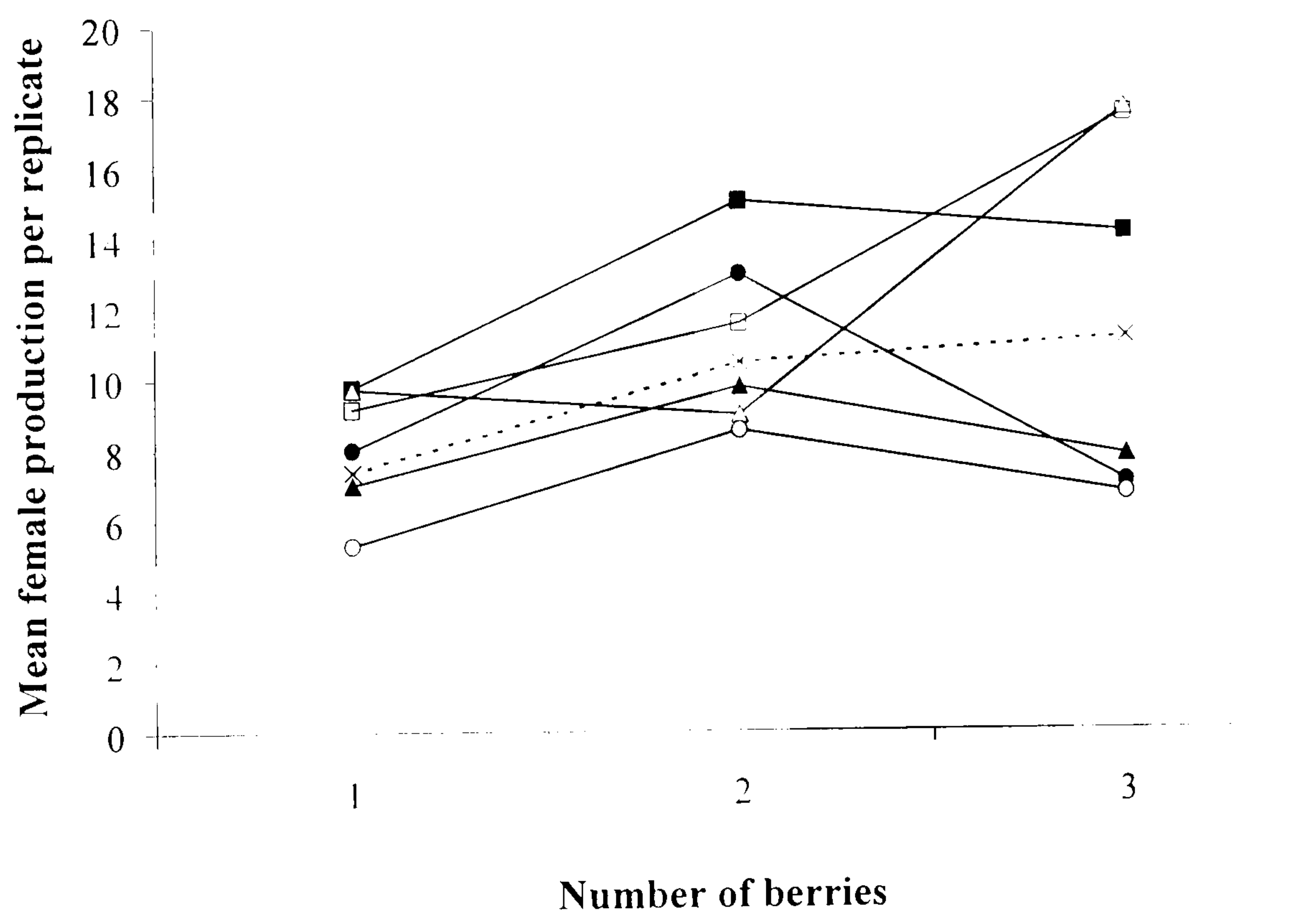


Figure 4.3 Intra- and Inter-specific competition. Mean female production per replicate, regardless of which species emerged. Foundress species; *C. stephanoderis* & *C. stephanoderis* □, *C. hyalinipennis* & *C. hyalinipennis* ○, *P. nasuta* & *P. nasuta* Δ, *C. stephanoderis* & *C. hyalinipennis* ●, *C. stephanoderis* & *P. nasuta* ■, *P. nasuta* & *C. hyalinipennis* ▲, All three species -x-.



combinations of foundress species, assessing the significance of the change in statistical fit after each simplification of the statistical model. All aggregations involving *C. hyalinipennis* did not lead to significant changes ( $P$  ranged between 0.7 and 0.13) and were thus retained. Species combinations not involving *C. hyalinipennis* also did not differ significantly and could be aggregated ( $P$  ranged between 0.93 and 0.32). Combining combinations with and without *C. hyalinipennis*, however, lead to a highly significant reduction in statistical fit ( $F_{1, 227} = 14.32$ ,  $P < 0.001$ ). Combinations with *C. hyalinipennis* had lower production of females than those in which *C. hyalinipennis* was not present (Fig. 4.4).

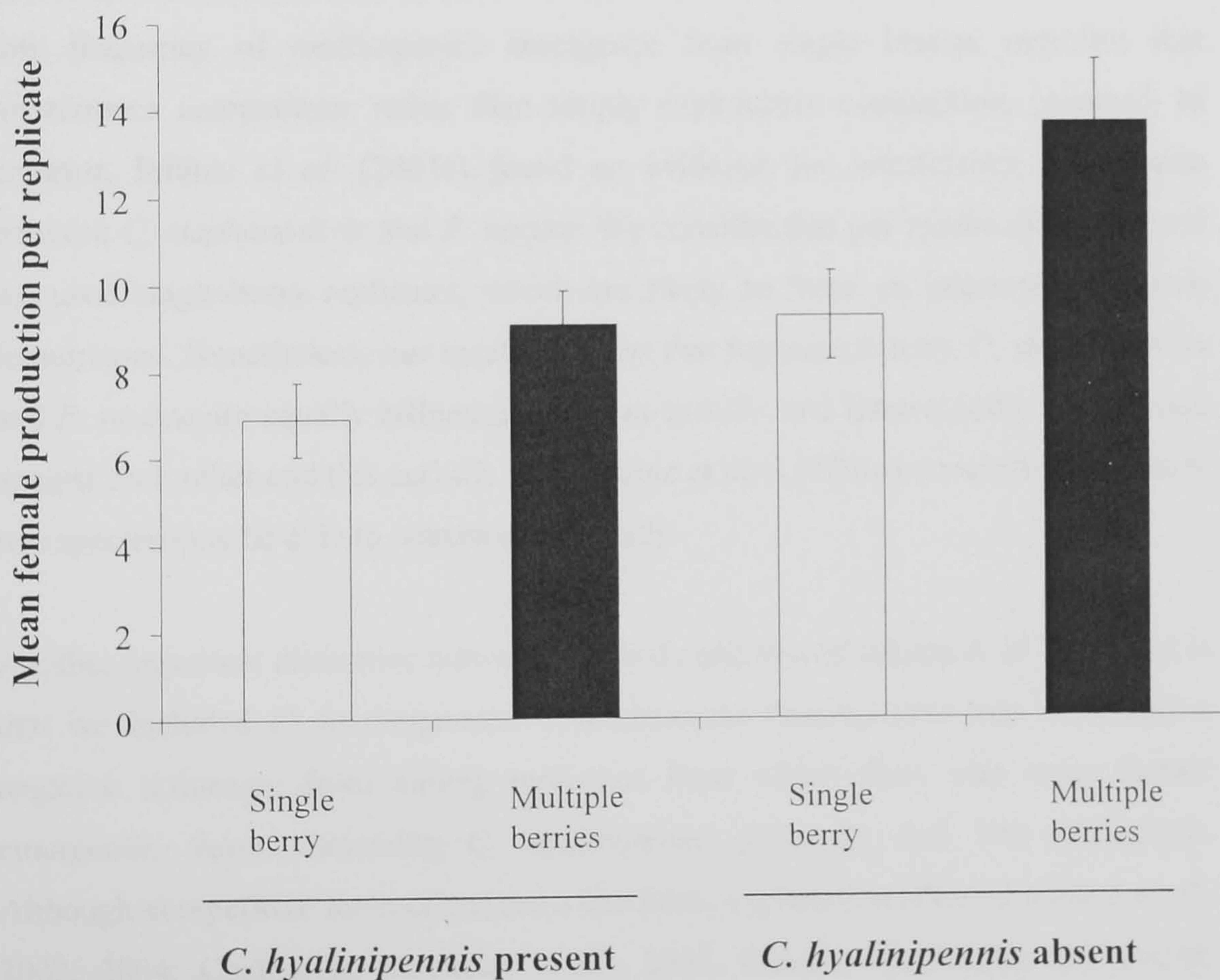


Figure 4.4 Mean production per replicate when there were single- or multiple-berries and *C. hyalinipennis* was present or absent from the replicate.



## 4.5 DISCUSSION

Overall, the most successful species, in terms of both emergence and female production, was *P. nasuta*. *Cephalonomia stephanoderis* was the species that emerged least often but *C. hyalinipennis* had the lowest production of female progeny.

Our results constitute evidence for both intra- and inter-specific resource competition: emergence and female production were often higher when multiple berries were provided. Inter-specific competition was sufficiently severe to make multiple-species emergence from single berries uncommon. Because berries generally contained sufficient hosts for more than one foundress to reproduce, the low frequency of multi-species emergence from single berries indicates that interference competition, rather than simply exploitative competition, occurred. In contrast, Infante *et al.* (2001a) found no evidence for interference competition between *C. stephanoderis* and *P. nasuta*. We consider that our results differ because we used single-berry replicates, which are likely to force an interaction between foundresses. Nonetheless, our results suggest that reproduction by *C. stephanoderis* and *P. nasuta* are equally influenced by intra-specific and inter-specific competition against each other and this accords with Infante *et al.*'s (2001a) conclusion that these two species may be able to coexist ecologically.

Another important difference between our study and that of Infante *et al.*'s (2001a) is that we included *C. hyalinipennis*. *Cephalonomia hyalinipennis* may have had a negative influence: from among replicates from which there was some female emergence, those containing *C. hyalinipennis* generally had low production. Although competitive interactions are a candidate explanation (Pérez-Lachaud *et al.*, 2002, 2004: Chapter 2; Batchelor *et al.*, 2005: Chapter 3), because the overall production (given that there was some emergence) of *P. nasuta* and *C. stephanoderis* in interspecific replicates was uninfluenced by the presence of *C. hyalinipennis*, it seems more likely that low production was due to a lower reproductive rate of *C. hyalinipennis* than the other species. *Cephalonomia hyalinipennis* may be less effective at reproducing within coffee berries than the other two species because its association with the CBB is relatively recent (the CBB was first recorded in Mexico



in 1978, [Baker, 1984]). Preliminary work investigating the time taken by each of the three bethylid species to enter an infested coffee berry in the laboratory indicates that *C. hyalinipennis* is slower than *P. nasuta* and *C. stephanoderis* (T.P. Batchelor, unpublished data).

These results derive from a relatively ‘holistic’ (albeit laboratory) experiment, with an emphasis on overall outcome rather than the processes by which outcomes are achieved and, taken in isolation, would imply that the most effective biological control agent would be *P. nasuta* since it emerged most often and had the highest production of female offspring. However, more ‘reductionist’ studies, that have examined aspects of the life-histories of the parasitoids and their interactions, have had different implications as to which of the three species would be the most effective biological control agent.

Population dynamic theory suggests that increasing the production of female offspring per host attacked will lead to greater host suppression (Murdoch and Briggs, 1996; Heimpel, 2000). All three species feed on some of the hosts they attack but *P. nasuta* and *C. stephanoderis* do so destructively while *C. hyalinipennis* frequently both feeds and oviposits on the same host individual (e.g. Lauzière *et al.*, 2001b; Pérez-Lachaud and Hardy, 2001). Furthermore, *P. nasuta* and *C. stephanoderis* almost always lay only one egg per host, while *C. hyalinipennis* lays 1-3 eggs (mean = 1.62) on CBB (Pérez-Lachaud and Hardy, 2001). In all three species, the probability of an egg surviving to adulthood is about 0.6-0.77, under the environmental conditions used in our study, and progeny sex ratios are broadly similar (proportion male ~ 0.11-0.25) (Infante *et al.*, 1992; Barrera *et al.*, 1993; Hardy and Mayhew, 1998; Pérez-Lachaud and Hardy, 1999, 2001; Infante, 2000). The reproductive biologies of these species, as estimated separately in ‘reductionist’ laboratory studies, therefore imply that *C. hyalinipennis* would be expected to produce the most emerging females per host and best suppress CBB populations. Additionally, due to having the greatest fundamental per capita rate of increase it might be expected to displace *C. stephanoderis* and *P. nasuta* from the agro-ecosystem, other factors being equal (e.g. Heimpel, 2000).

Additional 'reductionist' studies have investigated interspecific interactions between *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta*. Adult females of all three species have been observed to feed upon the immature stages of their allospecific bethylid competitors (Infante *et al.*, 2001a; Pérez-Lachaud *et al.*, 2004: Chapter 2). *Cephalonomia hyalinipennis* has further been shown to be a facultative hyperparasitoid of the immature stages of *P. nasuta* and *C. stephanoderis* in the laboratory (Pérez-Lachaud *et al.*, 2004: Chapter 2). The 'advantage of facultative hyperparasitism' again implies that *C. hyalinipennis* might eventually displace *C. stephanoderis* and *P. nasuta*. Intra-guild predation and hyperparasitism are generally thought to be detrimental to host population suppression in biological control programmes (e.g. Rosenheim *et al.*, 1995; Pérez-Lachaud *et al.*, 2004: Chapter 2).

A further aspect of interspecific interactions is that adult females may also interact via direct behavioural, and often fatal, contests for host resources. Laboratory studies found *C. stephanoderis* to be the most, and *P. nasuta* the least, successful competitor and that interspecific competition is stronger than intraspecific competition because intraspecific interactions are non-fatal (Pérez-Lachaud *et al.*, 2002; Batchelor *et al.*, 2005: Chapter 3). Taken in isolation, such results would imply that *C. stephanoderis* would displace the other species from the agro-ecosystem.

Reductionist studies have thus implied that, of the bethylids examined, either *C. stephanoderis* or *C. hyalinipennis* is likely to be the most successful biological control agent of the CBB and that *P. nasuta* is unlikely to be effective. In contrast, 'holistic' investigations have suggested that *P. nasuta* is likely to achieve greatest success (this study) or that *C. stephanoderis* and *P. nasuta* are able to coexist, with *C. stephanoderis* being more effective under the environmental conditions studied (Infante *et al.*, 2001a). These contrasting conclusions all derive from laboratory studies, each attempting to evaluate the suitability of these species, alone or in combination, as biological control agents. Such predictions can be compared with the establishment of each species in coffee agro-ecosystems. In southern Mexico *C. hyalinipennis* is regionally indigenous and is present but rare in coffee plantations. (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 1999) while *C. stephanoderis* and *P. nasuta* have been released since 1989 in a classical biological control programme (Barrera *et al.*, 1990b; Murphy and Moore, 1990). Observations suggest that *C.*



*stephanoderis* persists in low numbers after release (Barrera *et al.*, 1990b; Barrera, 1994; Damon, 1999; T.P. Batchelor, pers. obs.) whereas *P. nasuta* has consistently failed to establish (Infante *et al.*, 2001b; Infante *et al.*, 2003).

The high success of *P. nasuta* in holistic laboratory studies suggests that its failure to establish in Mexico is not likely to be due to competitive interactions with *C. stephanoderis* and *C. hyalinipennis*. The population densities of the two *Cephalonomia* species in the agro-ecosystem are lower than in our study and also than in the holistic study of Infante *et al.* (2001a) (Damon, 2000; Damon and Valle, 2002) and thus contests and other forms of interference competition are less likely to occur in the field than in the laboratory. In contrast to observations in Mexico, but not to our laboratory results, *C. stephanoderis* and *P. nasuta* are reported to coexist in Togolese coffee plantations, where both species are indigenous (Moore and Prior, 1988; Vega *et al.*, 1999). However, our finding that *P. nasuta* is more prevalent than *C. stephanoderis* is the opposite of field observations in Togo (Moore and Prior, 1988; Vega *et al.*, 1999). Climatically, African and Mexican coffee growing regions are broadly similar, being classified as 'tropical continental' or 'rainforest', and close to the equator with relatively invariant day length and sunlight. Within its native range, *P. nasuta* is more prevalent at higher altitude, particularly in East Africa (Barrera, 1994; Infante *et al.*, 2001a). The failure of *P. nasuta* to establish in Mexico may in part be due to predation by other arthropods (Infante *et al.*, 2003), yet predation also affects *C. stephanoderis* (Henaut *et al.*, 2001) and might be similarly expected to affect *C. hyalinipennis*, and remains unexplained.

The problems we must face are that different laboratory approaches to evaluating biological control agents and their interactions have generated different implications for best pest control practice and that there is no simple correspondence between laboratory results and (regionally inconsistent) field evidence. There are numerous reasons why such inconsistencies may arise. The parasitoids we have studied derive from medium to long-term laboratory cultures periodically augmented by field-caught individuals, and we consequently cannot be certain that differences in performance are due to genuine species differences rather than differences in culture size and genetic diversity. 'Reductionist' laboratory studies tend to focus on one or a few aspects of parasitoid life-history at a time and may overemphasise the

importance of the aspects studied for the overall performance of the species or the strength of an interspecific interaction. Even 'holistic' laboratory studies are unlikely to take into account all of the aspects that influence the performance of a biological control agent in the field. For instance, the relative foraging abilities of *C. stephanoderis*, *C. hyalinipennis* or *P. nasuta* have not been assessed as hosts were always provided within small tubes or jars, and further species of parasitoids and predators were not present.

Despite the varied expectations concerning the suitability and coexistence of *C. stephanoderis* and *P. nasuta*, the weight of evidence suggests *C. hyalinipennis* will not be beneficial to CBB control, due to a low population growth rate and/or negative affects on other biological control agents (contests, hyperparasitism). We have previously warned against the introduction of *C. hyalinipennis* into regions in which it is not indigenous because of its possible non-target effects on both herbivores and parasitoids outside the coffee agro-ecosystem (Pérez-Lachaud *et al.*, 2004: Chapter 2). The results of the present study lead us to further recommend that *C. hyalinipennis* should not be encouraged, for example through mass rearing, within its native range as it now appears to be an ineffective, and possibly detrimental, invader of the Mexican coffee agro-ecosystem.

While it is widely accepted that screening is an important component of the evaluation of potential biological control agents to reduce non-target effects (e.g. Hopper, 2001; Waage, 2001), screening usually focuses on evaluations of host specificity. In the case of CBB biological control using bethylids, the agents are either effectively monophagous (*C. stephanoderis* and *P. nasuta*, Pérez-Lachaud and Hardy, 2001) or native (Pérez-Lachaud, 1998) and the focus of 'screening' we have carried out has been on exploring potentially disruptive inter-agent interactions. It seems increasingly clear that intra-guild predation and other disruptive inter-specific interactions are generally common when more than one species are deployed in biological control programmes, yet at present there has been scant attention paid to this problem (Rosenheim *et al.*, 1995). Interspecific interactions are likely to be complex and multifaceted and untangling them may require a combination of



‘reductionist’ and ‘holistic’ approaches<sup>2</sup>. Even so, there may not be a simple correspondence between laboratory studies and the outcomes of actual biological control programmes and this remains a concern.

#### 4.5.1 Conclusions and recommendations

Our study is an attempt to evaluate inter-specific competition, and coexistence, within the context of the biological control of a pest with multiple-natural enemies. In the laboratory, despite ‘losing the battles’, in terms of offspring production per host, trophic interactions and direct contests, *P. nasuta* appears to ‘win the war’ in terms of the most crucial attribute: recruitment of female offspring to the next generation. Yet, in the field it has failed to establish where *C. stephanoderis* has established (Mexico) or coexists with *C. stephanoderis* but at lower densities (Togo). Since *C. stephanoderis* also performs well in holistic laboratory studies and in dyadic contests, and is apparently more successful than *P. nasuta* in the field where they coexist, it seems that *C. stephanoderis* will often be the more effective natural enemy. Turning to *C. hyalinipennis*, this species was recently discovered to occur naturally in the Mexican coffee agro-ecosystem and initial evaluations of its biology suggested it might be a promising agent for biological control. Investigations of its interactions with *C. stephanoderis* and *P. nasuta* have, however, demonstrated the potential for intra-guild predation, hyperparasitism and fatal contests. Such interactions do not in themselves suggest that *C. hyalinipennis* should not be encouraged within its native range because negative interactions may be uncommon in the field and *C. hyalinipennis* may still be a more effective biological control agent than the other two bethylids. The present study has, however, indicated that *C. hyalinipennis* has a low rate of offspring production and that its presence may have a negative effect on recruitment of female offspring to *C. stephanoderis* populations. Current evidence thus suggests that *C. hyalinipennis* should not be encouraged as a member of the guild of natural enemies of the CBB in Mexico or in other regions.

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<sup>2</sup> For a more detailed discussion of reductionist and holistic approaches in biological control see subsequent publication, Kidd and Jervis (2005).

## **Part Two: *Goniozus nephantidis* contests and chemical release**



# CHAPTER 5 - THE IMPORTANCE OF VALUING RESOURCES: HOST WEIGHT AND CONTENDER AGE AS DETERMINANTS OF PARASITOID WASP CONTEST OUTCOMES<sup>1</sup>

## 5.1 ABSTRACT

When two animals compete directly for an indivisible resource, the outcome may be influenced by differences in the contestants' intrinsic contest abilities and prior-owner status, collectively termed 'resource holding potential', and also by any difference in the values that contestants place on the resource. Using owner-intruder contests and owner-owner contests between females of the parasitoid wasp *Goniozus nephantidis*, we investigate the influence of resource value asymmetries generated by differences in contestant age and by the size of the host resource, while taking resource holding potentials into account. When owners and intruders compete, intruder success increases with both intruder age and relative body size, suggesting that larger females have greater contest ability and that older intruders value more greatly the opportunity to obtain a host. The size of the host did not influence the outcome of owner-intruder contests but in owner-owner contests, in which asymmetries in ownership status are absent, both larger females and owners of the larger, more valued, hosts were advantaged. We additionally document a novel observation of fatal fighting and provide analysis of factors influencing egg load and clutch size, since these in turn may affect contest outcomes via resource value asymmetries. Overall, studies of *G. nephantidis* show that asymmetries in both resource holding potentials and resource values interact to influence contest outcomes. Despite a large literature on animal contests, there have been few studies that have evaluated these influences both simultaneously and separately.

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<sup>1</sup> Submitted as HUMPHRIES, E.L., HEBBLETHWAITE, A.J., BATCHELOR, T.P. and HARDY, I.C.W. (submitted). The importance of valuing resources: host weight and contender age as determinants of parasitoid wasp contest outcomes. 2005.

## 5.2 INTRODUCTION

In diverse animal species, pairs of individuals compete for indivisible resources via agonistic contest behaviour (Huntingford and Turner, 1987; Riechert, 1998; Mesterton-Gibbons and Adams, 1998). Theoretical studies, using game theoretic modelling and the concept of evolutionarily stable strategies (Maynard Smith, 1982), have identified factors expected to influence the outcome of such dyadic contests. The two major categories are associated with the difference in the value placed on the resource by each contestant (resource value, RV: Parker, 1974; Maynard Smith and Parker, 1976; Enquist and Leimar, 1987) and with the difference in the abilities of the contestants to acquire and retain resources (resource holding potential, RHP: Parker, 1974; Maynard Smith and Parker, 1976; Hammerstein, 1981). Further, an individual's RHP can be thought of as consisting of a component derived from its intrinsic contest ability (e.g. fighting ability) and a component related to whether it is the prior owner of the resource or a non-owner (variously termed 'floater' or 'intruder'): these components are termed 'resource-uncorrelated' and 'resource-correlated' RHPs respectively (Fig. 5.1). In the absence of asymmetry in RV, a contest is predicted to be won by the individuals with the higher RHP, and in the absence of a difference in RHP, the contestant that places the greater value on the resource is expected to win. Asymmetries in RV and RHP may also influence contest outcome concurrently.

We explore factors influencing the outcomes of contests between adult female parasitoid wasps. Direct contest interactions between females occur in several parasitoid taxa (e.g. Lawrence, 1981; Field, 1992; Hughes, Harvey and Hubbard, 1994; Field and Calbert, 1999; Batchelor *et al.*, 2005): our study system is the bethylid wasp *Goniozus nephantidis* (Muesebeck), a gregarious ectoparasitoid of lepidopteran larvae. This species has previously been shown to engage in owner-intruder contests for host larvae that have been previously paralysed by the owner female (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). When such interactions occur during the host-guarding period, before the owner has oviposited, contests are generally won by the larger female, with body size interpreted as a component of resource-uncorrelated RHP. There is also an advantage associated with ownership, such that owners can win against intruders that are larger. While



## Resource Value (RV)

## Resource Holding Potential (RHP)

Component **correlated** with resource possession

( e.g. positional advantage)

Component **uncorrelated** with resource possession

( e.g. intrinsic contest ability)

**Figure 5.1 Schematic and theoretical overview of factors influencing contest outcomes.** A contested resource may have the same or different value to each contestant. In the absence of other asymmetries, the individual to which the resource has the most value (RV) is expected to win. In the absence of asymmetries in RV, contest outcomes are determined by difference in the ability to acquire or retain a resource (RHP). The RHP of each contestant is comprised of a component uncorrelated with whether or not it is in possession of the resource prior to the contest (resource-uncorrelated RHP) and a component related to resource possession (resource-correlated RHP). The individual with the largest total RHP is expected to win. Asymmetries in RV and RHP may operate simultaneously to influence the outcome of a contest.

ownership effects are potentially due to mechanistic advantages or evolved conventions, constituting resource-correlated RHPs, these do not seem likely explanations in *G. nephantidis* (Stokkebo and Hardy, 2000). An alternative explanation for owners winning contests is that owners place a higher value on the host than do intruders. A resource value asymmetry may arise because host ownership promotes maturation of unlaidd eggs making owners able to utilize (by ovipositing) the host resource more quickly. Stokkebo and Hardy (2000) found an influence of egg load asymmetry was undetected in owner-intruder contests, possibly because egg load is confounded with ownership status. When owner-owner contests between size-matched contestants, which remove or reduce asymmetries in RHP, were studied, it was found that egg load differences influenced contest outcomes strongly (Stokkebo and Hardy, 2000). These results suggest that in *G. nephantidis* ownership effects may be due to asymmetries in RV instead of, or as well as, in resource-correlated RHP.

In these two experimental studies the size of the host was held essentially invariant (*ca.* 35mg). However, host size is the most obvious, and probably most important, determinant of host quality for parasitoid wasps, as attested by a substantial literature on host-size related host acceptance, clutch size and sex ratio decisions (e.g. reviews by Schmidt, 1991; King, 1993; Godfray, 1994, and subsequent work by Zaviezo and Mills, 2000; West and Sheldon, 2002; Karsai, Somogyi and Hardy, 2005), including studies on *G. nephantidis* and other bethylids (e.g. Hardy *et al.*, 1992; Luft, 1993; Mayhew and Godfray, 1997; Mayhew and Hardy, 1998; Pérez-Lachaud and Hardy, 2001).

Here we investigate the influence of RV asymmetries on the outcome of contests in *G. nephantidis* by varying the size (weight) of the hosts contested. We study both classical owner-intruder contests and owner-owner contests, which are a useful way of studying contest behaviour in the absence of RHP asymmetries deriving from resource ownership (e.g. Stutt and Willmer, 1998). In owner-intruder experiments both resource-correlated and resource-uncorrelated components of RHP vary and RV is represented by the size of the initial owner's host, large or small: we hypothesized that prior owners will have a more accurate assessment of host quality than will intruders and that ownership effects would thus be stronger when owners have large hosts. The age difference between contestant females is explored as an additional influence on RV asymmetry: we hypothesized that older intruder females will value more greatly the opportunity to obtain a host and thus ownership effects would be weaker when intruders are older. The influence of host size is undetected in owner-intruder contests but we find an influence of age that implies that the value of hosts of a given size is greater to older females. In owner-owner experiments asymmetries in resource-correlated RHPs are absent, differences in resource-uncorrelated RHPs are quantified and RV asymmetries are represented by differences in the size of the host owned by females prior to the contests. Host value is additionally assessed by allowing some pairs of females to oviposit prior to the contest and exploring the influence of difference in clutch size. Our rationale was that the two owners should differ in their evaluation of the resource they defend against a perceived intruder; owners of larger hosts (or layers of larger clutches) would thus be expected to win. We find expected effects of both resource-uncorrelated RHP and RV. While an understanding of factors influencing contest outcomes is our primary objective, we



also provide analysis of factors influencing egg load and clutch size, since these in turn may affect contest outcomes via asymmetries in RV.

### 5.3 MATERIALS AND METHODS

We used the same culturing procedures and strains of parasitoids and hosts as the prior studies of Petersen and Hardy (1996) and Stokkebo and Hardy (2000): *G. nephantidis* were reared on larvae of the factitious host *Corecya cephalonica* (Stainton) (Lepidoptera: Pyralidae). Cultures and experiments were maintained at 26°C, and high relative humidity, under a 8:16 light: dark cycle.

General experimental procedures followed closely those of Petersen and Hardy (1996) and Stokkebo and Hardy (2000). Females used in contests were of known age, had no prior experience of contest interactions and were kept in isolation in glass vials before use in experiments following removal from their natal brood. Females in a contest dyad had developed in different broods and were marked with different colours on the dorsal surface of their thorax using a dot of bright yellow or bright red acrylic water-based paint. An anaesthetic (CO<sub>2</sub> gas) was used briefly to facilitate marking. Wasps were weighed to an accuracy of 0.01mg immediately after marking. Some females were provided with a host of known weight, and allowed to paralyse it, for 18h or more; we term these females ‘owners’. Other females were kept without a host and are termed ‘intruders’.

Prior to contest observations, females with hosts were placed individually into an isolated chamber within an opaque plastic block decked with Plexiglas, as described and illustrated in Petersen and Hardy (1996). This apparatus had three circular chambers (diam. 18mm, depth 6mm) set 10mm apart with a 1mm wide interconnecting slot, into which plastic barriers were initially inserted to divide each chamber. In owner-intruder experiments, the owner was placed in the central chamber and the intruder in one of the outer chambers while in owner-owner experiments, owners were placed in the central chamber either side of the barrier. We chose pairs of females to obtain a range of weight differences in contest dyads. The females were allowed to settle for 30 min after transfer to the apparatus. Then barriers were drawn back to the outer edge of the outer chamber allowing females

access to all three chambers. Events in the host-containing chamber were recorded on videotape for 90 minutes following barrier withdrawal. It was then noted which female was in possession of the host(s) and then each female was removed, re-weighed and then dissected and the number of mature eggs in the abdomen was noted. Immature eggs tend to be short, cloudy and surrounded by nurse cells. Mature eggs are elongated, opaque and are not surrounded by nurse cells (Stokkebo and Hardy, 2000). When observing the videotaped events in the central chamber, we particularly noted any behavioural interactions, which were classified as ‘fights’ (escalated conflicts), ‘chases’ (semi-escalated conflicts) or ‘non-aggressive’ (extreme close proximity or contact not resulting in agonistic behaviour) (as in Petersen and Hardy, 1996).

### **5.3.1 Experiment 1: owner-intruder contests**

There were a total of 53 owner-intruder replicates. In 31 of these, the owner and the intruder were of similar age (approx. 2–3 days) and the owner had been provided with either a ‘small’ or a ‘large’ host for approximately 48h and had paralysed it but not oviposited. We attempted to provide small hosts weighing 30mg and large hosts of 50mg but due to the difficulty of obtaining hosts to match exactly these criteria there was some variation in the weights of small and large hosts used (means  $\pm$  SDs:  $30.14 \pm 1.94$ mg and  $49.90 \pm 1.82$ mg respectively). In the 22 replicates with an age asymmetry between the females, owners were 2–3 days old and intruders 4–5 days old. In approximately half of the replicates the red-marked wasp was the owner and in the remainder it was the intruder.

### **5.3.2 Experiment 2: owner-owner contests**

We followed the general methodology above with some owners having been given a small host and others having been given a large host: in all cases contest interactions were between an owner of a small host and an owner of a large host. There was some variation in the weights of small and large hosts used (means  $\pm$  SDs:  $28.85 \pm 2.59$  and  $49.99 \pm 1.93$  respectively) leading to an average within-replicate host weight difference of 21.14mg. The smallest host-weight difference in a replicate was 15.40mg: host weight differences were thus always substantial.



There were 56 owner-owner replicates in total. In 28 of these, pairs of females were used that were of the same age, had each been provided with a host for 18 or more hours and had paralysed it but not oviposited. In the other 28 owner-owner replicates, females were held in vials with hosts for 95h or more to allow them to oviposit. Before being placed into the contest apparatus, the number of eggs (clutch size) laid by each female was counted. Clutch size provides an additional index of host quality as it reflects the value that the female herself places on the resource that is the host. In approximately half of owner-owner replicates the red-marked wasp was provided with the large host and in the remainder with the small host.

### **5.3.3 Statistical analysis**

Data were analysed using generalized linear modelling carried out in the GenStat statistical package. Our general approach was to use, where possible, parametric analyses in which the assumed distribution of residuals was matched to the data rather than transforming data to fit standard assumptions (see Wilson and Hardy, 2002). Further, we adopted the top-down approach (i.e. stepwise backward analysis) in which significance is assessed when explanatory variables are removed from an initially complex model (containing all main effects and interaction terms of interest) to obtain a parsimonious statistical description. When candidate explanatory variables are mutually and highly correlated interpretational problems can arise ('collinearity': Grafen and Hails, 2002; Quinn and Keough, 2002); this lead us to exclude egg load (correlated with body size, wasp age, host weight and whether or not a female had laid eggs, see below) from the analysis of contest outcomes. Except for initial binomial tests (Siegel and Castellan, 1988), to assess the influence of the colour of paint marks, contest data were explored using logistic analyses (Petersen and Hardy, 1996; Hardy and Field, 1998; Stokkebo and Hardy, 2000). Binary response variables were defined as whether there was an aggressive interaction between females, whether there was a contest between females, or, when a contest occurred, whether a given female won. The significance of explanatory variables and interactions was assessed by the change in deviance,  $G$ , (which approximates  $\chi^2$ ). Count data (egg load, clutch size) were explored using log-linear analyses in which the dispersion parameter was estimated empirically to take overdispersion (variances

greater than the mean leading to non-Poisson distributed residuals) into account, with significance assessed using *F*-ratio tests (Crawley, 1993).

## 5.4 RESULTS

Before analysing our data in terms of contest behaviour we explore factors influencing egg load and clutch size.

### 5.4.1 Egg load

Using data from owner-intruder contests (experiment 1), we explored the influences of wasp weight, wasp age, host ownership and host weight on egg load. For females aged 2-3 days, egg loads increased with increasing wasp weight ( $F_{1,56} = 32.46$ ,  $P < 0.001$ ) but were not significantly influenced by ownership ( $F_{1,56} = 0.22$ ,  $P = 0.641$ ). Intruders aged 5 days had higher egg loads than those aged 3 days ( $F_{1,49} = 4.53$ ,  $P = 0.039$ , Fig. 5.2a) and heavier intruders also had more eggs ( $F_{1,49} = 32.14$ ,  $P < 0.001$ , Fig. 5.2a). For owners, egg loads were uninfluenced by host weight ( $F_{1,49} = 0.16$ ,  $P = 0.688$ ).

Using data from owner-owner contests (experiment 2), egg loads were higher when females were provided with larger hosts ( $F_{1,110} = 8.97$ ,  $P = 0.003$ , Fig. 5.3) and when they had oviposited prior to the contest ( $F_{1,110} = 5.71$ ,  $P = 0.019$ , Fig. 5.3) but were uninfluenced by wasp weight ( $F_{1,110} = 3.01$ ,  $P = 0.085$ ). Before owner oviposition, host weight ( $F_{1,54} = 4.31$ ,  $P = 0.043$ ), but not wasp weight ( $F_{1,54} = 0.12$ ,  $P = 0.728$ ), influenced egg loads. Once owners had oviposited, egg loads were influenced by wasp weight ( $F_{1,54} = 5.57$ ,  $P = 0.022$ , Fig. 5.2b), but not host weight ( $F_{1,54} = 3.80$ ,  $P = 0.057$ ) or clutch size ( $F_{1,54} = 0.09$ ,  $P = 0.770$ ).

We repeated all analyses of egg load treating host sizes as a categorical (large or small) rather than a continuous variable (actual weight) and obtained identical interpretations. Because egg loads can be correlated with both our measure of resource-uncorrelated RHP (wasp size) and variables associated with RV (wasp age, host size, oviposition), we avoided collinearity (see above) by excluding egg load as an explanatory variable from the following analyses of contest behaviour.



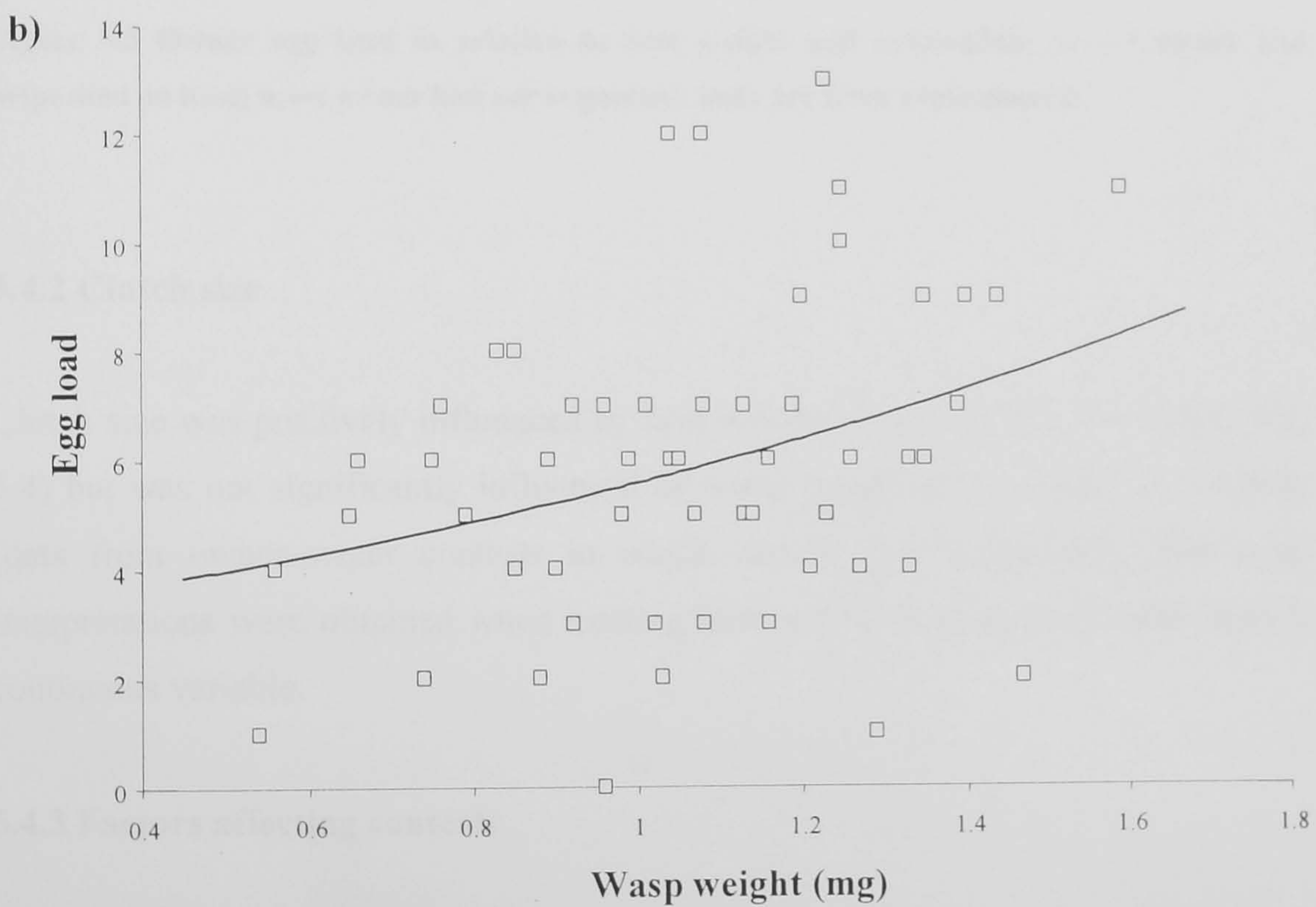
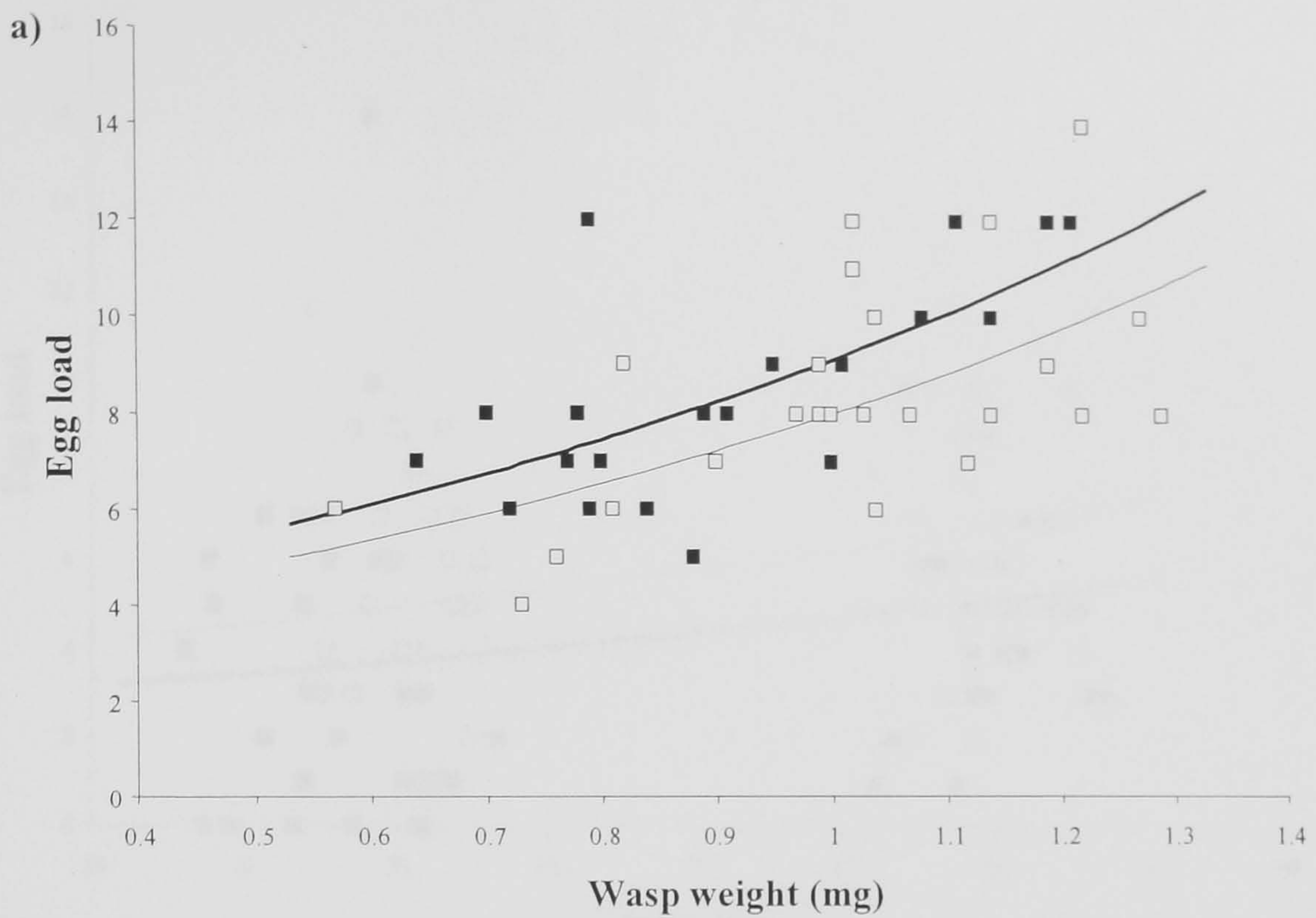


Figure 5.2 Egg load in relation to wasp weight. a) Intruder egg load in relation to wasp weight and age □, —: intruder 3 days old; ■, —: intruder 5 days old. Data are from experiment 1. b) Owner egg load in relation to wasp weight when owners had previously oviposited. Data are from experiment 2.



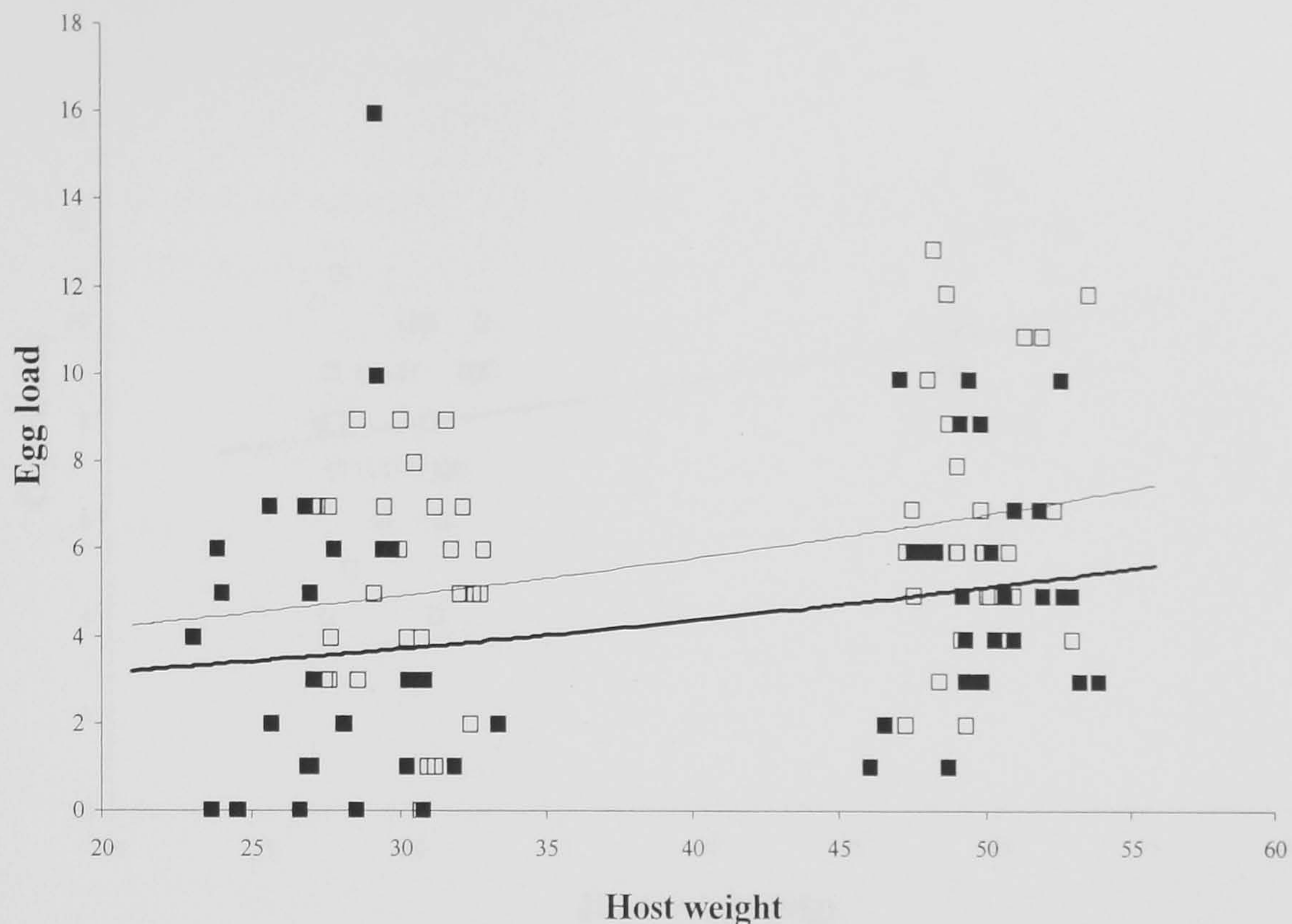


Figure 5.3 Owner egg load in relation to host weight and oviposition. □, —: owner had oviposited on host; ■, - - -: owner had not oviposited. Data are from experiment 2.

#### 5.4.2 Clutch size

Clutch size was positively influenced by host weight ( $F_{1,54} = 10.42$ ,  $P = 0.002$ , Fig. 5.4) but was not significantly influenced by wasp weight ( $F_{1,54} = 0.06$ ,  $P = 0.808$ ) (data from owner-owner contests in which owners had oviposited). The same interpretations were obtained when treating host size as a categorical rather than a continuous variable.

#### 5.4.3 Factors affecting contests

##### 5.4.3.1 Experiment 1: owner-intruder contests

There was a clear winner in 51 of the 53 contests. Both unresolved contests involved similarly aged females and in one the females both occupied the host until the end of



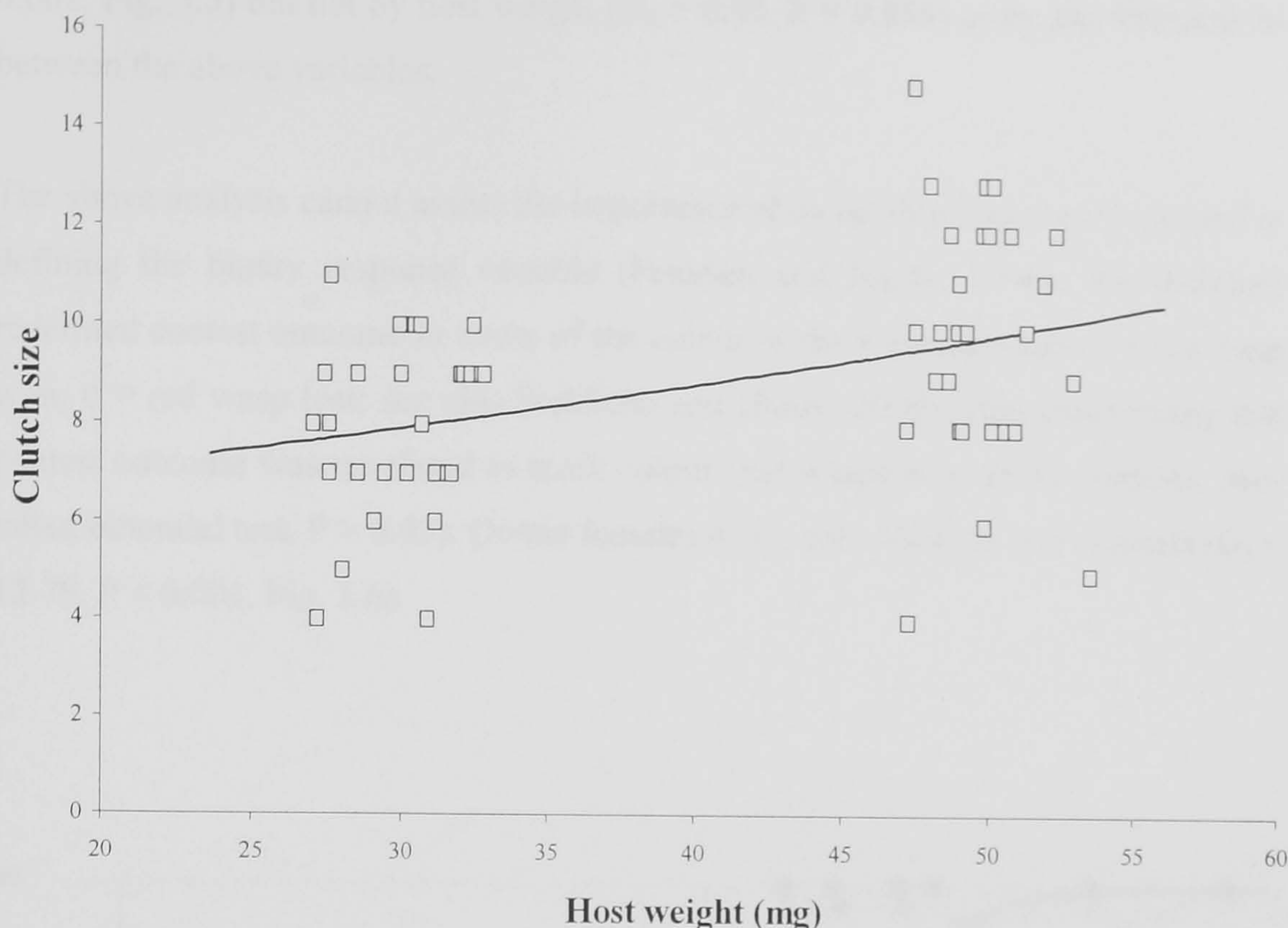


Figure 5.4 Clutch size of owners in relation to host weight. Data are from experiment 2.

the observation period. We restrict our analysis to contests with a clear winner: 29 between similar aged wasps and 22 in which intruders were older than owners. The same suite of behavioural interactions were observed as in previous studies [chasing, biting, stinging, grappling (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000)] and additionally we observed one instance of fatal fighting. In this case the intruder, which was older and 0.05mg heavier than the owner, was killed (apparently by stinging) after five fights, some lasting for up to 67 seconds.

We first defined contest outcome as a binary response: 1 = intruder won, 0 = intruder lost and explored the influence of the following explanatory variables: absolute wasp weight difference (intruder weight – owner weight), owner's host size (large or small), wasp age difference (same age or older intruder than owner) and their interactions. The probability of the intruder taking over the host was influenced by both weight difference (larger intruders were more successful,  $G_1 = 27.76$ ,  $P < 0.001$ , Fig. 5.5) and age difference (older intruders were more successful,  $G_1 = 9.99$ ,  $P =$



0.002, Fig. 5.5) but not by host weight ( $G_1 = 0.03$ ,  $P = 0.856$ ) or by any interactions between the above variables.

The above analysis cannot assess the importance of ownership because this is used in defining the binary response variable (Petersen and Hardy, 1996). We therefore redefined contest outcome in terms of the colour of the winning wasp (1 = red wasp won, 0 = red wasp lost; see also Stokkebo and Hardy, 2000), after establishing that contest outcome was unrelated to mark colour (red wasps won 25/51 contests, two-tailed binomial test,  $P > 0.05$ ). Owner females were more likely to win contests ( $G_1 = 12.79$ ,  $P < 0.001$ , Fig. 5.6).

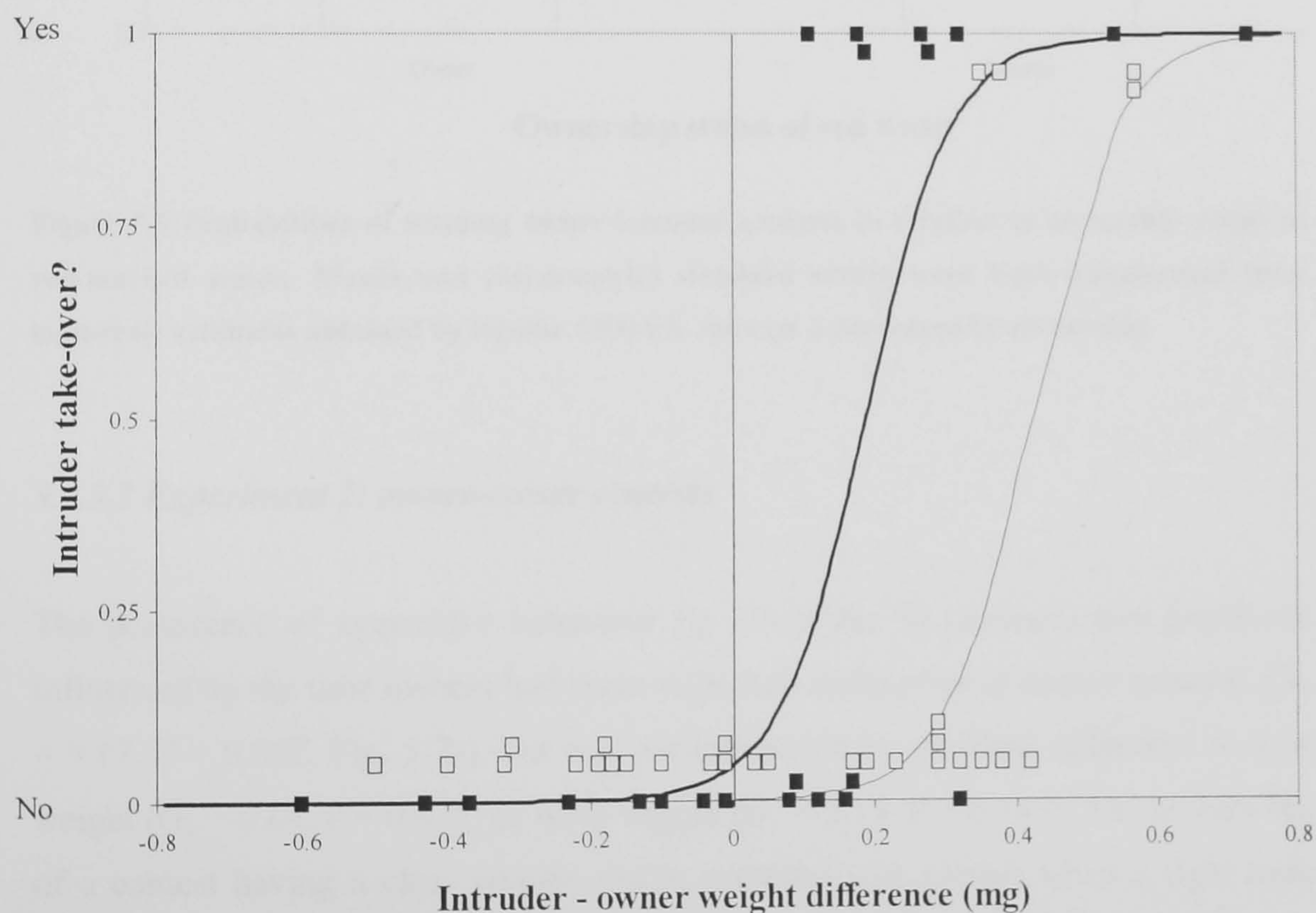


Figure 5.5 Owner-intruder contest outcome in relation to difference in wasp weight and age. The curves show the probability of the intruder winning the encounter estimated from logistic regression when the intruder was either the same age ( $\square$ , —) or older ( $\blacksquare$ , —) than the owner. Binary data are shown vertically displaced to show numbers of observations. Intruder success is increased by both greater body size and age.



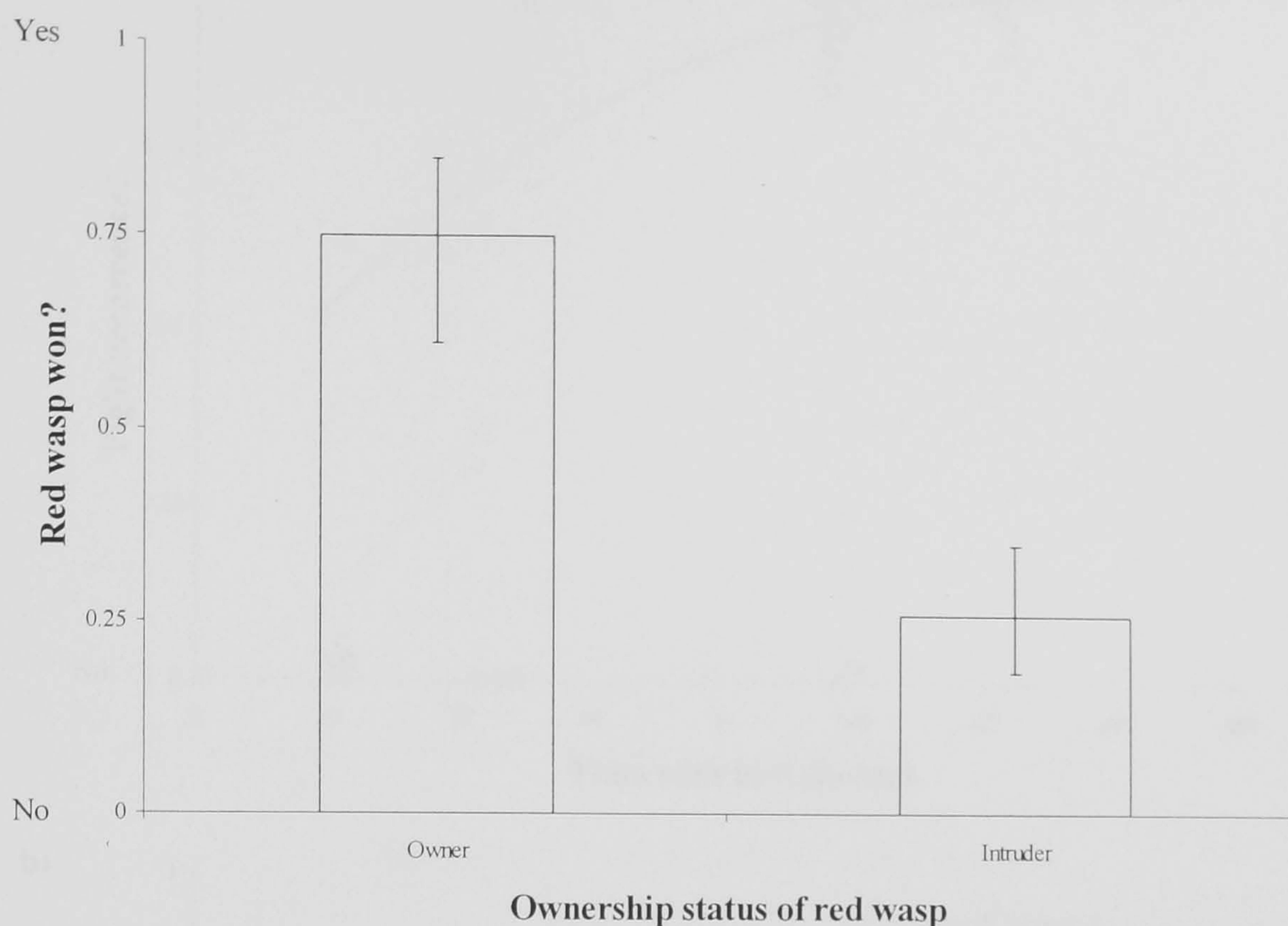
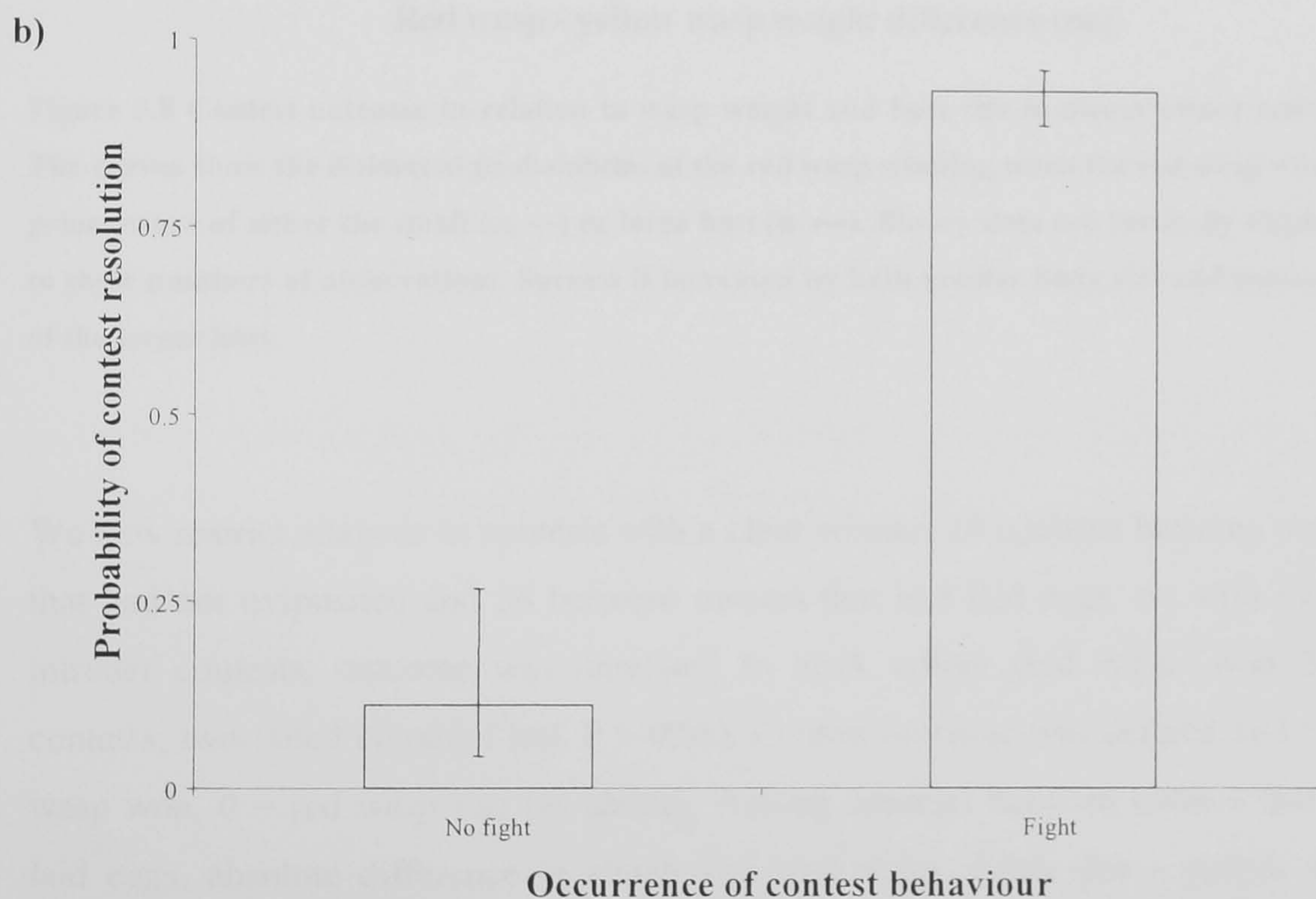
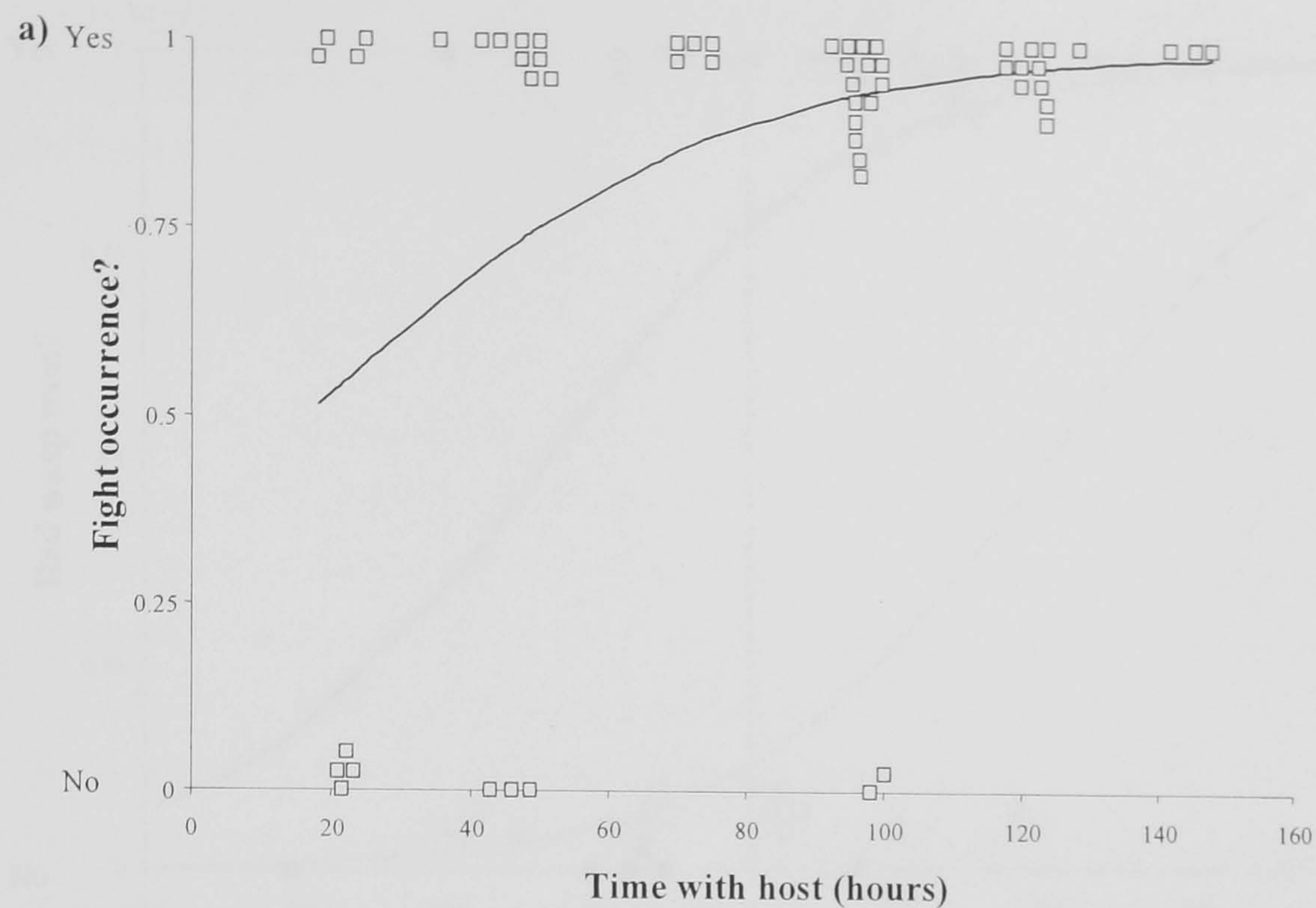


Figure 5.6 Probabilities of winning owner-intruder contests in relation to ownership status of red-marked wasps. Means and (asymmetric) standard errors were back-transformed from logit-scale estimates obtained by logistic ANOVA. Success is increased by ownership.

#### 5.4.3.2 Experiment 2: owner-owner contests

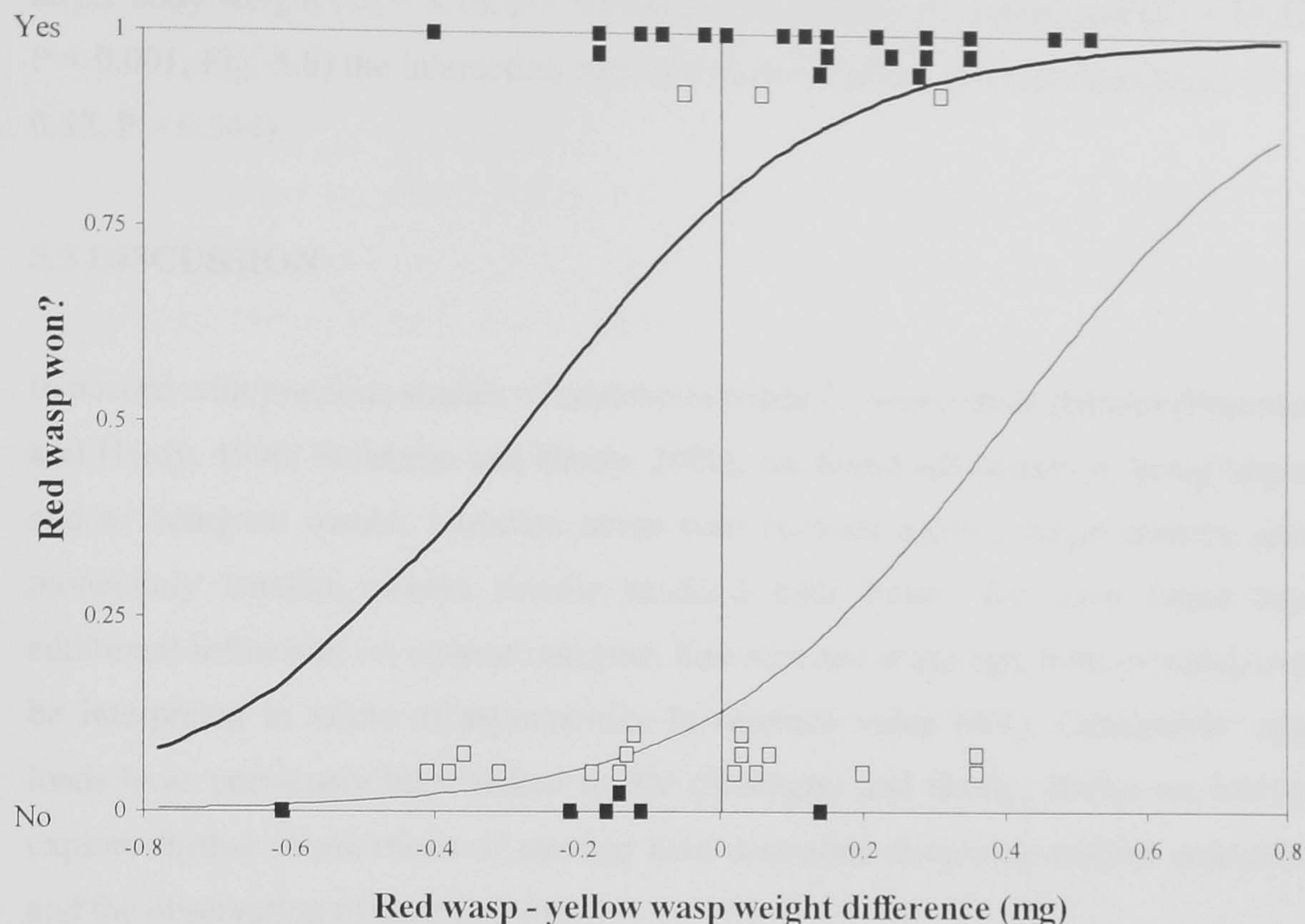
The occurrence of aggressive behaviour (in 47 of the 56 contests) was positively influenced by the time owners had spent with their hosts prior to barrier removal ( $G_1 = 9.17$ ,  $P = 0.002$ , Fig. 5.7a), but was not influenced by absolute difference in host weight ( $G_1 = 0.64$ ,  $P = 0.425$ ) or wasp weight ( $G_1 = 2.13$ ,  $P = 0.145$ ). The probability of a contest having a clear winner (46/56 contests) was greater when a fight took place ( $G_1 = 26.89$ ,  $P < 0.001$ , Fig. 5.7b); absolute difference in clutch size, wasp weight and host size, or their interactions, did not influence contest resolution (clutch size:  $G_1 = 2.41$ ,  $P = 0.121$ ; host weight:  $G_1 = 0.15$ ,  $P = 0.699$ ; wasp weight:  $G_1 = 0.14$ ,  $P = 0.708$ ).





**Figure 5.7** Fighting behaviour and contest resolution. a) The influence of the time that females had been with hosts prior to barrier withdrawal on the occurrence of at least one fight during the observational period. Data are binary (1 = fight occurred, 0 = no fight occurred) but are shown vertically displaced to illustrate numbers of observations. b) Relationship between the occurrence of fighting and whether a contest was clearly resolved. Means and (asymmetric) standard errors were back-transformed from logit-scale estimates.





**Figure 5.8** Contest outcome in relation to wasp weight and host size in owner-owner contests. The curves show the estimated probabilities of the red wasp winning when the red wasp was the prior owner of either the small ( $\square$ , —) or large host ( $\blacksquare$ , - -). Binary data are vertically displaced to show numbers of observations. Success is increased by both greater body size and possession of the larger host.

We now restrict analysis to contests with a clear winner: 20 contests between owners that had not oviposited and 26 between owners that had laid eggs. As with owner-intruder contests, outcome was unrelated to mark colour (red wasps won 24/46 contests; two-tailed binomial test,  $P > 0.05$ ). Contest outcome was defined as 1 = red wasp won, 0 = red wasp lost (as above). Among contests between owners that had laid eggs, absolute difference in clutch size (red wasp clutch size – yellow wasp clutch size) did not influence outcome ( $G_1 = 1.99$ ,  $P = 0.158$ ). For all owner-owner contest data together, the probability of red wasps winning was uninfluenced by whether eggs had been laid prior to the contest ( $G_1 = 0.11$ ,  $P = 0.736$ ). Finally, for all owner-owner data, we explored the influences of absolute difference in wasp weight (red wasp weight – yellow wasp weight) and host size (red wasp the owner of the large host or of the small host). The probability of winning a contest was increased by



larger body weight ( $G_1 = 8.16$ ,  $P = 0.004$ ) and by owning the larger host ( $G_1 = 17.32$ ,  $P < 0.001$ , Fig. 5.8) the interaction between these variables was non-significant ( $G_1 = 0.37$ ,  $P = 0.544$ ).

## 5.5 DISCUSSION

In accord with previous studies of contests between *G. nephantidis* females (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000), we found advantages to being larger and to being an owner: intruders never won contests against larger owners, and moderately smaller owners usually retained their hosts. We have found two additional influences on contest outcome, host size and wasp age, both of which can be interpreted in terms of asymmetries in resource value (RV). Contestants' egg loads have previously been linked to RV (Stokkebo and Hardy, 2000): we briefly explore further implications of our egg load data after discussing contest outcomes and the observation of fatal fighting.

### 5.5.1 Resource value and contest outcome

#### 5.5.1.1 Host size

We found an influence of host size on the outcomes of owner-owner contests: the female with the larger host usually won. We interpret this as an outcome dictated by difference in RV. We predicted that owners of large and of small hosts would differ in their evaluation of their host resources. Parasitoids generally assess host quality before oviposition (e.g. Schmidt, 1991; Godfray, 1994) and gregarious parasitoids generally lay larger clutches on larger hosts (e.g. le Masurier, 1987; Luft, 1993; Godfray, 1994; Mayhew, 1998; Hardy *et al.*, 1998; Zaviezo and Mills, 2000). *Goniozus nephantidis* is no exception: when provided with larger hosts, larger broods of ultimately larger-sized offspring are produced (Hardy *et al.*, 1992; this study). It is entirely consistent with these life-history characteristics that owners should value larger hosts more than smaller hosts and compete more strongly to retain them. To our knowledge, this is the first report of host size affecting contest outcome in parasitoids, and also the clearest example of RV asymmetries operating in parasitoid



contests more generally (Hughes *et al.*, 1994; Field and Calbert, 1998, 1999; Stokkebo and Hardy, 2000).

In general, owners are expected to have a better estimate of RV than intruders, which would lead to instances of smaller owners winning against larger intruders (Enquist and Leimar, 1987; Dugatkin and Biederman, 1991; Hack, Thompson and Fernandes, 1997; Nijman and Heuts, 2000). Despite the strong effect on *G. nephantidis* owner-owner contests, an influence of host size was not found in owner-intruder contests, in which an asymmetry in resource-correlated RHP (ownership) was present. Similarly, Stokkebo and Hardy (2000) only detected an influence of egg load asymmetry (interpreted in terms of RV) on contest outcome when removing or minimising asymmetries in both resource-correlated and resource-uncorrelated RHPs (both contestants were owners and were size-matched). In both cases, the studied difference in RV is confounded with resource-correlated RHP, because owners generally have more eggs (Stokkebo and Hardy, 2000) and, by definition, more host resource (this study) than intruders. Despite a large literature on animal contests, there have been few studies that have evaluated the influence of resource value as well as resource correlated and uncorrelated components of RHPs. However, there are some analogous studies that have found resource-size related RV effects in other taxa (e.g. Verrell, 1986; Lindström, 1992; Hack *et al.*, 1997; Lindström and Pampoulie, 2005). For instance, in sand gobies smaller owners of a nest site challenge their larger opponents more actively when defending a large (high RV) nest than when defending a small (low RV) nest (Lindström, 1992) and individuals that take over large nests are larger than those that take over small nests (Lindström and Pampoulie, 2005), suggesting that a greater asymmetry in resource-uncorrelated RHP (fighting ability) is needed for intruder take-over when RV is high.

#### **5.5.1.2 Contestant age**

There was an influence of contestant age on the outcome of owner-intruder contests: intruders which were older than owners generally required a smaller size advantage to take-over the resource than did intruders aged the same as owners. Combatant age has been found to influence contest outcome in a range of other invertebrate species, for example female Mediterranean fruit flies (Papaj and Messing, 1998), adult male

butterflies (Kemp, 2000, 2002, 2003) and male crab spiders (Hu and Morse, 2004). Age could potentially affect contests via correlations between age and RHP and/or between age and RV. For instance, if individuals engage in multiple contests, older individuals may be favoured by enhanced contest ability (RHP) generated by their prior experience (Hsu and Wolf, 2001; Kemp and Wiklund, 2004). Alternatively, older individuals may have reduced RHP due to a relatively poor nutritional stage (no opportunity to host feed while foraging) or may have reduced weaponry as they age and develop (as in the larvae of some species of endoparasitoids, e.g. Lawrence, 1988; Mackauer, 1990; Marris and Casper, 1996). These RHP-based explanations cannot, however, explain the observed influence of *G. nephantidis* age as individuals had no prior contest experience and in terms of contest success were favoured, not hindered, by greater age. The observed effect of age is more likely due to differences in RV: it is consistent with the hypothesis that ownership effects would be more often overridden when intruders are older because older intruders value more greatly the opportunity to obtain a host (see also Parker, 1974; Grafen, 1987; Enquist and Leimar, 1990; Korona, 1991). An analogous result from studies of parasitoid foraging behaviour is that acceptance of lower quality (previously parasitized) hosts is more common when the foraging female is older (Visser *et al.*, 1992; Goubault *et al.*, 2005).

#### 5.5.1.3 Fatal fighting

In contests between *G. nephantidis* females, escalation has frequently been observed to include combinations of chasing, biting, grappling and attacks with stingers (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000) but the current study is the first to observe a fatal fight. In this instance, the intruder was killed during an owner-intruder contest in which the resource-uncorrelated RHP and RV estimates of the competitors may have been similar: the intruder was older and marginally (0.05mg) heavier and the owner was defending a large host. It is notable that this fatal interaction involved a series of fights, some lasting more than one minute, whereas previously reported fight durations in *G. nephantidis* are around 1-3 seconds (Petersen and Hardy, 1996). Fatal intraspecific fights are generally rare amongst adult parasitoids (Batchelor *et al.*, 2005; Chapter 3) but occur commonly in a few species, such as fig wasps and species of *Melittobia*, where males compete for



extremely limited mating opportunities (Cook *et al.*, 1999; West *et al.*, 2001; Abe *et al.*, 2003). Fatal fights have also been commonly observed in interspecific contests between bethylid females, even though fatalities are not observed when the same species compete with conspecifics (Pérez-Lachaud *et al.*, 2002; Batchelor *et al.*, 2005; Chapter 3).

### 5.5.2 Egg load and clutch size

Egg loads varied greatly but were positively related to body size and age (as previously found by Stokkebo and Hardy, 2000). Additionally, owners of larger hosts had more eggs (this study), which is akin to the finding that, before oviposition, owners have higher egg loads than non-owners (Stokkebo and Hardy, 2000). Egg load thus confounds with variables associated with both RHP (body size, ownership) and RV (host size, wasp age, ability to exploit hosts quickly). Unexpectedly, we further found that owners that had already oviposited had higher egg loads than same-aged owners that had not yet laid any eggs. *Goniozus nephantidis* clutch size is positively correlated with host size and females are able to produce multiple clutches if provided with a succession of fresh hosts (Hardy *et al.*, 1992). The maturation of excess eggs thus suggests both that females anticipate future reproductive opportunities and that being gravid is not greatly detrimental to subsequent foraging ability (Luft, 1993). The more hosts females generally parasitize, the stronger the trade-off between current and future reproduction is likely to be. This may weaken the argument that *G. nephantidis* might be expected to produce 'Lack' clutch sizes, maximising fitness per brood, although clutch-size independent brood guarding periods would still select towards brood fitness maximisation (Hardy *et al.*, 1992). While it is unknown how many hosts *G. nephantidis* females normally parasitize in the field (Cock and Perera [1987] have suggested 1-2), our egg load data suggest that anticipated future reproduction may explain why *G. nephantidis* clutch size is smaller than the Lack clutch size calculated from laboratory-estimated fitness parameters and the assumption of semelparity (Hardy *et al.*, 1992). Other, contest-behaviour related, possibilities are explored in Petersen and Hardy (1996) and Mesterton-Gibbons and Hardy (2004).

### 5.5.3 Conclusion

We have explored influences on the value of resources (RV) to *G. nephantidis* females. Our data support the prediction that owners of large hosts defeat owners with small hosts due to an asymmetry in RV. There is also support for the hypothesis that ownership effects will be weaker when intruders are older because older females estimate the RV of the host more highly. Females' egg loads were highly variable and confounded with other variables that may influence contest outcomes via asymmetries in RV and RHP. The high egg loads of females that have recently oviposited suggest that subsequent reproductive opportunities may be common and clutch size optima may consequently be reduced compared to under the assumption of semelparity. Overall, studies of *G. nephantidis* show that asymmetries in both RHPs and RVs interact to influence contest outcomes. Despite a considerable body of literature on the effects of host size on parasitoid behaviour, we believe that this is the first study to show an effect of host size on parasitoid contest outcomes.



## CHAPTER 6 - CHEMICAL RELEASE BY BETHYLIDS DURING AGONISTIC ENCOUNTERS

### 6.1 ABSTRACT

The bethylid literature contains two reports of species that release volatile chemicals, possibly during agonistic interactions. We use mass spectrometry to investigate whether volatile chemicals are released by eight bethylid parasitoid species and relate these to their phylogeny. We find that, under stressful conditions, male and female members of two species in the Bethylid sub-family Bethylinae release a spiroacetal, 2-methyl-1,7-dioxaspiro[5.5]undecane, and that males and females of five out of six studied species from the Bethylid sub-family Epyrinae release a chemical of 131Da, believed to be 3-methylindole (skatole). Releases are discrete events under active control by the wasps. We then carry out real-time chemical analyses of agonistic interactions during dyadic contests between *G. nephantidis* females. Contests were set-up between pairs of wasps with either one or both of them in possession of a host. Release during contests was not observed in the majority of cases of female–female interaction, implying discrimination in its release. The real-time technique permits the determination of the exact timing and relative quantity of chemical releases, in tandem with behavioural observations of individuals. Thus, it allows new and interesting opportunities to study the reasons for, and effects of, chemical releases in small terrestrial animals.

## 6.2 INTRODUCTION

Animals often employ chemicals in their behavioural interactions with conspecifics. These chemicals may have diverse functions, including sexual attraction (Wyatt, 2003; Ardeh *et al.*, 2004; Nojima *et al.*, 2005), social organisation (Hurst *et al.*, 1996; Billen and Morgan, 1998; Wyatt, 2003; Cuvillier-Hot *et al.*, 2004; D'Ettore *et al.*, 2004), territorial marking (Gosling *et al.*, 1996; Gosling and Roberts, 2001; White, Swaisgood and Zhang, 2002; Wyatt, 2003) and signals or weapons used during contests (Gosling *et al.*, 1996; Breithaupt and Eger, 2002; Nevison *et al.*, 2003; Wyatt, 2003; Bergman, Martin and Moore, 2005). Studying the identity, timing and effects of chemicals released in these interactions can be experimentally challenging. Much prior research has been restricted to the collection of volatile substances onto absorbent blocks for chemical analysis (e.g. by Gas Chromatography-Mass Spectrometry) subsequent to, but not during, behavioural studies (e.g. Hernández, Cabrera and Jaffe, 1999; Gómez *et al.*, 2005). Other investigations have employed physiological techniques, such as electroantennography (Wyatt, 2003, p 29; e.g. Zhu *et al.*, 2000; D'Ettore *et al.*, 2004; Nojima *et al.*, 2005) which monitors the nervous impulses in detached antennae in response to chemicals but cannot assess behavioural responses of the whole organism. As a result, many previous studies have only been able to conclude that an organism produced a specific chemical or that an individual responds to a particular chemical stimulus but could not relate this accurately to a dynamic behavioural event. With very few exceptions (Breithaupt and Eger, 2002; Monnin *et al.*, 2002; Bergman *et al.*, 2005) analyses of dyadic interactions involving both behavioural events and chemical exchange have therefore not been possible. Here we develop and use a method that addresses this problem in the study of the behaviour of small terrestrial animals.

Here our principal study organism is the parasitoid wasp *Goniozus nephantidis* (Hymenoptera: Bethyridae, subfamily Bethylinae) in which females are known to engage in dyadic contests for reproductive opportunities (Petersen and Hardy, 1996; Stokkebo and Hardy 2000; Chapter 5). Two bethyrid species, *Cephalonomia gallicola* and *C. stephanoderis*, in the sub-family Epyrinae have been reported to release a volatile chemical (skatole, a methylindole) when experimentally stressed (Kuwahara, 1984; Gómez *et al.*, 2005). We first investigate whether volatile



chemicals are released by *G. nephantidis* and by further bethylid species: we find that a spiroacetal is released by artificially stressed *G. nephantidis*. We then monitor in real-time the release of this chemical during female-female contest interactions, using Atmospheric Pressure Chemical Ionisation – Mass Spectrometry (APCI-MS). This method allows us to continuously record the timings, identities and relative quantities of chemicals released whilst simultaneously recording the behavioural interactions between individuals.

### 6.2.1 Contest behaviour

Dyadic contests for limited resources take place in a wide variety of taxa, and these interactions have been the focus of ongoing interest for animal behaviourists (see reviews in Maynard Smith, 1982; Huntingford and Turner, 1987; Mesterton-Gibbons and Adams, 1998; Riechert, 1998; Chapter 3, 5). Individuals may compete for feeding and reproductive opportunities and contests can vary from non-aggressive interactions settled by convention (which thus avoid injury for both competitors) through to full escalation that can result in injurious, and even fatal, fighting (Grafen, 1987; Enquist and Leimar, 1990; Chapter 3). Contests can be settled due to asymmetries in the value the contestants place on the resource (resource value; RV) or the competitors' abilities to gain or retain possession of the resource (resource holding potential; RHP) (Parker, 1974; Maynard Smith and Parker, 1976). Asymmetries in resource-uncorrelated RHP (fighting ability) or resource-correlated RHP (attributes associated with prior ownership) may be assessed by individuals before or during a contest, through the use of a range of behaviours, which can include chemical releases.

### 6.2.2 Contests in *Goniozus nephantidis*

*Goniozus nephantidis* (Muesebeck) (Hymenoptera: Bethyridae) is a gregarious ectoparasitoid of larvae of the coconut pest *Opisina arenosella* (Walker) (Lepidoptera: Oecophoridae) (Cock and Perera, 1987). Adult female *G. nephantidis* paralyse their host before laying a clutch of up to 18 eggs on the host (Hardy *et al.*, 1992). Females then guard their brood against conspecifics and allospecifics (Hardy and Blackburn, 1991; Petersen and Hardy, 1996). Guarded hosts can be encountered

by further females, which leads to the classic owner-intruder contests considered by many game-theoretic models and in studies of many other animal taxa. In owner-intruder contests in the bethylid *G. nephantidis*, owners of a host usually win, which may be interpreted as due to asymmetries in resource-correlated and resource-uncorrelated RHP but also in terms of resource value (RV) asymmetries due to differences in the number of unlaidd ripe eggs (physiological state) carried by the contestants (Stokkebo and Hardy, 2000). Absolute body size asymmetry also influences a female's success in contests (Petersen and Hardy, 1996; Chapter 5) and further manipulative experiments show that owner-owner contests are influenced by host value and owner-intruder contests are influenced by wasp age (both RV asymmetries, Chapter 5). While the above studies have provided understanding of factors influencing contest outcomes, none have considered the potential role of chemicals in female-female contests.

## 6.3 MATERIALS AND METHODS

### 6.3.1 Atmospheric Pressure Chemical Ionisation - Mass Spectrometry

Atmospheric Pressure Chemical Ionisation - Mass Spectrometry (APCI-MS) can be used for the real-time monitoring of gas phase volatiles (Linforth and Taylor, 1998; Taylor *et al.*, 2000; Taylor and Linforth, 2003). The gas phase is continuously drawn into the MS with no chromatographic separation, hence there is no sample loss or discrimination during sampling. The low dead volume and response rate of the system allow rapid changes to be tracked (up to 50 observations per second) with good sensitivity (limit of detection ca. 0.1mg/m<sup>3</sup>).

Gas phase samples were continuously sampled into an Atmospheric Pressure Chemical Ionisation source (MS Nose<sup>TM</sup>, Waters Manchester UK) mounted on an LCZ quadrupole mass spectrometer (Waters). The sample stream (20ml/min) was conducted through a heated (160°C) transfer line via a deactivated fused silica tube (1m × 0.53mm ID). The analytes entering the source were ionized by a 4kV corona discharge (that results in a cascade of charge transfer), typically resulting in their protonation to form MH<sup>+</sup>. For full scan analysis the mass spectrometer acquired 1 scan per second over the mass range 25 to 260Da, with the cone voltage set to either



18 or 28V. For selected ion mode the mass spectrometer monitored  $m/z$  171 only with a dwell time of 0.02s and a cone voltage of 18V.

### **6.3.2 Temporal dimension and quantity of releases**

The analytical system was originally designed (Linforth and Taylor, 1998) to study changes in gas phase composition with minimal delays between a change taking place and its detection. Consequently it is also possible to observe and quantify (given appropriate standards) the rise in level of the chemical on release and its decay. Parameters such as the width of the peak at half maximum height (typically used to assess chromatographic peaks), and the time taken to reach maximum height can be used to describe the shape of the volatile release events. Estimation of the gas phase concentration of the chemical released, 2-methyl-1,7-dioxaspiro[5.5]undecane, was achieved using the calibrants tetrahydropyran and 1,7-dioxaspiro[5.5]undecane (Aldrich, Gillingham UK) both of which have structural similarities to 2-methyl-1,7-dioxaspiro[5.5]undecane, which was unavailable. Known amounts of the calibrants were continuously introduced into the source make-up gas of the APCI-MS in a dilute solution of cyclohexane (Fisher, Loughborough UK). The gas phase concentration was estimated by comparison of the height of the release obtained for the calibrants, with that of the 2-methyl-1,7-dioxaspiro[5.5]undecane. The height of the release is proportional to the amount of a compound entering the source of the MS, and hence in the case of the gas phase, its concentration (given the sampling flow rate).

### **6.3.3 Chemical release by bethylids**

We investigated whether bethylid wasps release chemicals when individuals were crudely squashed with forceps close to the sampling point of the APCI-MS. Subsequently, experiments were performed where the air around the wasp was sampled and then, after a minute, a fine paintbrush was introduced into the glass tube and used to stress the wasp by repeated prodding of the wasp. The level of the volatile was continuously monitored to see if it was present before and after the stress treatment.

Using the stressing approach, we investigated the molecular weights of the chemicals released by males and females of eight bethylid species: two members of the sub-family Bethylinae (*G. nephantidis* and *G. legneri*) and six members of the sub-family Epyrinae (*Cephalonomia hyalinipennis*, *C. stephanoderis*, *C. tarsalis*, *C. waterstoni*, *Laelius pedatus* and *Prorops nasuta*). This gives us an indication of whether the Bethylinae and Epyrinae release the same, or similar, chemicals when stressed. Both *Goniozus* species are reared in our laboratory; *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* were supplied by Juan Barrera and Gabriela Pérez-Lachaud at ECOSUR, Tapachula, Mexico; *C. tarsalis* and *C. waterstoni* were supplied by Ralf Howard, USDA-ARS, Manhattan, Kansas, USA; *L. pedatus* was supplied by Peter Mayhew, University of York, UK.

#### 6.3.4 Gas Chromatography – Mass Spectrometry (GC-MS)

To identify the volatile chemical released by *G. nephantidis*, 10 individuals were partially crushed and placed in a 100mL flask. 300mL of headspace from the flask were displaced using a stream of nitrogen through a Tenax trap over a 5min period. The Tenax trap was desorbed at 240°C for 10 min using a CHISA injector (SGE, Milton Keynes, UK), onto a 40cm long region of the column (25m × 0.22mm ID, BP-1, 1.0µm film thickness; SGE), which was cooled by immersion in liquid nitrogen, which formed a cold trap. After transfer of the volatile compounds onto the column, the liquid nitrogen was removed and the gas chromatograph (Hewlett Packard 5890, Wokingham, UK) temperature program started (carrier gas helium, 20psi). The initial temperature (30°C) was held for 2min and then increased at 5°C/min to 100°C and then at 10°C/min to 200°C. Spectra were recorded using a Fisons MD800 mass spectrometer (Loughborough, UK), scanning from  $m/z$  25 to 200 at 2 scans/s.

#### 6.3.5 Real time analysis of contests between *G. nephantidis* females

*Goniozus nephantidis* was reared on a factitious host, the stored product pest *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) due to the difficulty of rearing its natural host, *O. arenosella*, in the laboratory. Parasitoid cultures were maintained at 26±2°C and a 8 L:16 D photoperiod.



All parasitoids used were mated females. Contests were between two owners or an owner versus an intruder (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). Owners were classed as individuals that had been supplied with a single host of *C. cephalonica* and had been left with that host in a glass vial (7.5cm × 2.4cm), which was stoppered with cotton wool and nylon gauze fabric bung, for between 2-4 days. Owners paralysed their host during this period and in some circumstances also laid eggs on the host; in either case they were considered 'in possession' of the host. Intruders had not had any previous contact with a host, but were kept in an empty glass vial (7.5cm × 2.4cm), which was stoppered with a cotton wool and nylon gauze fabric bung, also for 2-4 days. No wasps had previous experience of a contest.

Interactions between females were observed using apparatus consisting of an opaque plastic block with three chambers linked by a slot (through which parasitoids can pass) and covered with clear Plexiglas (see Fig. 1 in Petersen and Hardy, 1996). Using sliding barriers, chambers can either be isolated or connected. Putative intruders were initially placed in a separate chamber from the owner and the host. When chambers were connected, intruders could enter the chamber containing the host of their own volition, rather than being forced into a contest situation. To monitor chemical release a small hole was drilled into the side of the block through to the central chamber. The end of the MS transfer line was then introduced to the central chamber to allow monitoring of chemical release during contests. A small piece of nylon gauze was placed into the hole between the end of the transfer line and the central chamber to prevent wasps entering or contacting it directly. The chemicals emitted by females were monitored by connecting the chamber to the mass spectrometer for direct analysis of volatiles in the gas phase.

Owner wasps were introduced to the central compartment of the contest block and allowed to settle for 5 minutes prior to experiments. Intruders were placed in an outer chamber and separated from owners by a barrier, also for 5 minutes. A total of 20 owner-owner contests and 20 owner-intruder contests were undertaken. Temperature was the ambient temperature in the laboratory (range  $19 \pm 2^{\circ}\text{C}$ ).

The ion trace was started with the probe unattached to the block for 1 minute. The block was then connected to the probe for between 1-2 minutes to obtain a reading for the concentration of the chemical in the block before the parasitoid interaction occurred. The observation period was started on withdrawing the barriers to allow the intruder access to the central chamber, or in owner-owner contests to allow owners to come into contact. A video camera was used to record behavioural events in the central chamber during the 30-45 minute contest period. After every contest we assessed the ability of each contestant to release 2-methyl-1,7-dioxaspiro[5.5]undecane by squeezing them individually next to the APCI-MS intake.

On viewing videos, all behavioural interactions between contestant females and their timings were recorded: this information could then be related to chemical events recorded on the ion trace. Behaviour was classified into the following categories – non-aggressive behaviour, interaction with host, chasing, biting, an attack with stinger and fighting. Patterns of behaviour could include components of each of these behavioural categories. Repeated measures ANOVA was conducted on the 40 replicates to assess whether there was a relationship between the behaviours observed and pheromone release.

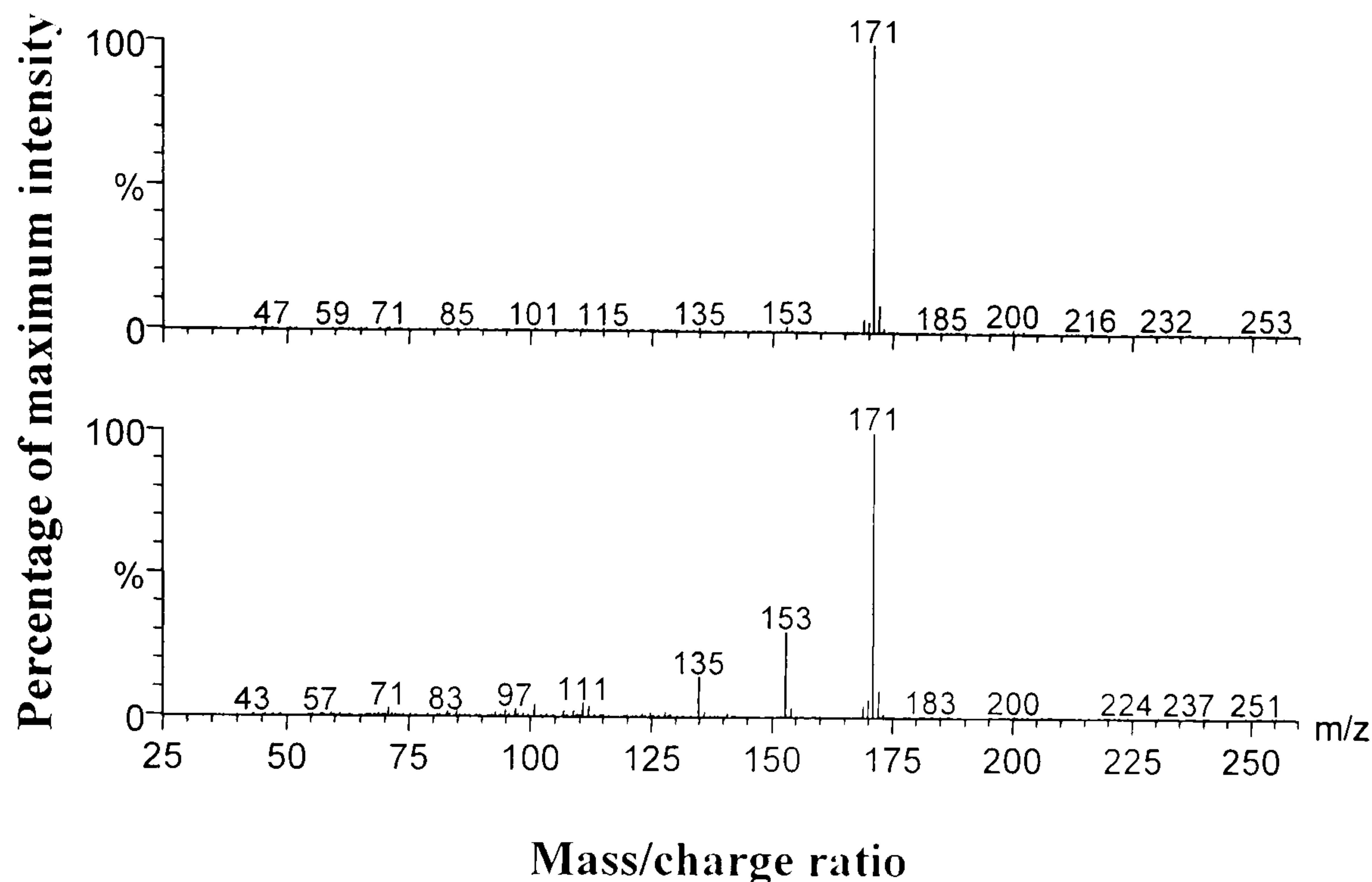
## 6.4 RESULTS

### 6.4.1 Initial screening of volatiles emitted by bethylid species using APCI-MS

Due to limited biological material, simplified analyses were conducted on seven of the bethylid species (except *G. nephantidis*). Live male (n = 1-3) and female (n = 3-8) wasps were stressed individually with a fine paintbrush next to the APCI-MS probe. In the Epyrinae, only *L. pedatus* did not produce a noticeable peak when stressed (n = 1 male, 3 females). Males and females of *C. hyalinipennis*, *C. stephanoderis*, *C. tarsalis*, *C. waterstoni* and *P. nasuta* all produced a single major ion at m/z 132, which is likely to be skatole (3-methylindole) (Gómez *et al.*, 2005).

Full scan analysis of *G. nephantidis* and *G. legneri* showed that, when the gas phase from around partially crushed individuals was sampled, one major ion was observed





**Figure 6.1** APCI-MS spectra produced from full-scan analysis of the gas phase around *G. nephantidis* at cone voltages of 18V (upper) and 28V (lower). Increasing the cone voltage of the mass spectrometer to 28V induced weak fragmentation of the molecule.

in the spectrum at  $m/z$  171 (Fig. 6.1). The molecular weight of the main compound detected in each species was thus thought to be 170Da (which had been protonated to form  $m/z$  171). Increasing the cone voltage of the mass spectrometer can induce weak fragmentation of the molecule. When the cone voltage was increased from 18 to 28V, further ions were observed at  $m/z$  153 and 135 in both species (Fig. 6.1), indicating the loss of one or two water molecules respectively. This indicated that the compound contained two oxygen atoms. These results, together with prior reports, allowed us to compare the chemicals released by each species with their phylogeny (Fig. 6.2).

#### 6.4.2 Identification of volatiles emitted by *G. nephantidis* using GC-MS

Additional GC-MS analyses were performed on *G. nephantidis* to identify the compound released. The main peak observed on the GC chromatogram was at 21.16min, all other peaks were less than 20% of the intensity of this peak. The mass

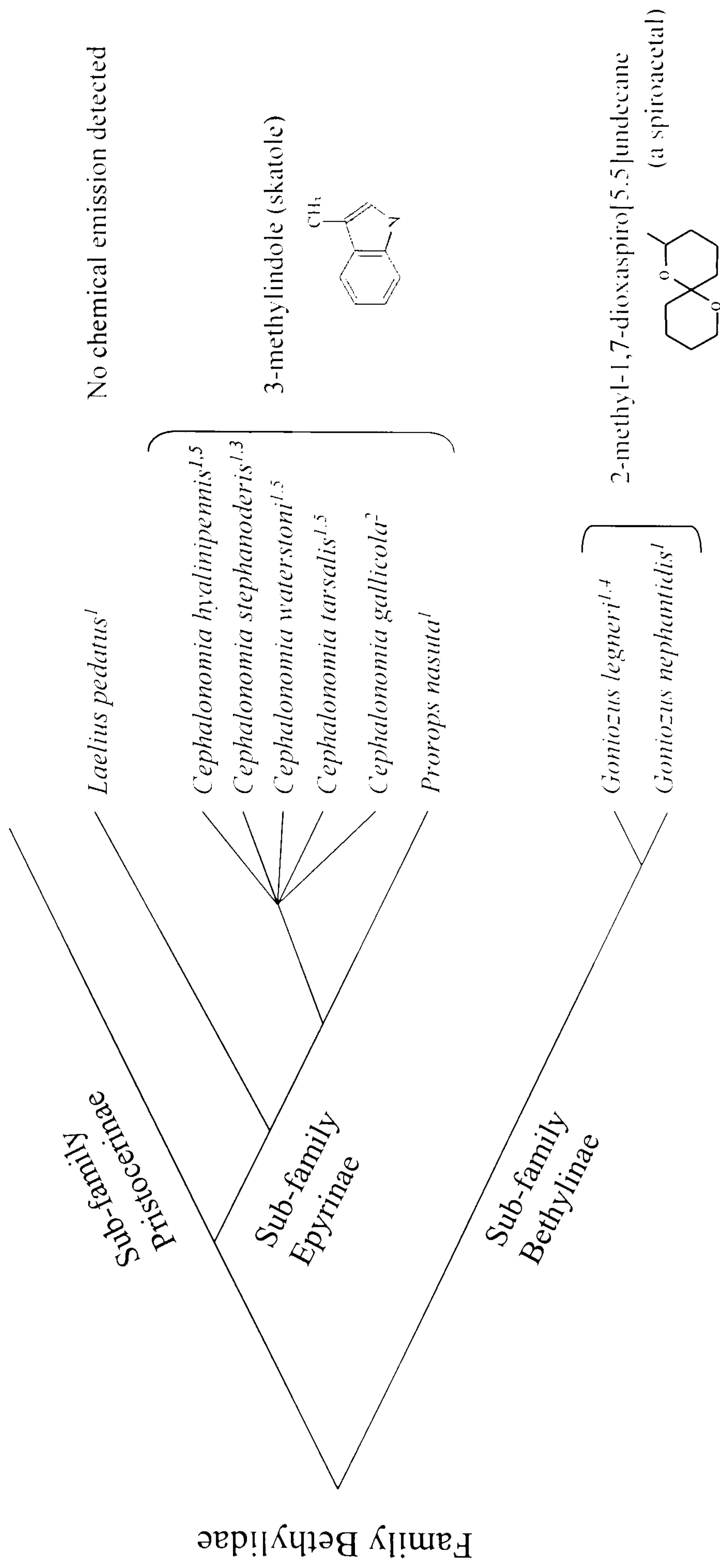
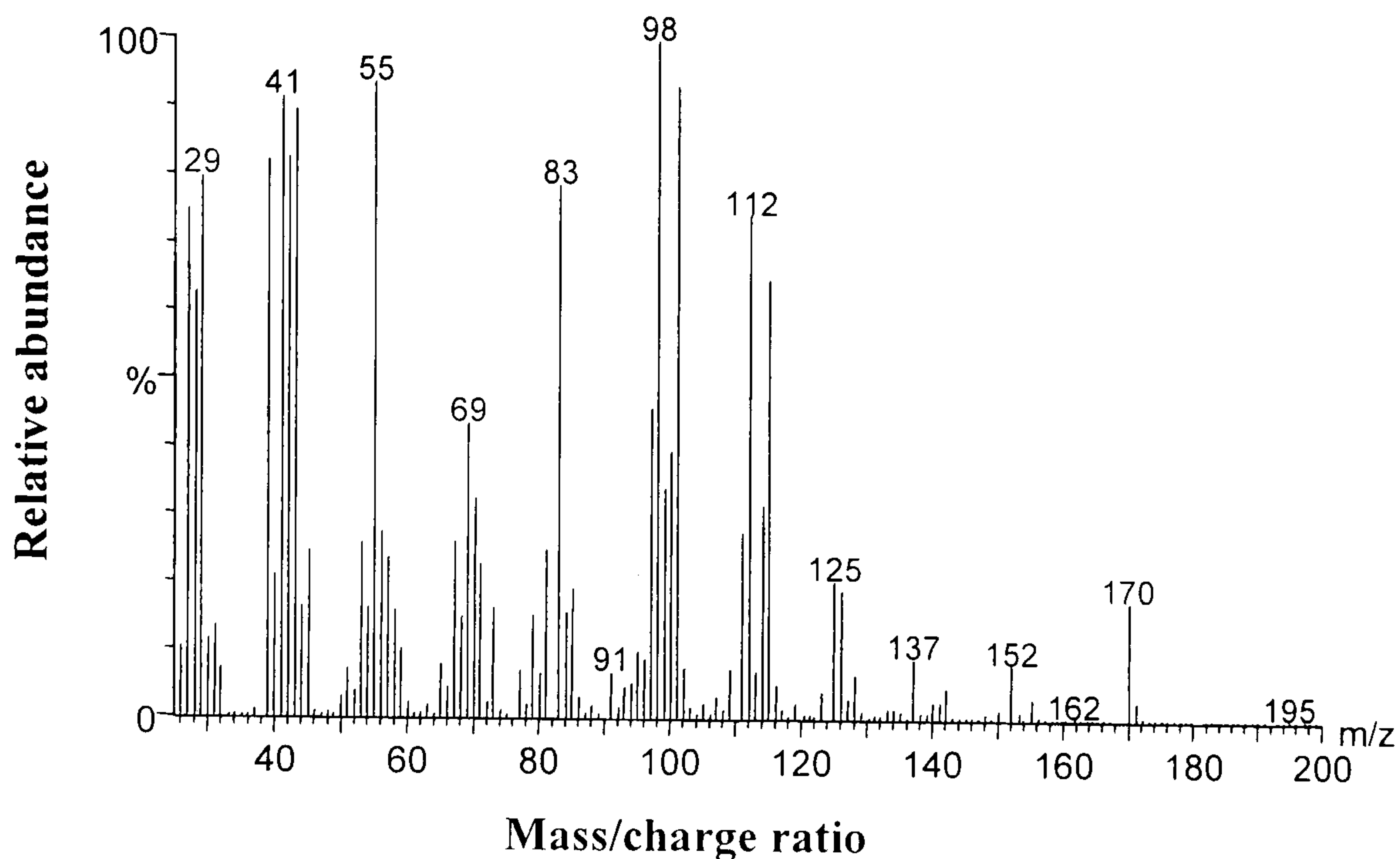


Figure 6.2 Phylogenetic relationships and chemical production of bethylid species studied. Branch lengths are schematic and not intended to show divergence times. The phylogeny is based on a simplified version of Fig. 2 in Hardy and Mayhew (1998). Results obtained by <sup>1</sup>this study, <sup>2</sup>Kuwahara (1984), <sup>3</sup>Gomez *et al.*, (2005), <sup>4</sup>Goubault *et al.*, (submitted) and <sup>5</sup>R. Howard (pers. comm.).





**Figure 6.3** GC-MS spectra of the main peak observed in the analysis of *G. nephantidis* headspace. This corresponds to the spectrum for *G. legneri* (Goubault *et al.*, submitted: Appendix).

spectrum (Fig. 6.3) was consistent with that of 2-methyl-1,7-dioxaspiro[5.5]undecane, a spiroacetal with two 6-membered rings and one methyl group attached to an alpha-carbon. This compound has a molecular weight of 170Da and would be expected to protonate to give an  $MH^+$  ion at  $m/z$  171, as observed in the APCI spectrum. The molecule also contains two oxygen atoms, which is consistent with the loss of the two water molecules from  $m/z$  171 to form  $m/z$  153 and 135. There was no significant presence of other compounds with a molecular weight of 170Da that would be detected at  $m/z$  171 by APCI-MS, and hence confused with 2-methyl-1,7-dioxaspiro[5.5]undecane. The changes in 2-methyl-1,7-dioxaspiro[5.5]undecane concentration could therefore be followed by monitoring  $m/z$  171. Further analyses were thus performed in selected ion ( $m/z$  171) mode to maximize sensitivity.

Initial experiments on *G. nephantidis* involved crushing the entire insect to observe chemical release. However, separation, by dissection, and crushing of the major segments of the body under the sampling point of the mass spectrometer indicated

that the chemical was stored in, and released from, the head, but not the thorax or abdomen.

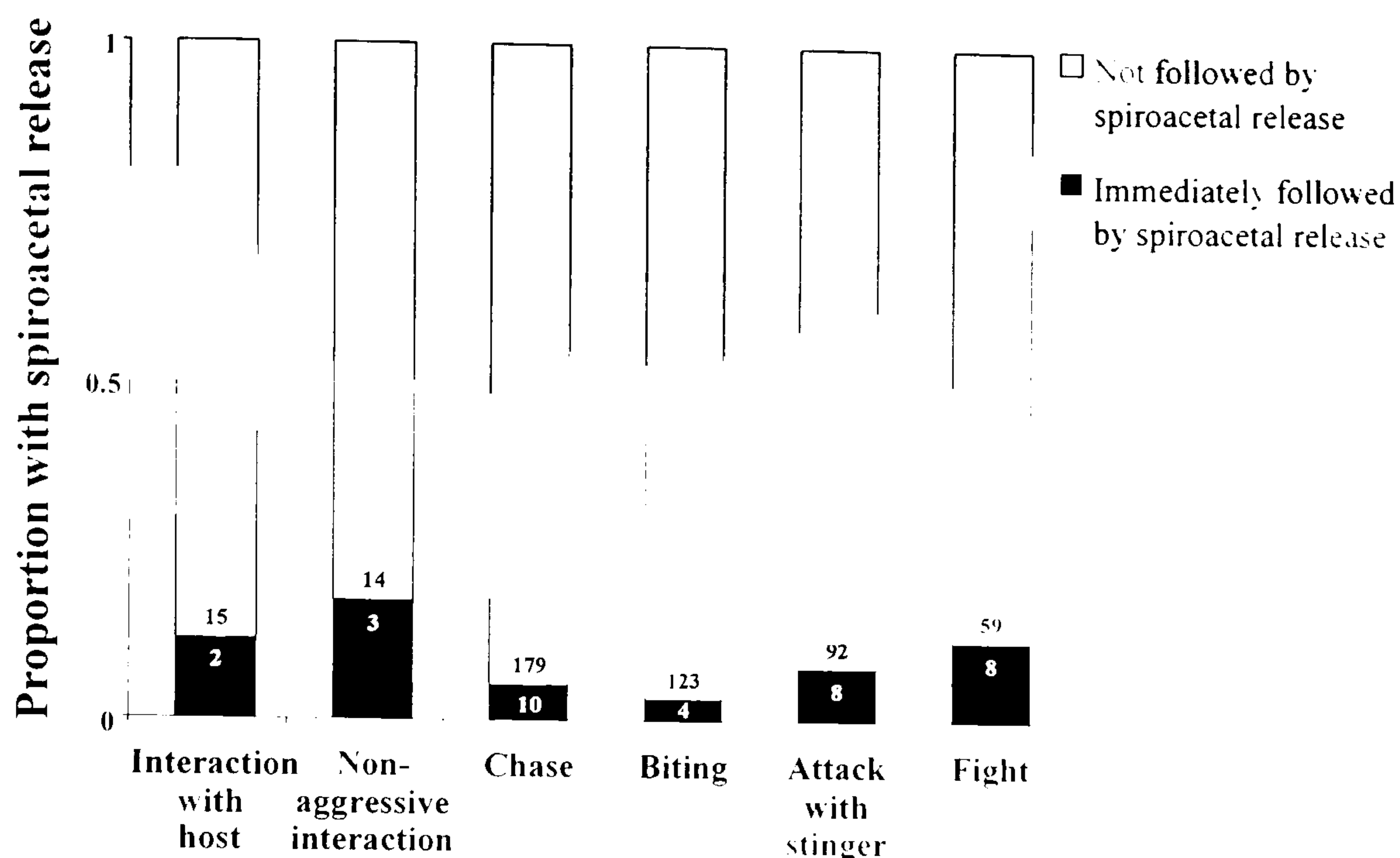
#### 6.4.3 Volatile release in relation to *G. nephantidis* behaviour

With the capability for real-time monitoring of volatile chemical release it was possible to study the timing and use of the chemical. Monitoring of the gas phase inside the vials containing the wasps did not detect 2-methyl-1,7-dioxaspiro[5.5]undecane. This suggested that it is not continuously released into the immediate environment. Release was always elicited when agitating males and females with a fine paintbrush whilst simultaneously monitoring the gas phase above them by APCI-MS. Thus, the chemical is not sex specific and its release is a discrete event under active control by the wasp. The question remained as to when and why *G. nephantidis* release 2-methyl-1,7-dioxaspiro[5.5]undecane. Further study focussed on interactions between females.

##### 6.4.3.1 Contests between *G. nephantidis* females

Overall, twelve of the 40 replicates involved chemical release, with 20 separate volatile releases occurring in total. A chase was the behaviour most commonly associated with volatile release (ten instances) followed by a fight and an attack with a stinger (eight instances of each) (Fig. 6.4). On several occasions it was not possible to attribute a volatile release to a single behaviour but rather to a mixture of behaviours. Volatile release was an infrequent event and chases, bites, attacks with a stinger and fights were significantly more likely to be unaccompanied by volatile release than by volatile release (Repeated measures ANOVA: chase:  $F_{1,39} = 12.4$ ,  $P = 0.0011$ ; biting:  $F_{1,39} = 8.3$ ,  $P = 0.0065$ ; attack with stinger:  $F_{1,39} = 10.7$ ,  $P = 0.0023$ ; fight:  $F_{1,39} = 11.1$ ,  $P = 0.019$ , Fig. 6.4). Non-aggressive interactions between females and contact with the host did not significantly influence whether volatile release occurred or not (Repeated measures ANOVA: non-aggressive interaction:  $F_{1,39} = 3.47$ ,  $P = 0.070$ ; interaction with host:  $F_{1,39} = 3.52$ ,  $P = 0.068$ , Fig. 6.4). Figure 6.5 shows a characteristic ion trace involving two volatile release events. The vast majority of interactions, whether apparently aggressive or not, did not result in a chemical release (Fig. 6.4). Despite this, all 80 contestant females were found to





**Figure 6.4** Frequency of agonistic interactions with or without an immediate associated volatile release. Results are summed across all 40 replicates combined and behaviours are ranked in order of increasing ‘escalation’ from left to right.

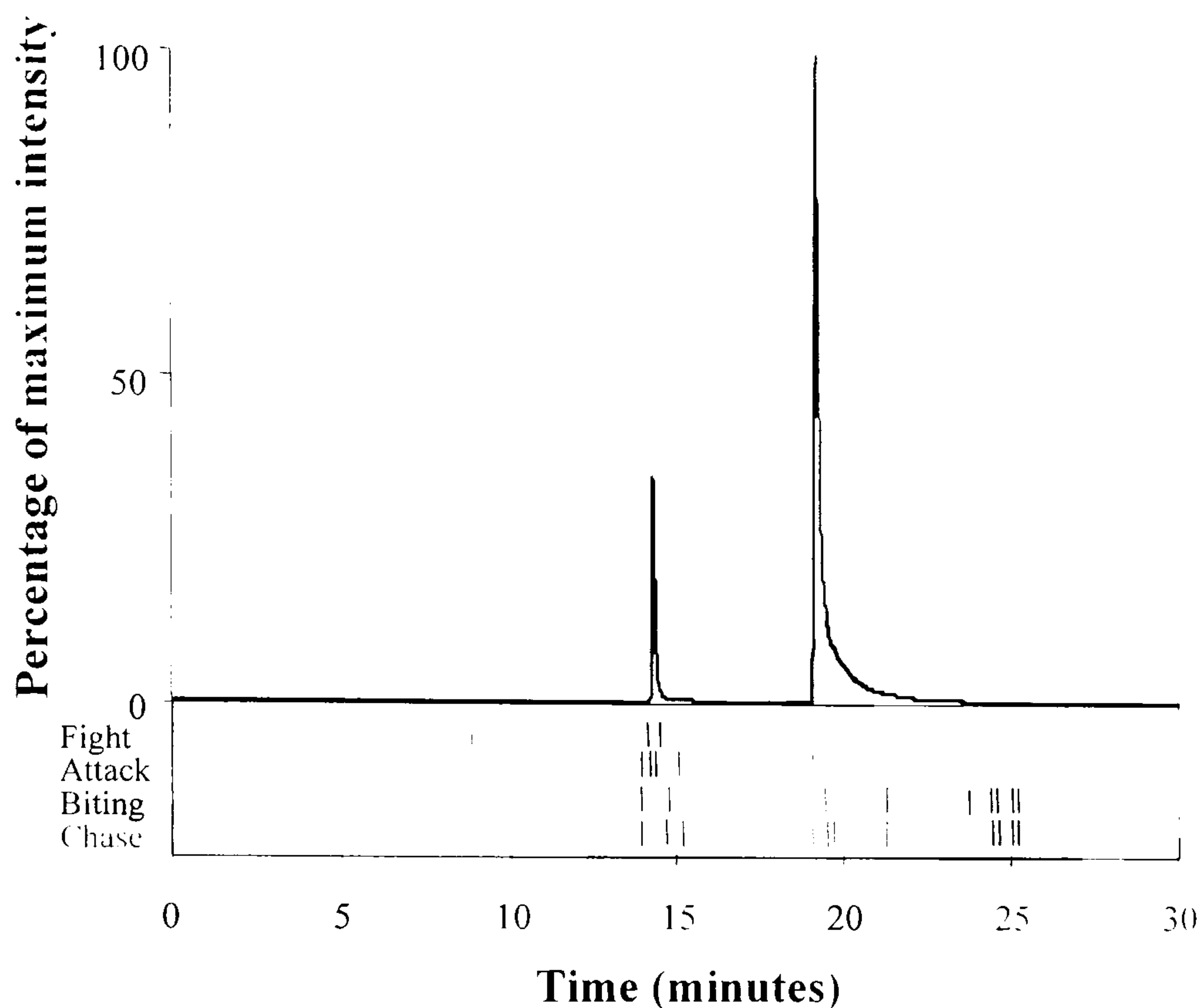
contain 2-methyl-1,7-dioxaspiro[5.5]undecane when squashed after the ‘contest’.

#### Owner-owner contests

Eight of 20 owner-owner repetitions included a chemical release producing 14 marked chemical release events in total. Fifteen of the 20 repetitions involved some interaction between the wasps, 15 had biting and 13 contests involved direct fights, chases and an attack with a stinger. On seven occasions volatile release appeared directly associated with a fight, seven times with chasing, three times with biting, six times an attack with stinger, and three times no aggressive interaction (one non-aggressive contact between wasps, 1 contact with larva, 1 not viewable).

#### Owner-intruder contests

Four of 20 owner-intruder repetitions included a chemical release producing six marked volatile release events in total. Eight repetitions involved an interaction



**Figure 6.5** Example of an ion trace tracking spiroacetal release during an owner-owner contest. The first release event took place after 14.25 min and coincided with a fight and attack by one individual on the other with its stinger. The second, larger peak occurred after 19.15 min and co-occurred with a chase and attack with a stinger. In addition, there were a series of interactions involving combinations of fights, attacks with stingers, chases and biting which did not co-occur with chemical release.

between the wasps, eight had chases and an attack with a stinger, seven involved biting and six contests involved direct fights. One fight coincided with volatile release. On three occasions chemical release coincided with a chase, once with biting and twice with an attack with a stinger. There were two instances of chemical release associated with apparently non-aggressive interactions between females and one instance of emission when a female bit the host.

#### 6.4.4 The temporal dimension and quantity of volatile release

The half peak widths of the releases during contests were relatively short (mean, SD  $40 \pm 25$ s). The time to reach peak chemical release was also short (mean, SD  $12 \pm 7$ s). The overall arena volume was approximately 4.7ml and would have been slowly purged by the sampling flow rate (20ml/min). The shape of the three chambers,



combined with the narrow corridors which connected each chamber in the contest block, are effectively a series of dead volumes and were not ideal for purging with air to remove any released chemical. Despite this, clearance of the chemical was fairly rapid, further indicating that the discharge was a single, short release event as opposed to a continued chemical release.

On calibrating the release of 2-methyl-1,7-dioxaspiro[5.5]undecane, we found releases through stressing with a fine paintbrush (mean, SD  $22.3 \pm 14.6$  mg/m<sup>3</sup>) or squashing (mean, SD  $37.5 \pm 25.5$  mg/m<sup>3</sup>) were generally higher than those during contests (range 1.8 – 20.6 mg/m<sup>3</sup>).

## 6.5 DISCUSSION

We have found that two members of the Bethyloid sub-family Bethylinae, *G. nephantidis* and *G. legneri*, release a chemical of 170Da and have identified this as 2-methyl-1,7-dioxaspiro[5.5]undecane in *G. nephantidis* (subsequent work by Goubault *et al.* [submitted] has confirmed that this volatile is released by *G. legneri*; see Appendix). In addition, we have shown that five members of the Bethyloid sub-family Epyrinae release a chemical of 131Da, which matches the peak of skatole, a chemical that has previously been identified in *C. gallicola* (Kuwahara, 1984) and *C. stephanoderis* (Gómez *et al.*, 2005), as well as in *C. hyalinipennis*, *C. tarsalis*, and *C. waterstoni* (R. Howard pers. comm.).

The two volatiles released by bethylids, 2-methyl-1,7-dioxaspiro[5.5]undecane and skatole, are not chemically similar: 2-methyl-1,7-dioxaspiro[5.5]undecane is a spiroacetal with two connected six-atom cyclical rings, each ring of which contains five carbons and an oxygen atom (Fig. 6.2), skatole is a methylindole with one six-atom cyclical carbon ring connected to one five-atom cyclical ring, the latter of which contains one nitrogen and four carbon atoms (Fig. 6.2). These two chemicals are unlikely to be biochemically related (R. Howard, pers. comm.).

The phylogenetic pattern of chemical release (Fig. 6.2) suggests that further members of the Bethylinae are likely to release 2-methyl-1,7-dioxaspiro[5.5]undecane and further members of the Epyrinae may release skatole. However, one member of the

Epyrinae tested in this study, *L. pedatus*, did not release any volatile chemical when stressed or squashed. We may have failed to detect chemicals that were present due to limited biological material, so further investigation is warranted. We would expect that, if *L. pedatus* does release a chemical, it would be skatole but this does not preclude the possibility that *L. pedatus* releases a different chemical.

Among the Hymenoptera, chemicals are used extensively for sexual attraction, alarm responses, defence and attack. Recent research in 'chemical ecology' has focused on cuticular hydrocarbons in social species, such as research on ants and paper wasps (Cuvillier-Hot *et al.*, 2004; D'Ettorre *et al.*, 2004; Dapporto *et al.*, 2004). Additional research has investigated the chemicals used by parasitoids for host finding or marking (Howard and Pérez-Lachaud, 2002; Howard and Baker, 2003; Olson, Hodges and Lewis, 2003; Manrique *et al.*, 2005), mate finding (Sullivan, 2002; Ardeh *et al.*, 2004) and competitor avoidance (Nakashima *et al.*, 2004). Our work has shown that volatile chemical release also occurs during some dyadic contests between female *G. nephantidis*.

The spiroacetal release could be associated with stress, defence, alarm, attack or resistance. 2-methyl-1,7-dioxaspiro[5.5]undecane has previously been observed in mandibular secretions of the Anthophorid bee, *Epeolus cruciger* (Pz.) (Tengö *et al.*, 1982), cephalic secretions of workers of the stingless social bee, *Melipona bicolor* (Schröder, [1985] in Francke and Kitching, [2001]) and in the sternal abdominal gland of both sexes of the longhorn beetle, *Agapanthia villosoviridescens* (Meyer, [1993] in Francke and Kitching, [2001]). Dettner *et al.* (1992) found that the spiroacetal E-2-methyl-1,7-dioxaspiro[5.5]undecane acted as an insecticidal fumigant against *Drosophila melanogaster* in the laboratory and is likely to have a narcotizing effect on other arthropods under natural conditions. Despite this evidence, the biological significance of spiroacetals is unknown in most species, but they have been associated with behavioural mediating properties (Francke and Kitching, 2001). In several species, spiroacetals appear to be components of secretions considered as typical defence agents. They are known to be used as pheromones (intraspecifically) but also interspecifically as kairomones (benefit the receiver of the chemical but not the releaser) and possibly allomones (benefit the releaser of the chemical but not the receiver) (Francke and Kitching, 2001).



Our finding that both males, which are thought not to engage in contests for mating opportunities, and females of *G. nephantidis* can release 2-methyl-1,7-dioxaspiro[5.5]undecane indicates that the volatile is not specific to contests, is unlikely to be a sex pheromone and may, in fact, be more often used as defence against conspecifics and predators. The chemical may be used as a pheromone but is also likely to have a function as an allelochemical (a chemical that mediates an interspecific interaction) because it was also released when stressed with a fine paintbrush and twice when interacting with a *C. cephalonica* larva. Similarly, skatole, which was found in males and females of five species of the Epyrinae studied here, has also been suggested to be an allomone used for attack and defence (Kuwahara, 1984) and may function as an alarm pheromone that triggers dispersal (Gómez *et al.*, 2005).

Our results cannot currently elucidate whether skatole or 2-methyl-1,7-dioxaspiro[5.5]undecane are released by bethylids primarily for attack or as an active defence. The location of the release structure for the spiroacetal and skatole is the head of the Bethylinae and Epyrinae, respectively. Skatole has previously been confirmed to be housed in the head, in glands close to the mandibles, of the bethylids *C. gallicola* (Kuwahara, 1984), *C. stephanoderis* (Gómez *et al.*, 2005) *C. hyalinipennis*, *C. tarsalis* and *C. waterstoni* (R. Howard pers. comm.). We suspect that this is also the case for *P. nasuta* and that 2-methyl-1,7-dioxaspiro[5.5]undecane may be located in, or around, the mandibular glands of *G. nephantidis* (R. Romani, M. Goubault and I.C.W. Hardy, unpublished data). Mandibular gland secretions are reported to be mainly for defence and alarm in a wide variety of species, particularly social ants and bees (Billen and Morgan, 1998), and many alarm substances appear to have evolved from compounds originally released for defence by fighting or injured conspecifics (Wyatt, 2003). The effect of alarm substances can be diverse, including triggering alertness, aggregation, attraction or dispersal (Billen and Morgan, 1998).

There has been very little prior research conducted on the stimuli for, and effects of, such volatile releases by bethylids. Gómez *et al.* (2005) have published the most comparable chemical release work but they could only detect that skatole is released by male and female *C. stephanoderis* when jars containing many wasps are shaken

and that exposure to skatole triggers increased activity in *C. stephanoderis* individuals; they were unable to track events in real-time. Consequently, no research comparable to the current study (together with Goubault *et al.*, submitted: Appendix) has been conducted on chemical release during parasitoid contests. The most similar contest studies are the investigations by Breithaupt and Eger (2002) and Bergman *et al.* (2005) who observed urine release during intraspecific fights between crayfish, *Astacus leptodactylus* and *Orconectes rusticus* respectively, by marking their urine with the dye Fluorescein. In both species, urine release occurred at high levels of aggression and the individual releasing the urine was more likely to win the contest (Breithaupt and Eger, 2002; Bergman *et al.*, 2005). Breithaupt and Eger (2002) suggest that urine contains information about the fighting ability or aggressiveness of the releaser in crayfish contests. Other contest research involving chemical signals has predominantly focussed on higher-vertebrates and the marks they deposit throughout the environment (Gosling *et al.*, 1996; Hurst *et al.*, 1996; Humphries *et al.*, 1999; Gosling and Roberts, 2001; White *et al.*, 2002; Nevison *et al.*, 2003), particularly at territorial boundaries. Thus, few studies have been able to evaluate real-time chemical release during direct encounters.

Although the work of this study has incorporated this real-time analysis, we have not found a clear relationship between any particular behaviour and volatile release by *G. nephantidis*. The majority of behavioural interactions did not involve spiroacetal release but when releases did occur they were generally related to aggressive interactions. This could indicate that 2-methyl-1,7-dioxaspiro[5.5]undecane is a chemical released by an attacker as a weapon, as a signal of fighting ability or as a defence signal or weapon to give the loser time to escape from its attacker or to avoid further escalation and potential damage to contestants. We have been unable to identify which individual releases the chemical during a contest, an issue that has been addressed by Goubault *et al.* (submitted: Appendix) using females that are reared on hosts injected with deuterated saline which thus assimilate heavy isotopes, and produce structurally identical molecules with greater molecular mass. Despite the shortcomings of the current study, there were 14 release events in owner-owner contests, a situation where we would expect more intense competition to occur, compared with six in owner – intruder contests. The release of the volatile may be energetically costly (although chemical signals are likely to have low cost: Bradbury



and Vehrencamp, 1998, p 551; Wyatt, 2003, p12). so may only be released when contests escalate and/or by individuals that place a high RV on the hosts. Owners are likely to place a higher RV on their own hosts than intruders do (Parker, 1974; Enquist and Leimar, 1987; Stokkebo and Hardy, 2000: Chapter 5) and so may be more willing to expend extra energy fighting for the resource.

Recent research on *G. legneri* has shown that it is always the loser of an individual agonistic interaction that releases the spiroacetal (Goubault *et al.*, submitted: Appendix). This appears to also be the case for *G. nephantidis* (M. Goubault, pers. comm.), but the two species do not necessarily have the same contest dynamics, as evidenced by the observation that *G. legneri* appear to have an 'intrudership advantage' in intra-specific contests (Goubault *et al.*, submitted: Appendix; T. Hull, pers. comm.), whereas there is an advantage to being a prior owner in *G. nephantidis* (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000; Chapter 5). Thus, all else being equal, for *G. legneri*, owners are more likely to lose a contest, so are more likely to release the volatile, but in *G. nephantidis* owners are more likely to win so possibly less likely to release the chemical. This requires further investigation.

The frequency of volatile release in *G. nephantidis* was also not consistent in time. We observed contests over five separate time periods. The last two periods (in August and November 2003) of experimentation involved no detectable chemical release during contests, despite the observation that the parasitoids engaged in aggressive interactions and released the volatile when stressed with a fine paintbrush after the contests. This might imply that alternative factors such as atmospheric pressure may be important. This observation of extreme selectivity of the parasitoids in the release of 2-methyl-1,7-dioxaspiro[5.5]undecane and the predictions above leave great scope for further investigation as to why the chemical is released, which individual releases it and the chemical's effect on an opponent.

The technique of APCI-MS offers much scope for further study on the factors that influence volatile release and its relation to contest resolution. Within bethylids, such as *G. nephantidis* and *G. legneri*, we can study the importance of wasp age, size, experience, ownership, egg loads, clutch size and host size on chemical release, but

the technique could also be employed in a wide variety of small terrestrial taxa to evaluate the impact chemicals have on animal behaviour.



## CHAPTER 7 – GENERAL DISCUSSION AND CONCLUSIONS

Each of the preceding chapters have their own discussion: the aim of this chapter is thus to integrate the components of this thesis. I briefly recap the objectives of the work, discuss whether bethylid species are generally suitable for biological control, and of the CBB in particular, consider the variables that influence the outcome of bethylid contests for host resources and discuss the possible relationships between chemical releases and bethylid behaviour.

Part one (Chapters 2-4) investigated, in the laboratory, the inter- and intraspecific effects of the three bethylid species that attack the CBB. These interactions included intra-guild predation and facultative hyperparasitism (Chapter 2), inter- and intra-specific interference competition (Chapters 3 & 4) and inter- and intra-specific exploitation competition (Chapter 4).

Part two (Chapters 5 & 6) investigated contest interactions between adult female *G. nephantidis* within the laboratory and pheromone release by bethylids. The attributes investigated that may have influenced contest outcome were ownership, competitor size, wasp age, host size and clutch size (Chapter 5). Chemical release was investigated in eight bethylid species, with a focus on chemical release during competitive interactions between *G. nephantidis* females (Chapter 6).

### 7.1 BETHYLID INTRA-GUILD INTERACTIONS IN CBB BIOLOGICAL CONTROL

There is wide overlap in the CBB host stages that *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* feed upon (eggs, occasionally adults) and parasitize (large larvae, prepupae and pupae). This implies that there is little interspecific resource partitioning and that release of all three species into the same coffee agro-ecosystem may instigate competition, which could result in the better competitor(s) establishing, to the detriment of any competitively inferior species.

The results of chapters 2-4 provide contrasting indications as to which of the bethylids would be most suitable for controlling the CBB and whether multi-species releases would be beneficial. Fatal fighting during contest interactions suggests that the superior competitor, *C. stephanoderis*, may exclude weaker opponents and thus result in the persistence of only this species in coffee agro-ecosystems (Chapter 3). Intra-guild predation and hyperparasitism observations imply that there may be inter-specific, and some intra-specific, interference competition, which could reduce the probability of persistence of multiple species in coffee environments (Chapter 2). Finally, the more holistic approach of providing parasitoids with CBB infested coffee berries and evaluating recruitment by each species, without observing the interactions that take place within or between the species, suggests that coexistence between species may be possible (Chapter 4).

The investigation in which CBB infested berries were provided (Chapter 4) resulted in two observations that have implications for multi-species releases. Firstly, intra- and inter-specific replicates which contained *C. hyalinipennis* had low production, suggesting that this species would be the least suitable for CBB control and should not be encouraged. Secondly, there was no apparent difference in production from intra- and inter-specific replicates involving *C. stephanoderis* and *P. nasuta*. This indicates that, in the conditions of the investigation, although coexistence may be possible, there is neither an advantage nor disadvantage to releasing both of these bethylid species to control the CBB. The fact that there are negative behavioural and trophic interactions suggests that the single 'best' species should be released, and that the most suitable species is either *C. stephanoderis* (Chapter 3) or *P. nasuta* (Chapter 4).

These recommendations are based on relatively limited laboratory-based research and would need considerable further investigation in field environments to assess whether the patterns observed are replicated in natural conditions. For example, all of the investigations forced bethylids to compete for limited CBB resources, whereas the CBB is a pest in natural conditions in Mexican coffee agro-ecosystems and has high population density. This should reduce the prevalence of inter- and intra-specific competition involving any bethylids released, as there are more than sufficient hosts for each female to parasitize. Competitive exclusion between the



bethylids following release would thus be unlikely (Keller, 1984). The three bethylid species may, however, come into direct competition in coffee berries if there are large parasitoid densities relative to pest density, such as during inundative releases (Damon and Valle, 2002), or during periods of low density of suitable host stages to parasitize, such as the inter-harvest period.

In practice, records of establishment and parasitism rates of the CBB by *C. stephanoderis* or *P. nasuta* after release in Mexico have, however, indicated that they exert only very weak influences on CBB populations (Damon, 2000; Infante *et al.*, 2001b; Damon and Valle, 2002). In addition, *C. hyalinipennis* has rarely been found in coffee plantations since its original discovery. Therefore the opportunities for direct competition between the bethylid species in established populations appear to be low. If the three bethylids begin to exert effective control, bringing CBB populations to equilibrium levels, there would then be more inter- or intraspecific contact. At this time, coexistence between multiple natural enemies becomes an important consideration because detrimental effects of multiple natural enemies on each other become more prevalent and could disrupt the pest-parasitoid equilibrium. In this situation, selection will favour those individuals that maximise their own fitness by winning direct fights, hyperparasitizing and preying upon their opponents, dispersing to find berries with unguarded hosts, specialising on different niches or using alternative strategies to avoid direct competition for CBB hosts.

These behaviours could explain why *C. stephanoderis* and *P. nasuta* coexist in some African countries, such as Togo where *C. stephanoderis* is more prevalent (Moore and Prior, 1988; Vega *et al.*, 1999a). Their coexistence may be promoted by niche differentiation. For example, whilst these bethylid species were once considered monophagous, they have both been reared on alternative hosts in the laboratory (Pérez-Lachaud and Hardy, 2001). This suggests that they could be oligophagous in their natural ranges, enabling some niche differentiation if they attack several different African host species; CBB may not be their primary host (Damon, 1999). This is supported by the observation that parasitism of the CBB by *C. stephanoderis* in Togo only attained high prevalence during the inter-harvest season (Damon, 1999). This is also apparent in Chiapas, Mexico, where *C. stephanoderis* populations

are only evident in surveys leading up to the inter-harvest period, and then only in low numbers.

Observations in the African natural ranges of *C. stephanoderis* and *P. nasuta* indicate that they are not frequently found sympatrically so they may actually have narrow niche or geographical overlap. For example, several studies have suggested that the optimal conditions for the development of *P. nasuta* tend to be at lower temperature and perhaps higher altitude than for *C. stephanoderis* (Barrera, 1991; Infante *et al.*, 2001a; Murphy and Moore, 1990). Their coexistence in Togolese coffee plantations (Vega *et al.*, 1999) suggests that competition may not be the primary factor influencing their distribution and that preferences for other biotic or abiotic conditions may be more important. Their coexistence may actually be possible due to the establishment of metacommunities over a regional scale (Leibold *et al.*, 2004; Amarasekare *et al.*, 2004). Thus, coexistence between the species over a restricted range may be possible, but release of the species together may be inappropriate because at least one species may not be suitably adapted to the release environment.

This could explain why *P. nasuta* has repeatedly failed to establish in Mexican coffee plantations (Infante *et al.*, 2001b, 2003), despite its persistence in several other countries of release, such as Colombia (Bustillo-Pardey *et al.*, 1996; Jaramillo *et al.*, 2005) and Brazil (Hempel, 1934; Moore and Prior, 1988). The *P. nasuta* cultures that were used for releases in Mexico from 1992 actually originated from field collections of established individuals in Brazil, and the same stock was used for successful releases, in terms of establishment, in Colombia. This suggests that the failure to establish in Mexico is more likely to be a result of poor suitability to the release environment rather than their becoming too well-adapted to laboratory conditions.

#### **7.1.1 Recommendations for future research on CBB biocontrol**

Research to date on control of the CBB has mainly focused on laboratory investigations that have used reductionist criteria to assess the potential success of biological control agents (Kidd and Jervis, 2005). These criteria have included fecundity, feeding behaviour, host specificity, competition and ease of culturing of



bethylids of the CBB. Future investigations using the bethylids of the CBB need to adopt a more holistic approach, releasing agents within field environments. This would allow observations of the parasitism levels of the CBB by each of the species when alone or in combination with other bethylid species without quantifying the exact interactions which take place. More realistic predictions could then be made as to whether single or multiple bethylid species releases would have the greatest impact on CBB suppression over a wider scale.

In light of these suggestions, and the results of this thesis, I believe that experimental effort should now focus on the interactions that could take place between the three bethylid species and also the eulophid *Phymastichus coffea*, which is currently the subject of release programmes around the world (Baker *et al.*, 2002; Jaramillo *et al.*, 2005). The predictions of a model by Gutierrez *et al.* (1998) suggest that *P. coffea* may be capable of CBB population suppression. It seems unlikely, however, that economic control will be achieved by *P. coffea* because it has low parasitism levels in West Africa, where it is indigenous (Feldhege, 1992; Vega *et al.*, 1999). An additional prediction of the model of Gutierrez *et al.* (1998) was that the release of *C. stephanoderis* or *P. nasuta* in combination with *P. coffea* could suppress CBB populations more than *P. coffea* would alone. There is evidence of their compatibility in the study of Vega *et al.* (1999) who found *C. stephanoderis*, *P. nasuta* and *P. coffea* coexisting in very low numbers (0-6 individuals of each species per site) in Togolese coffee plantations and larger numbers of *C. stephanoderis* (10-277 per site) and *P. coffea* (1-90 per site) together in the Ivory Coast. In addition, Feldhege (1992) reported that direct competition was not observed between *P. coffea* and the bethylids *C. stephanoderis* and *P. nasuta* in Togo.

The life-history of *P. coffea* supports the suggestion that it is unlikely to compete directly with bethylids. For example, *P. coffea* does not enter coffee berries, but parasitizes CBB females that are beginning to bore into the berry, before dispersing to find another CBB female to parasitize. In contrast, the bethylids need to enter CBB infested berries that provide suitable host stages for parasitism. *Phymastichus coffea* sterilises the adult CBB on parasitism, so any berry that is being attacked by a parasitized adult CBB will only suffer superficial damage and the endosperm, where CBB stages need to develop to allow bethylid attack, will remain intact. Thus, *P.*

*coffea* attacks CBBs before any economic damage occurs to the bean and therefore before there are suitable host stages for bethylids to parasitize. *Phymastichus coffea* is consequently able to exploit the CBB earlier in the season than can the bethylids, giving *P. coffea* a competitive advantage. This advantage may, however, be compromised if the bethylids can practice IGP by hyperparasitizing *P. coffea* immatures or paralyzing and consuming a parasitized adult CBB, killing the developing *P. coffea* endoparasitoid. Previous investigations have suggested that *C. stephanoderis* and *P. nasuta*, but not *C. hyalinipennis*, will consume adult CBB, although *C. hyalinipennis* could still interfere if it can act as a facultative hyperparasitoid of *P. coffea* immatures. However, there may be little advantage for a bethylid to attack a single adult CBB in an uninfested berry if numerous alternative infested berries, containing a range of CBB developmental stages, are available. In addition, the exoskeleton of a parasitized CBB may effectively act as a barrier to hyperparasitism.

#### 7.1.2 Bethylids as biological control agents

The bethylid species that attack the CBB have thus far had little impact on CBB populations where they have been released. Furthermore, they often have little control over CBB population levels in their African natural ranges, for example CBB infestations of 80% of coffee berries in Uganda (Hargreaves, 1926) 90% in Tanzania (Le Pelley, 1968), and up to 92% in Kenya (Barrera, 1991) have been reported.

*Cephalonomia hyalinipennis*, *C. stephanoderis* and *P. nasuta*, like many bethylids, may appear to have life-history characteristics that would make them efficient biological control agents, for example brood guarding which can ensure low brood mortality. However, Force (1972) suggests that the best species to release as biological control agents in novel environments may actually be those that exhibit *r*-strategies (based on having high intrinsic rates of increase, *r*), rather than *K*-strategists (based on predictions that population density of species that are competitively superior in stable environments will stabilise at their carry capacity, *K*) (MacArthur and Wilson, 1967; Pianka, 1970). Thus, because *r*-strategists are expected to have high reproductive capacity, good dispersal and a broad tolerance to more extreme environmental conditions (MacArthur and Wilson, 1967; Pianka,



1970), they may be more effective than *K*-strategists in initially reducing pest population density. Although few insect species could be considered as true *K*-strategists, bethylids do display characteristics, such as brood guarding behaviour and relatively low fecundity, which may be considered as *K*-strategies. In particular, natural enemies are often sought against large populations of pest species. Control of such large populations will often require natural enemies that can adapt to a wide range of conditions to bring the pest population to lower levels. Once at these lower levels, *K*-strategists may then have more impact on pest populations.

This situation may have occurred with the only bethylid that is currently considered to be a successful biological control agent, *G. legneri*, which was first released in 1979 and 1980 against the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), an almond and pistachio pest in California. At this time, navel orangeworm infestation levels were less than 9% in Californian almond orchards due to previous releases of the polyembryonic parasitoid *Copidosomopsis plethorica* Caltagirone (Hymenoptera: Encyrtidae) in the 1960s (Legner and Gordh, 1992). On release, *G. legneri* acted as one of a suite of natural enemies, helping to reduce navel orangeworm infestations to less than 4% of almonds damaged at harvest, a level desired by the Californian almond industry (Legner and Gordh, 1992). *Goniozus legneri* continues to act as a reasonably effective natural enemy of the navel orangeworm in California but annual inoculative releases are required to augment established populations after the winter period (Legner and Gordh, 1992; Bentley, 1999).

## **7.2 CONTESTS AND CHEMICAL INTERACTIONS BETWEEN BETHYLIDS AND RECOMMENDATIONS FOR FUTURE RESEARCH**

Whilst resource value (RV) and resource-correlated and resource-uncorrelated resource holding potentials (RHP) have been independently studied in a wide range of animal species (Riechert, 1998), few investigations have assessed their combined influences on the outcome of contests. The few experiments that have addressed this issue include research on birds (Petrie, 1984), newts (Verrell, 1986), fish (Lindström, 1992; Lindström and Pampoulie, 2005) spiders (Hack *et al.*, 1997; Hoefler, 2002) and parasitoids (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000; Chapter 5).

Thus, there is a dearth of information on the interplay of RVs and RHP influences on contest interactions throughout the animal kingdom. For this reason, contest research studies now need to assess the role each of these influences have in contest interactions, either when assessed alone or when in combination with each other.

Contests between females of *G. nephantidis* provide a model system in which the influence of asymmetries in RV and RHPs can be explored. Previous research on *G. nephantidis* evaluated the influence of ownership, brood stage and asymmetries in body size and egg load between competitors, on contest outcome (Hardy and Blackburn, 1991; Petersen and Hardy, 1996; Stokkebo and Hardy, 2000). Investigations in Chapter 5 have added the variables wasp age and host size. To assess the influence of individual, or multiple, variables on contest outcome, asymmetries between those variables associated with resource-correlated RHP, resource-uncorrelated RHP and RV that are not the focus of the investigation can be removed, or reduced. Females can be matched for body size, age, ovipositional experience, ownership, host size and clutch size. Egg load asymmetries are less easy to control because they are assessed by invasive procedures, which are only possible after experiments (Stokkebo and Hardy, 2000; Chapter 5). However, egg load is influenced by age, body size, ownership, host size and ovipositional experience (Stokkebo and Hardy 2000; Chapter 5) so by removing asymmetries in these variables the size of asymmetries in egg loads between contestants should be reduced.

A female's egg load is also likely to be related to her clutch size and comparisons of egg loads and clutch sizes in *G. nephantidis* (Figs. 5.2 & 5.3 compared with Fig. 5.4, respectively) suggest that mean egg loads and mean clutch sizes appear to be similar (Chapter 5). Clutch size is also predicted to be influenced by the prevalence of parasitoid competition and contest behaviour (Petersen and Hardy, 1996; Visser and Rosenheim, 1998; Mesterton-Gibbons and Hardy, 2004). When population densities of competitors are perceived to be high, or host resources are limited, contests for hosts may become common. When large size confers a competitive advantage, such as in *G. nephantidis*, an ovipositing female increases her fitness if she produces larger offspring. In this situation, if clutch size influences body size (see Traynor and Mayhew, 2005), a female laying fewer eggs than her competitors on a given sized



host provisions more resource to each of her offspring, thus producing larger progeny (Petersen and Hardy, 1996; Mesterton-Gibbons and Hardy, 2004). To assess experimentally whether female *G. nephantidis* alter clutch size in response to higher perceived levels of competition, owners females can be exposed to varying numbers of intruders (A. Mack, M. Goubault and I.C. W. Hardy, unpublished data). If females reduce clutch size in response to increasing contest competition, smaller clutches would be expected as the number of females intruding on a guarded host increases.

Clutch size has also been shown to be positively influenced by host size across a number of bethylid species, including *G. nephantidis* (Hardy *et al.*, 1992; Luft, 1993; Mayhew and Godfray, 1997; Mayhew and Hardy, 1998; Pérez-Lachaud and Hardy, 2001; Chapter 5), indicating that larger hosts can be considered as a higher quality resource than smaller hosts. Owners of larger hosts also generally win dyadic contests against owners of smaller hosts (Chapter 5), which could be interpreted as due to asymmetries in the RV placed on their respective hosts. A female's RV estimate for a given host may also be positively influenced by how much reproductive effort she has invested in the resource (Hardy and Blackburn, 1991). Further laboratory research on *G. nephantidis* has investigated the influence of asymmetries in brood developmental stage between two owner females (D. Scott, D. Heron and I.C.W. Hardy, unpublished data). Owner females can be in possession of a paralysed host, a paralysed host with her own eggs on or a paralysed host bearing her own larval brood. Contests can then be observed between two owners that are defending hosts at different stages of parasitism, so that one owner has invested more reproductive effort in her own host than has her opponent on its own host. This host-parasitism-stage asymmetry may result in more aggressive defence by the owner female whose brood is at the furthest developmental stage.

Similarly, in owner-intruder contests, we may expect owners to defend more aggressively a host bearing their brood than they would an unparasitized host, and thus observe that it becomes progressively more difficult for intruders to take over the resource as the brood matures (Hardy and Blackburn, 1991). This prediction would only be valid to the point at which the host is still suitable for an intruder to use for oviposition or the owner still needs to defend the brood. Intruders that encounter a host with eggs on will commit ovicide and then oviposit on the host if

the owner is absent (Hardy and Blackburn, 1991). whereas larval broods rapidly consume the host and intruders encountering these do not tend to perform larvicide, making them less likely to fight for possession of a host that already supports larvae of a conspecific. Defence may remain important to owners, however, because the larval brood may still be vulnerable to hyperparasitism, multiparasitism and predation by allospecifics (Hardy and Blackburn, 1991).

All previous research on *G. nephantidis* fighting behaviour has used females that had no previous experience of contests (Petersen and Hardy, 1996; Stokkebo and Hardy, 2000; Chapter 5). Contest experience may increase a female's fighting ability (resource-uncorrelated RHP) through learning and improving fighting strategies, as is the case in crab spiders (Hoefler, 2002), or reduce their fighting ability as they accrue injuries (Taylor and Jackson, 2003). Experience of winning and/or losing contests may also influence the aggressiveness of females, and hence their future success in contests, as is predicted by theory (Mesterton-Gibbons, 1999) and has been found in fish (Hsu and Wolf, 2001). Additionally, *G. nephantidis* and *G. legneri* have been found to release a spiroacetal, 2-methyl-1,7-dioxaspiro[5.5]undecane, during some contest interactions (Chapter 6). Goubault *et al.* (submitted; Appendix) found that, in *G. legneri*, the chemical is only released by losers of contest interactions but that contests can also be resolved without release. Further, losers of individual interactions involving chemical release could still return to win the contest overall, taking possession of the host<sup>1</sup>, although such events were observed only occasionally. Thus there is scope for further investigation into why chemicals are released by losers of an interaction, the response of the 'releaser' and 'receiver' after chemical release and the influences contest experience and/or chemical release have on current and future fighting success in *G. nephantidis* and *G. legneri*.

Chemical and contest behaviour research could also be incorporated into investigations of the three bethylids involved in CBB control attempts. The

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<sup>1</sup> Identification of which individual released the chemical was enabled by injecting the factitious host, *C. cephalonica*, with saline containing a heavy isotope (deuterium). *Goniozus legneri* offspring that develop on these hosts assimilate the heavy isotope and hence produce volatiles with a mixture of molecular weights at ion peaks  $m/z$  171, 172, 173 and 174, rather than mainly the  $m/z$  171 of undeuterated females.



identification of skatole in *C. stephanoderis* and *C. hyalinipennis* (with *P. nasuta* to be confirmed in the future) could not only facilitate investigations into its release during contests but also its effect on the success of biological control releases. Damon and Valle (2002) first reported a characteristic smell was produced when jars of numerous *C. stephanoderis* were transported to the field for release against the CBB. They suggested that chemical release may be induced by interference competition and stress, could be costly to the energy budget of parasitoids and may reduce host-patch searching efficiency, and thus parasitism levels, by inducing females to disperse away from the release area. They improved parasitism levels on the CBB by releasing *C. stephanoderis* whilst they were still immatures within coffee berries, so that no, or reduced, chemical interference occurred on release.

Gómez *et al.* (2005) have since identified the chemical produced by *C. stephanoderis* as skatole and suggested that it may function as an alarm pheromone because it induces agitated running behaviour by females. Skatole may actually function differently at varying concentrations. For example, when observing the methods for culturing *C. hyalinipennis*, *C. stephanoderis* and *P. nasuta* at ECOSUR, females were often temporarily stored together with large numbers of conspecifics (>200) in glass tubes (1.5 cm diameter × 7.3 cm height) for several hours prior to their introduction to 11 jars containing CBB infested coffee berries. Females, chosen arbitrarily, that were transferred from the crowded glass tubes to individual tubes for observation appeared to be sluggish, continued to be lethargic on subsequent days and appeared to have short longevity (T.P. Batchelor, pers. obs.). This could be because skatole release is energetically costly. Alternatively, skatole may act as an insecticidal fumigant in high concentrations (within confined spaces, such as a coffee berry, or if released in high quantities, such as when collected together in jars). Similarly, *G. legneri* and *G. nephantidis* release a spiroacetal, 2-methyl-1,7-dioxaspiro[5.5] undecane (Goubault *et al.*, submitted: Appendix: Chapter 6), which is suggested to have narcotizing effects on other arthropods in field conditions and can act as an insecticidal fumigant in the laboratory (Dettner *et al.*, 1992).

Further research could thus develop the work of Gómez *et al.* (2005). Chapter 6 and Goubault *et al.* (submitted) to evaluate the effects skatole and 2-methyl-1,7-

dioxaspiro[5.5]undecane releases have on the behaviour and life-histories of bethylids that attack the CBB and *Goniozus* spp., respectively.

### 7.3 OVERALL CONCLUSIONS

The life-histories of bethylids provide valuable study systems for research into competitive interactions, particularly in dyadic contests. Many variables can be manipulated to investigate how contests between females for host resources can be resolved and why these contests can escalate to aggressive interactions, including fatal fighting. The prevalence of these escalated contests between bethylids in natural environments remains to be investigated. If hosts are abundant, as is often the case where pest populations are targeted with biological control releases, and females know population density is high, we should expect individuals to spend little time engaged in contest interactions because there are numerous available un-guarded hosts. This may stimulate the dispersal of individuals over a wider area where the pest is a problem and could increase the parasitism levels of the biological control agents, thus improving pest control. It seems, however, that the competitive life-histories of bethylids may make them more suitable for investigations on contest interactions than as biological control agents.



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# VOLATILE EMISSION BY CONTEST LOSERS REVEALED BY REAL-TIME CHEMICAL ANALYSIS

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Outcomes of direct behavioural contests for resources are commonly influenced by competitor asymmetries in intrinsic contest ability, prior ownership and resource value, as predicted by classic game-theoretic models (Maynard Smith, 1982; Mesterton-Gibbons and Adams, 1998; Riechert, 1998). Attention has also focussed on interactions between opponents within contests (Payne, 1998; Riechert, 1998; Briffa and Elwood, 2002; Maynard Smith and Harper, 2003). While it is known that interactions often involve chemicals, particularly via olfaction, their role has largely been studied in species that continuously display or deposit relatively involatile compounds (Gosling and Roberts, 2001; Hurst et al. 2001; Nevison et al. 2003; Wyatt, 2003): there has been little evaluation of chemical release during contests themselves (Breithaupt and Eger, 2002; Monnin et al. 2002). Here we report findings from a novel method, Atmospheric Pressure Chemical Ionisation - Mass Spectrometry (APCI-MS) coupled with manipulation of molecular-mass achieved by rearing contestants on deuterium-enhanced nutrients. This allows real-time monitoring of the occurrence, quantity and identity of volatile chemicals released by each of two competitors, in tandem with behavioural observations. We show that during female-female contests in the parasitoid wasp *Goniozus legneri* the losing individual emits a spiroacetal, 2-methyl-1,7-dioxaspiro[5.5]undecane, usually following escalated fighting. Emission is most common in more behaviourally aggressive contests, which occur when prior resource owners successfully resist take-over by similar-sized intruder females. Aggression in contests is reduced after spiroacetal release. We suggest that the spiroacetal functions as a weapon of rearguard action. We anticipate that APCI-MS, which is non-intrusive, rapid



**and relatively inexpensive, will be widely applied in studies linking chemistry and behaviour.**

Chemicals are the medium of much communication between organisms (Wyatt, 2003). Chemicals may be continually exhibited or broadcast (Gosling and Roberts, 2001; Hurst et al. 2001; Nevison et al. 2003; Wyatt, 2003) or chemical exchange may have a stronger temporal dynamic (Breithaupt and Eger, 2002; Monnin et al. 2002). Studies of chemical exchange between interacting organisms have usually been constrained to trap volatile substances onto adsorbent blocks prior to analysis (e.g. by Gas Chromatography-Mass Spectrometry (Wyatt, 2003; Gómez et al. 2005), see Supplementary Materials), hence missing the exact correspondence between chemical release and behavioural events. Other studies have used physiological techniques, such as electroantennography, to monitor nervous signals in sensory organs exposed to chemical stimulants (Wyatt, 2003); these miss the interactive-behavioural dynamics of intact whole organisms. To date, it has thus been possible to show that chemicals are produced by one organism and detected by another but not, in general, to follow ‘a chemical conversation’ with accurate determination of the timing and behavioural associations of chemical emission.

An area in which assessment of chemical exchange should prove especially valuable is the study of dyadic contests for indivisible resources (Maynard Smith, 1982). Although theoretical attention has been paid to both signal evolution and intra-contest assessment (Payne, 1998; Maynard Smith and Harper, 2003), there is little empirical evidence concerning the importance of chemicals as temporally dynamic signals, or as weapons, during animal contests (Breithaupt and Eger, 2002; Monnin et al. 2002). We provide such assessment using APCI-MS (Taylor and Linfoth, 2003) to track olfactory events by continuously sampling the airspace around freely moving and competing individuals. We explore the chemical behaviour of *Goniozus legneri* Gordh (Hymenoptera: Bethylidae, subfamily Bethylinae), a parasitoid of lepidopteran larvae that tunnel into the tissues of crops such as pistachio nuts and almonds (Steffan, Daane and Mahr, 2001). The adult female wasp paralyzes a host approximately one day before laying a clutch of eggs onto its surface. In common with other bethylids (Petersen and Hardy, 1996) ‘owner’ females aggressively defend paralysed hosts against conspecific intruders, resulting in classic owner-intruder

contests (Fig. 1). Several species in the bethylid sub-family Epyrinae emit a methylindole from the cephalic region when stressed (Gómez et al. 2005) (R.W. Howard, pers. comm., T.P.B, R.S.L & I.C.W.H. unpublished data) but there are no prior reports of emissions by bethylid species. We first investigated the presence and composition of emissions by stressed *G. legneri* and then, using real-time analysis, their occurrence during contests between chemically distinguishable females.

As an initial screen, we stressed 10 adult *G. legneri* by squashing between forceps under the sampling point of an APCI mass spectrometer in full-scan mode (see Methods). This showed that one major ion was observed in the spectrum at  $m/z$  171 (Fig. 2a). Standard analysis using Gas Chromatography - Mass Spectrometry (GC-MS) (see Supplementary Materials) then showed one major peak on the chromatographic trace which corresponded to 2-methyl-1,7-dioxaspiro[5.5]undecane, a spiroacetal with two 6-membered rings and one methyl group attached to an alpha-carbon (Fig. 2a). This molecule has a molecular weight of 170Da and is expected to protonate to give an  $MH^+$  ion at  $m/z$  171 when tracked by APCI-MS. Heads, thoraxes and abdomens of 10 freshly dissected females were then crushed separately, showing that the spiroacetal was released from the head, but not from the thorax or abdomen. The spiroacetal was not detected in the gas phase within vials containing individual unstressed adults or females attacking hosts (which react violently to stinging) but release could readily be elicited by harassing males or females with the hairs of a fine paintbrush: we conclude that chemical release is a discrete event under the active control of the wasp (see Supplementary materials).

We generated individuals that emitted 2-methyl-1,7-dioxaspiro[5.5]undecane at higher molecular weights (additional neutrons) by rearing wasps on hosts injected with deuterated saline (see Methods). In the spectra of chemicals emitted by deuterated females, the relative proportion of the  $m/z$  172 ion was 40-50% of the height of  $m/z$  171 (Fig. 2b) instead of the 8-12% observed in standard females (Fig. 2a). Manipulation of the spiroacetal thus enables clear distinction between emissions of females, termed 'deuterated' and 'undeuterated', during contests.



To set up female-female contests, we used 4-5 day old adult females of known weight and with red or yellow marks (see Methods). Half of the females were 'owners', each provided with a host larva for 24h which they had paralyzed but not laid eggs on, the other 'intruder' females had had no previous contact with a host. Pairs of non-sibling females were selected to obtain a range of weight differences. Within a pair, there was always an owner and an intruder, a red and a yellow marked female and a deuterated and an undeuterated female. Contests were staged, following established methods (Petersen and Hardy, 1996) that neither force behavioural interactions nor prevent loser retreat (see Methods), within a chamber in purpose-made apparatus that allows video-recording of behavioural interactions (principally chases, bites, attempted attacks with stinger, attacks and fights) and possession of the paralysed host. Chemical release was continuously monitored in real-time by APCI-MS (Fig. 3a). After the contest, each female was individually harassed using a paintbrush to check whether they were able to release volatile chemicals: all ( $n=102$ ) released the spiroacetal and the spectra of deuterated and undeuterated females were clearly distinguishable.

Contest outcomes were influenced by competitor asymmetries in weight, prior ownership and deuterium treatment (Fig. 4). Larger body weight was advantageous while prior ownership of the host reduced female contest success (Fig. 4). Deuterated females were disadvantaged compared to similar sized undeuterated females but the effect of deuterium was smaller than that of ownership, indicating that experimental manipulation of wasp chemical composition need not obscure relationships of interest. Contests were generally won by the female that instigated the most agonistic interactions ( $G_1=26.28$ ,  $P<0.001$ , see Supplementary Materials). When intruders took over the host, there was no relationship between intruder-owner weight asymmetry and the number of agonistic interactions but when prior owners successfully defended their hosts, aggressive behaviour was more common when contestants were of similar weight (Fig. 5a).

The spiroacetal was released during 21% (40/189) of aggressive encounters. While release was associated with all classes of agonistic behaviour, its probability increased with the aggressiveness of the interaction (Fig. 3b): release was seldom associated with chasing (repeated measures ANOVA:  $F_{1,50}=22.09$ ,  $P<0.0001$ ) and

biting ( $F_{1,50}=4.40$ ,  $P=0.04$ ), the numbers of attempted and successful attacks with and without release were similar ( $F_{1,50}=2.92$ ,  $P=0.09$  and  $F_{1,50}=0.00$ ,  $P=1.00$ , respectively) whereas fights, in which females violently grapple for 0.40-8.68 seconds until one breaks away and retreats (Fig. 1, and Supplementary Materials), usually co-occurred with release ( $F_{1,50}=9.66$ ,  $P=0.003$ ). Overall, the probability of release was higher when agonistic behavioural interactions were more common and further depended on whether the prior owner or the intruder eventually won the contest (Fig. 5b). Without exception, the spiroacetal was released by females when losing an individual agonistic encounter, and in 93.6% (44/47) of instances of release the female that emitted was also the ultimate loser of the contest overall. In two contests both females emitted spiroacetal, with the last to emit being the ultimate loser. Within individual contest replicates, the occurrence of biting and fighting were reduced following spiroacetal release (Wilcoxon Signed-Ranks Test:  $T=42$ ,  $n=19$ ,  $P=0.03$  and  $T=11$ ,  $n=14$ ,  $P=0.007$  respectively) whereas occurrences remained similar for other agonistic behaviours (Chase:  $T=104$ ,  $n=21$ ,  $P=0.70$ ; Attempted attack:  $T=12.5$ ,  $n=8$ ,  $P=0.46$ ; Attack:  $T=30.5$ ,  $n=11$ ,  $P=0.83$ ; see Supplementary material).

Contests occur commonly in numerous and diverse animal taxa (Maynard Smith, 1982; Mesterton-Gibbons and Adams, 1998; Riechert, 1998; Briffa and Elwood, 2002; Maynard Smith and Harper, 2003). The advantage associated with large body size in *G. legneri* accords with observations on a congener, *G. nephantidis* (Petersen and Hardy, 1996), and the animal contest literature in general. The lower success of prior owners does not, but is corroborated by independently gathered data on this species (T. Hull pers. comm.) and may be expected from theory under some conditions (Mesterton-Gibbons and Adams, 1998). Our experiments further reveal that *G. legneri* produce and emit a spiroacetal, 2-methyl-1,7-dioxaspiro[5.5]undecane. We have also detected this compound in *G. nephantidis* (unpublished data) and it is reported in mandibular or cephalic secretions of several bee species and in the abdominal glands of a beetle (Francke and Kitching, 2001). Other spiroacetals are produced by other aculeate hymenopteran species, as well as by a number of beetles and flies (Francke and Kitching, 2001). Bethyrid species belonging to the Epyrinae, however, emit a different mandibular product: the structurally distinct skatole (3-methylindole) from mandibular glands, possibly as an



alarm pheromone (Gómez et al. 2005) (R.W. Howard, pers. comm., T.P.B, R.S.L & I.C.W.H. unpublished data). In insects, spiroacetals appear to function variously as sex, aggregation or repellent ‘spacer’ pheromones and as components of defensive secretions (Francke and Kitching, 2001). In *G. legneri*, spiroacetal release is clearly associated with agonistic intra-specific interactions, particularly fully escalated fighting, but release by artificially stressed males (which are not known to fight with conspecifics) and females suggests that it may also deter predators or allospecific competitors. Spiroacetal release during female-female contests could reduce subsequent aggression by functioning as a signal (Maynard Smith and Harper, 2003) of submission. The resolution of contests without emission, however, indicates that any signalling function is not always necessary. More likely is that the spiroacetal functions as a weapon. Several minutes of exposure to a high concentration (*ca.* 240 mg m<sup>-3</sup>) of 2-methyl-1,7-dioxaspiro[5.5]undecane is fatal to some insects (Dettner et al. 1992). While the concentrations detected during our experiments using continually flushed chambers were approximately one-tenth of those evaluated by Dettner et al. (1992) (see Supplementary materials), concentrations may be much higher when emission occurs within small cavities and tunnels excavated by the host. We thus suggest that the spiroacetal functions as a weapon used by losers during tactical withdrawals from behaviourally intense agonistic interactions, with the effect of temporarily and partially incapacitating the winner that remains within the confines of the host’s tunnel.

Although numerous studies have focused on animal contests and communication (Maynard Smith, 1982; Riechert, 1998; Maynard Smith and Harper, 2003), technical constraints (see above) have meant that very few prior studies have been able to evaluate the temporal dynamics of chemical release associated with contests. Without reliable correspondence between observable behaviour and chemical exudation, such as exists when some ants compete (Monnin et al. 2002), it may be possible to use a manipulative technique, such as injecting Fluorescein dye into crayfish to make visible subsequent emissions of urinary signals during aquatic contests (Breithaupt and Eger, 2002). For small, terrestrial species, the APCI-MS technique can continuously sample the air around intact and freely moving animals and has great potential to be widely applicable. In addition to the timing of release, this technique can identify the chemical composition of emissions, tracking

simultaneously a number of different compounds, and quantify their relative proportions, whether they are released individually or as a cocktail. APCI-MS is much less behaviourally intrusive and less technically demanding than electroantennography (Wyatt, 2003) yet analytical results are immediately available, unlike GC-MS (Wyatt, 2003; Gómez et al. 2005). We envision that APCI-MS will be employed to facilitate the study of many other types of chemically related behaviour, such as mating interactions (Wyatt, 2003), insect attraction to induced plant volatiles (Turlings, Davison and Tamò, 2004) and, when coupled with chemical manipulation, mark-recapture studies (Steffan et al. 2001) in which standard techniques are invasive, time consuming, technically challenging or more expensive (Steffan et al. 2001; Turlings et al. 2004). Using APCI-MS, the current study has discovered a hitherto unknown component of parasitoid contest interactions: a volatile chemical that is emitted by losers.

## **Material and Methods**

### **Real-time volatile analysis**

We used APCI-MS (Taylor and Linfoth, 2003) in which the gas phase is continuously drawn into the MS with no chromatographic separation, hence there is no sample loss or discrimination during sampling. The low dead volume and response rate of the system allow rapid changes to be followed (up to 50 data points  $s^{-1}$ ) with good sensitivity (limit of detection  $\approx 0.1 \text{ mg m}^{-3}$ ). The APCI source was mounted on a Platform II mass spectrometer (Waters Manchester UK). The sample stream ( $25 \text{ ml min}^{-1}$ ) was conducted through a heated ( $160^\circ\text{C}$ ) transfer line via a deactivated fused silica tube ( $1 \text{ m} \times 0.53 \text{ mm ID}$ ). The analytes entering the source were ionized by a 4kV positive ion corona discharge (resulting in a cascade of charge transfer), typically resulting in protonation to form  $\text{MH}^+$ . For full-scan analysis, the mass spectrometer acquired 1 scan  $s^{-1}$  over mass range 25-260Da, with the cone voltage set to 18V. For selected-ion analysis, we monitored  $m/z$  171, 172 and 173 with a dwell time of 0.02s and cone voltage of 18V. Estimation of the gas phase concentration of (the commercially unavailable) 2-methyl-1,7-dioxaspiro[5.5]undecane was achieved using tetrahydropyran (Aldrich, Gillingham UK) which has similarities in both structure and ionization efficiency: signal height



for these two compounds is very similar for equimolar concentration. The gas phase concentration was estimated by comparison of the height of the signal obtained for the calibrants, with that of the 2-methyl-1,7-dioxaspiro[5.5]undecane.

### **Chemical marking**

*Goniozus legneri* were reared on 30-40mg larvae of the host *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) at  $27\pm 2^{\circ}\text{C}$ , 70% R.H. and a 9L:15D photoperiod. Host larvae were placed individually in glass vials with an adult female wasp. After paralysis by the wasp, host larvae were removed and injected, using a fine hypodermic needle inserted through the dorsal integument just posterior to the head, with 5 $\mu\text{L}$  of phosphate buffered saline (PBS) (solution 10 $\times$ DNase RNase and protease free) in either 90% water ( $\text{H}_2\text{O}$ ) or 90% deuterated oxide, ‘heavy water’ ( $\text{D}_2\text{O}$ ). Host larvae were returned to the vial and subsequently parasitized by the wasp: the resultant offspring matured around 14 days later and females were used in contest experiments.

### **Contest experiments**

Females were marked with a dot of acrylic paint on the dorsal surface of the thorax and weighed to an accuracy of  $10^{-2}$  mg. Between 9 and 15 replicates were obtained for each of the possible ownership, colour and chemical mark combinations within a pair of contestant females, giving a total of 51 contest replicates. Contests were observed within the central of three chambers, connected by a slot, in an opaque plastic block covered with clear plexiglass (Petersen and Hardy, 1996) and maintained at  $28^{\circ}\text{C}$ . Owners and their hosts were placed into the contest chamber and an intruder into a peripheral, and initially separated, chamber. After 30 minutes, barriers filling the slot were withdrawn sufficiently to inter-connect the three chambers. Wasp behaviour was recorded from above using a digital video camera for 30 min from when the intruder entered the central chamber. Chemical emissions were continuously monitored, with the MS transfer line drawing gas phase volatiles via a small hole in the side of the central chamber into an APCI-MS in selected ion mode. The exact starting time of experiments was recorded on both the videotape

and the ion trace to reveal correspondence between behavioural events and chemical release.

### Statistical analysis

Generalized linear modelling (in GenStat, version 7.2.0.208) was used to obtain parsimonious statistical descriptions via stepwise backward analyses. Logistic analyses were used for (proportional) contest data (Petersen and Hardy, 1996): in analysis of contest outcome (Fig. 4) the binary response (1=won, 0=lost) was defined by red female success (Petersen and Hardy, 1996) after checking that outcomes were not influenced by mark colour (red wasps won 19/51 contests; binomial test,  $P=0.092$ ). Analysis of count data (number of aggressive interactions) employed log-linear models with the dispersion parameter estimated empirically to take overdispersion into account, and significance assessed using  $F$ -ratio tests. Non-parametric analyses were used when error variances did not conform to parametric assumptions.

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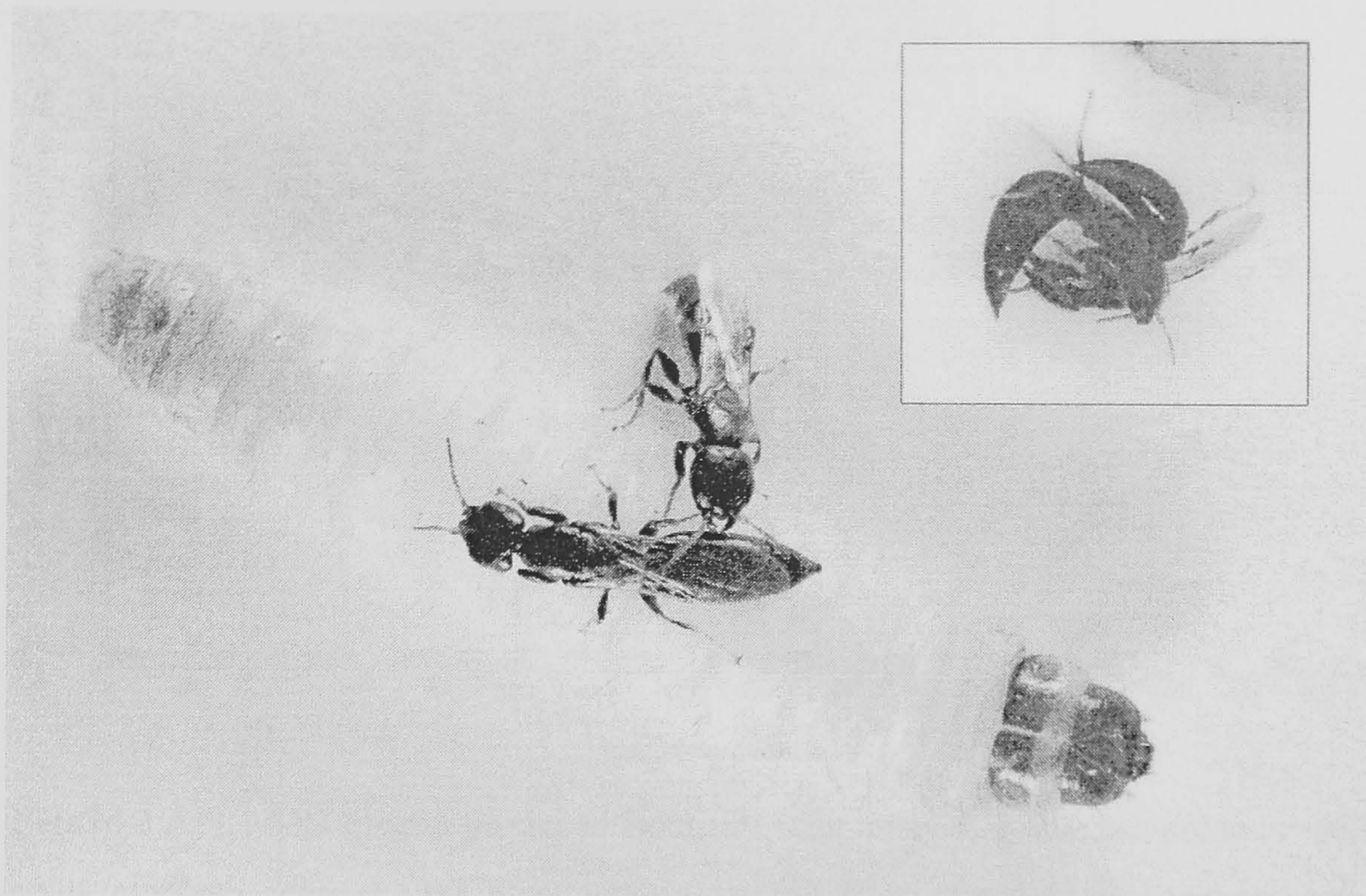
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**Author contributions** Experimental work was carried out by M.G. Analysis and writing were carried out by M.G. and I.C.W.H. All authors contributed to developing the methodology, discussed the results and commented on the manuscript.

**Author information** The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to I.C.W.H. ([ian.hardy@nottingham.ac.uk](mailto:ian.hardy@nottingham.ac.uk)).

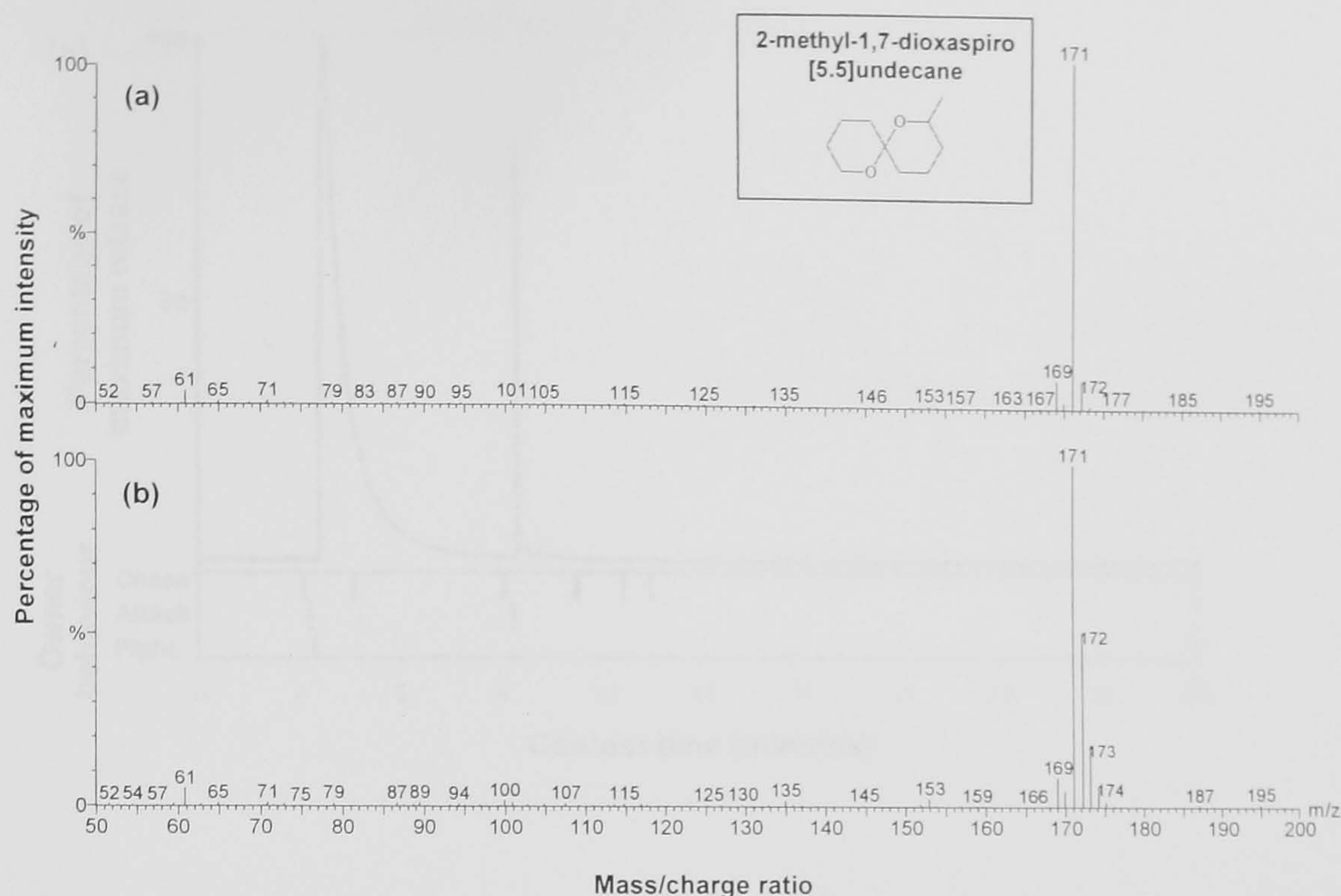


## FIGURES



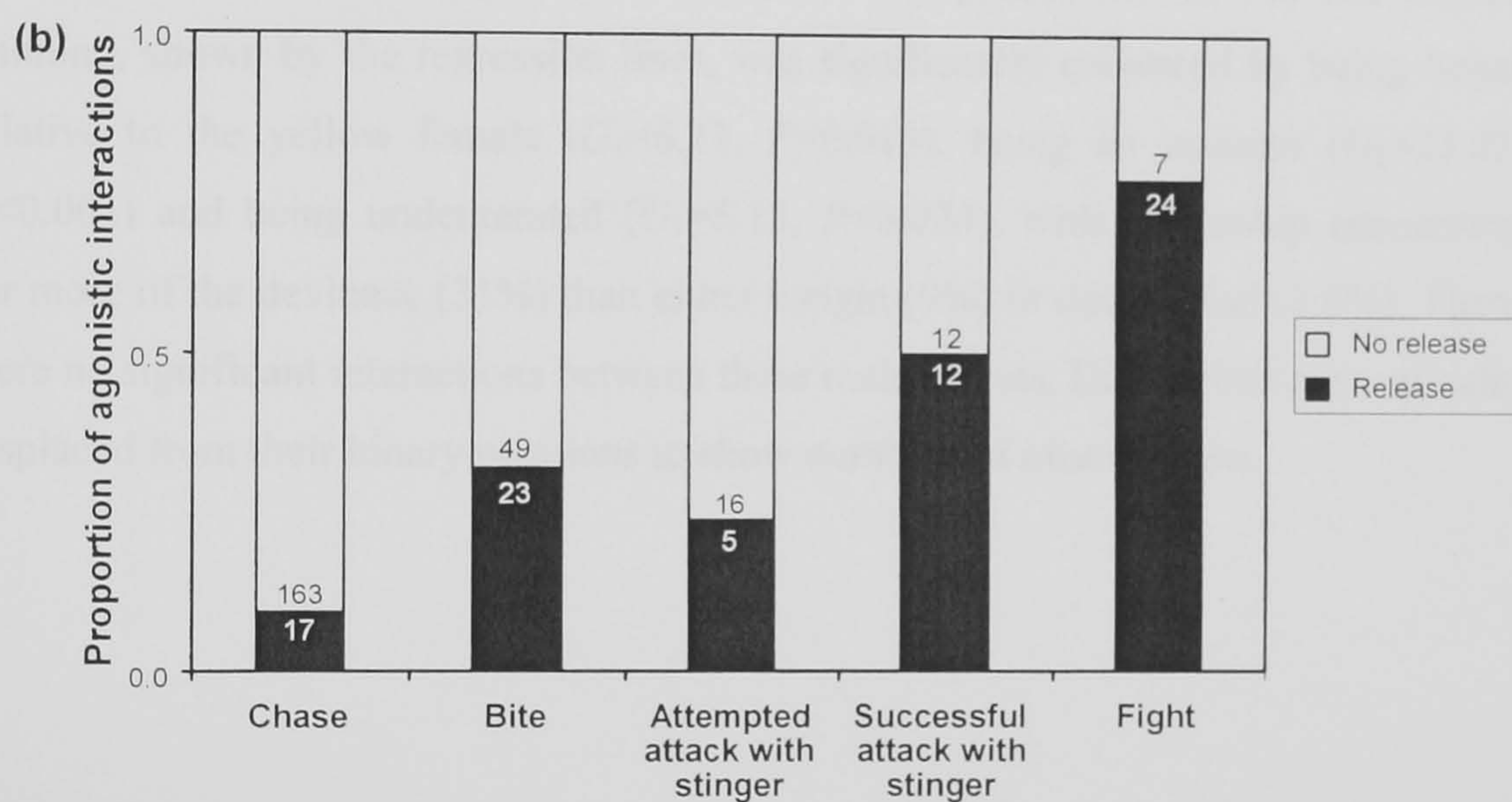
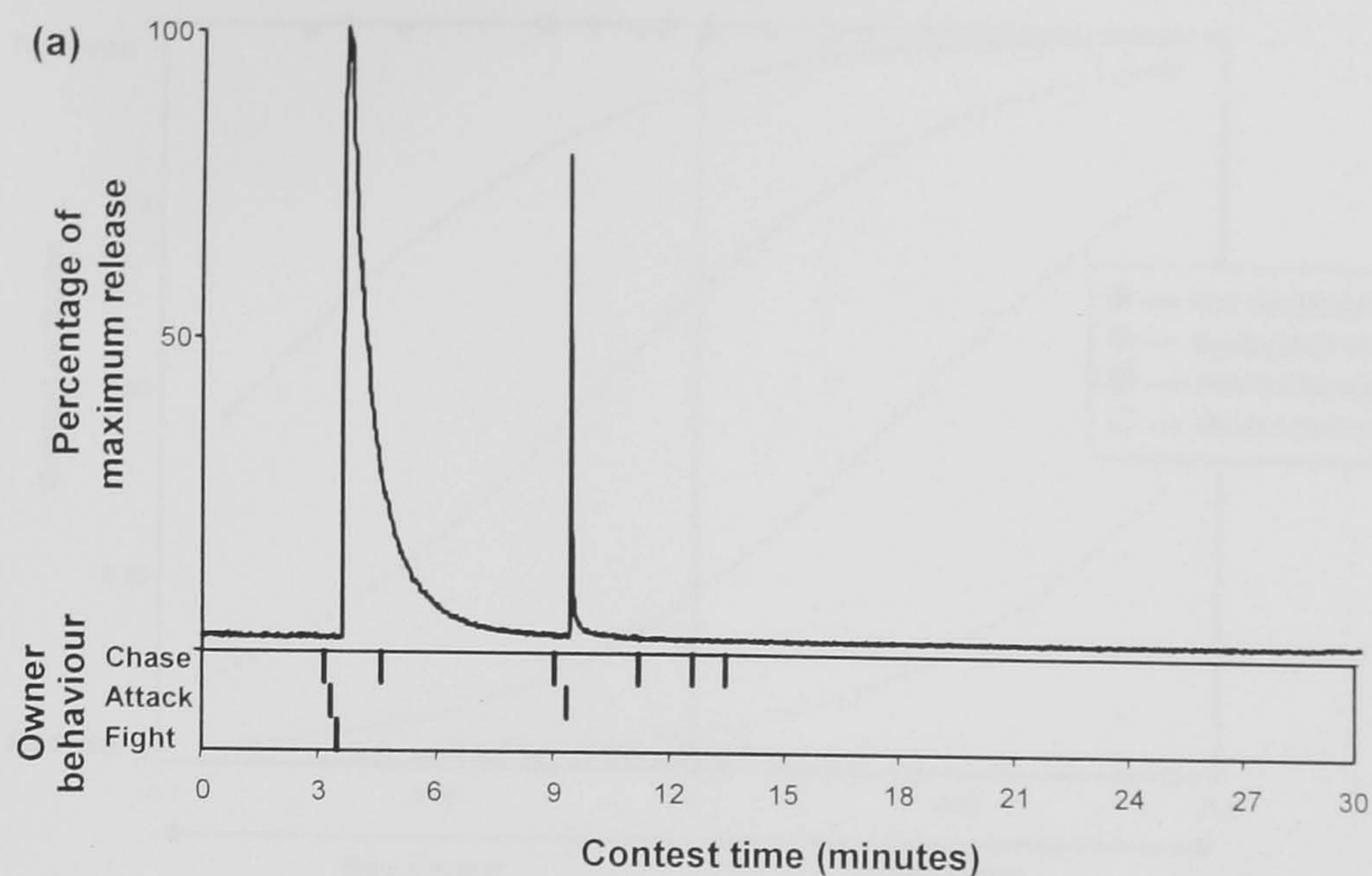
**Figure 1.** *Goniozus legneri* females competing for a host. One female is biting her opponent's abdomen. Such behaviour often leads to full fighting (inset) (Photos: Sonia Dourlot).





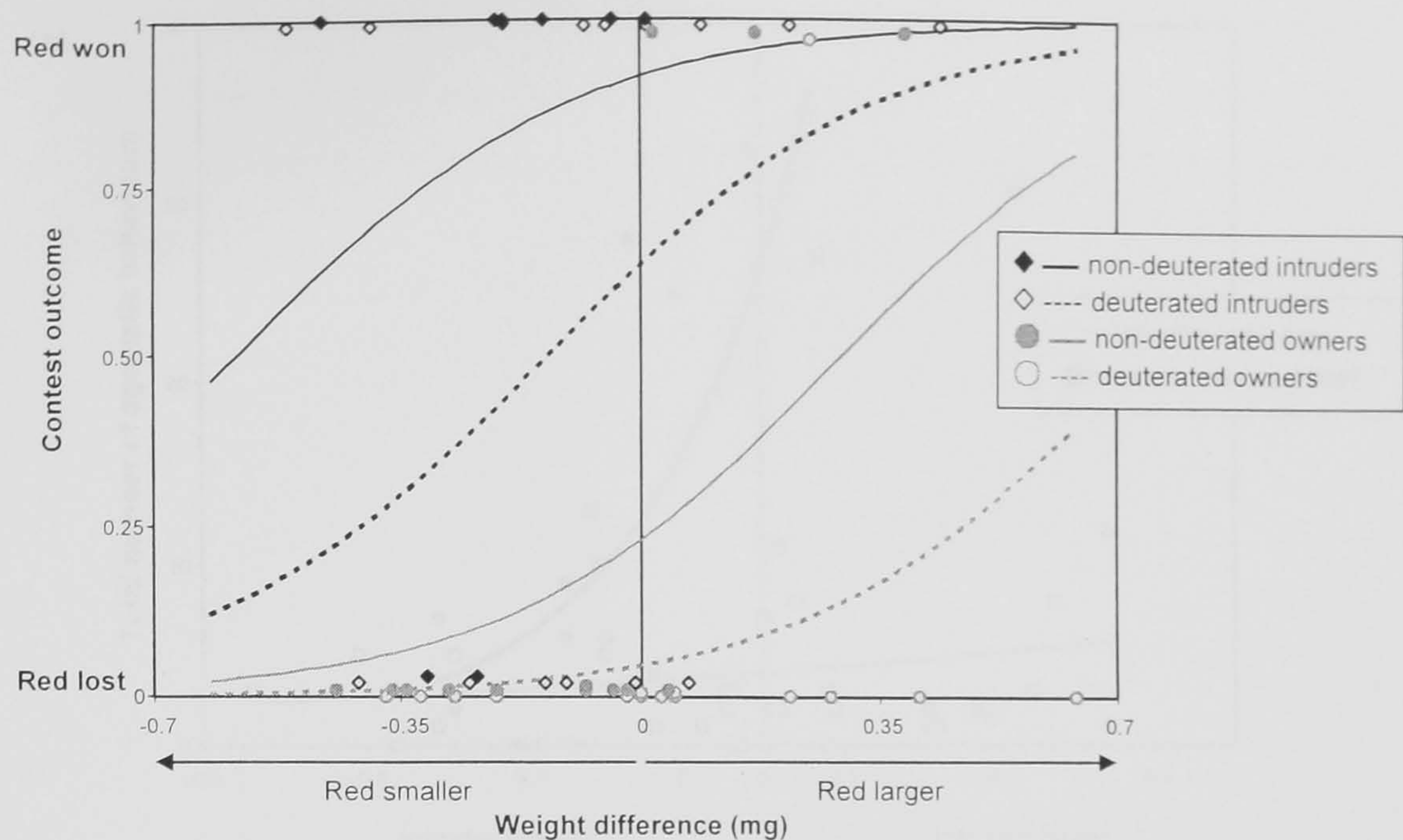
**Figure 2** APCI-MS spectra produced from full-scan analysis of the gas phase around adult female *G. legneri*. **(a)** Undeuterated wasp **(b)** Wasp reared on host injected with deuterated saline showing enhanced and additional peaks at m/z 172-174, corresponding to 2-methyl-1,7-dioxaspiro[5.5]undecane molecules (see insert for structure) containing 1-3 deuterium atoms. The small peak at m/z 172 in (a) is likely due to naturally occurring C<sub>13</sub>.





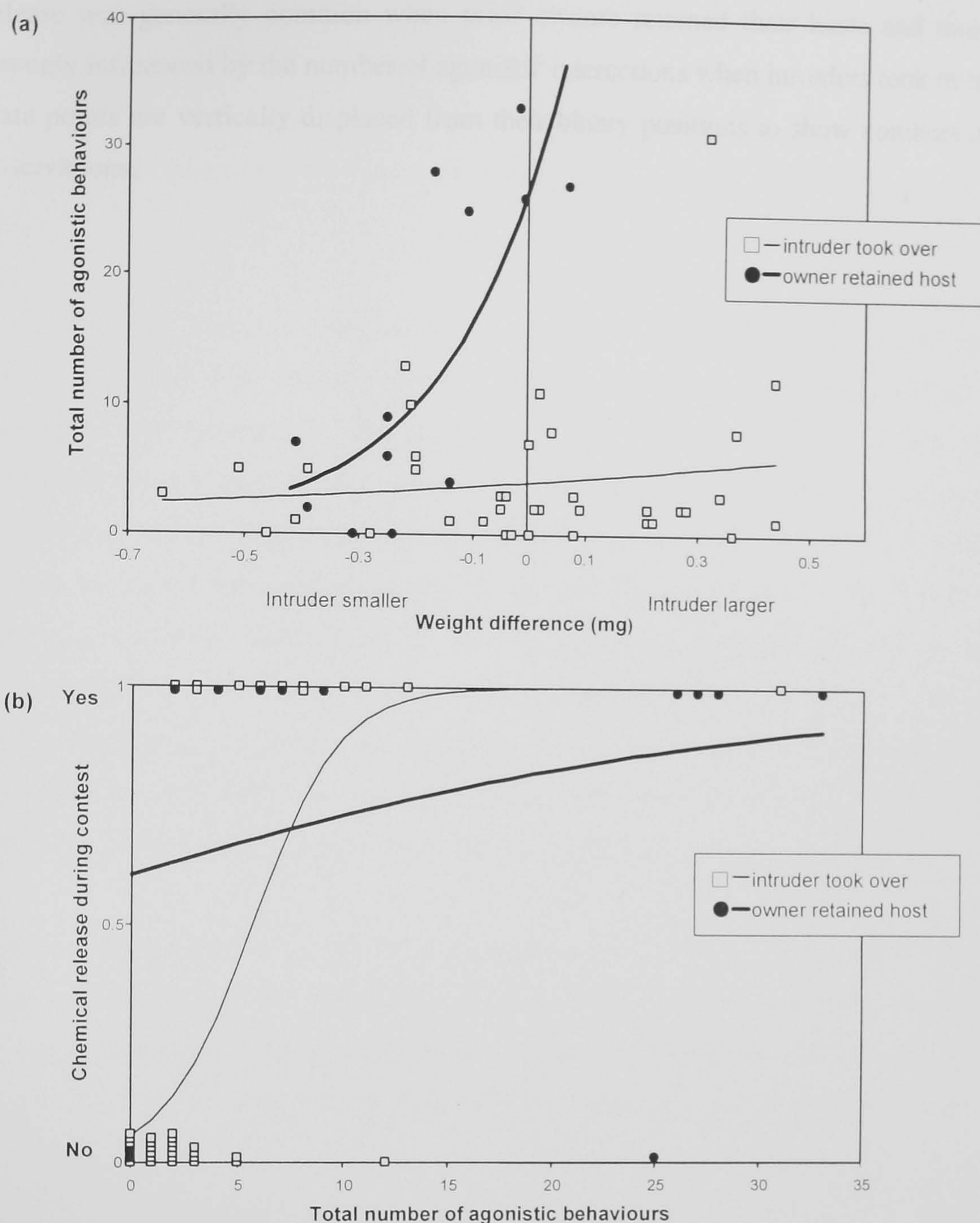
**Figure 3** Associations between chemical release and behaviour. (a) Typical ion trace during a contest. The intruder released twice in response to owner aggression. Some chases were not accompanied by release. The intruder did not initiate aggressive behaviour in this case (see Supplementary materials for video). (b) Frequencies of agonistic interactions, in order of apparently increasing escalation, with and without associated spiroacetal release. Results are summed across 51 replicates.





**Figure 4** Determinants of contest outcome. The probability of the red female winning, shown by the regression lines, was significantly enhanced by being larger relative to the yellow female ( $G_1=6.13$ ,  $P=0.013$ ), being an intruder ( $G_1=21.22$ ,  $P<0.001$ ) and being undeuterated ( $G_1=5.13$ ,  $P=0.024$ ), with ownership accounting for more of the deviance (31%) than either weight (9%) or deuteration (7.6%). There were no significant interactions between these main effects. Data points are vertically displaced from their binary positions to show numbers of observations.





**Figure 5** Contest outcome, agonistic behaviour and chemical release. **(a)** Number of agonistic behaviours during each contest in relation to owner-intruder weight difference and winner identity. Level of aggression was not influenced by size asymmetry when intruders took possession of the host (log-linear analysis,  $F_{1,37}=1.29$ ,  $P=0.26$ ) but was strongly related to weight asymmetry when owners retained the host ( $F_{1,10}=11.44$ ,  $P=0.007$ ). **(b)** Probability of spiroacetal release in relation to frequency of agonistic behaviour and winner identity. Release was more likely when agonistic interactions were more common (logistic analysis,  $G_1=17.23$ ,  $P<0.001$ ) and was further influenced by the winner's identity ( $G_1=7.76$ ,  $P=0.005$ ) and its interaction with the number of agonistic interactions ( $G_1=7.79$ ,  $P=0.005$ ):

release was generally common when prior owners retained their hosts and more strongly influenced by the number of agonistic interactions when intruders took over. Data points are vertically displaced from their binary positions to show numbers of observations.

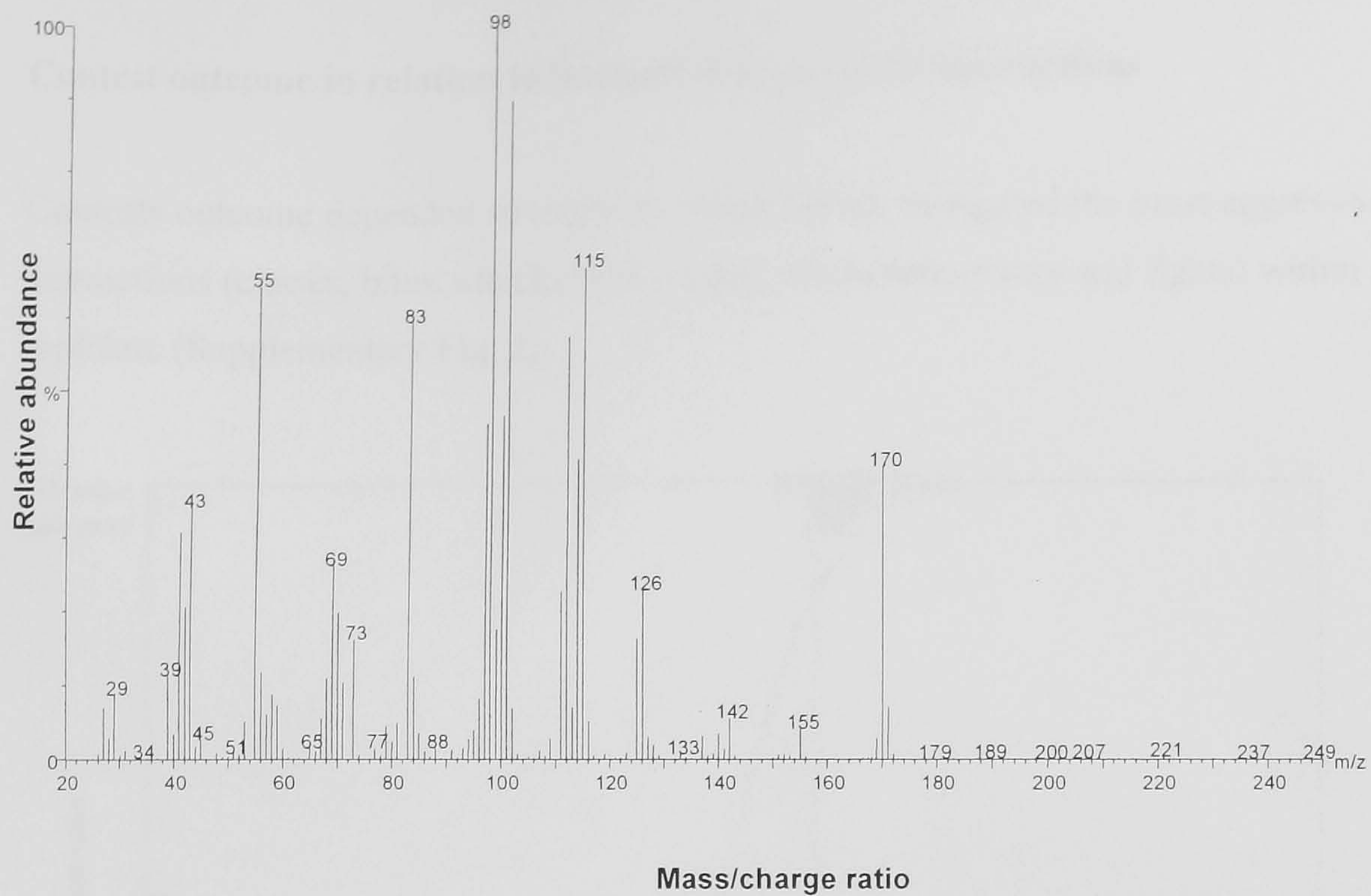


## SUPPLEMENTARY TEXT AND FIGURES

### Chemical identification by Gas Chromatography - Mass Spectrometry (GC-MS)

To identify the chemical released by *G. legneri*, 10 females were squashed and placed in a 20mL flask sealed with a PTFE lined septum. A SPME fibre (50 30µm, assembly Divinylbenzene/Carboxen/Polydimethylsiloxane; Supelco, Bellefonte, USA) was exposed in the flask headspace for 5min at 22°C. The volatile compounds on the fibre were subsequently desorbed in the injector at 240°C for 1 min. After transfer of the volatile compounds onto the column (30m×0.25mm ID, BP-5, 1.0µm film thickness; SGE, Milton Keynes, UK), the gas chromatograph (Trace GC Ultra, Thermo, Austin, USA) temperature program started (carrier gas helium, constant flow 1.5ml/min). An initial temperature of 50°C was held for 2min and then increased at 10°C min<sup>-1</sup> to 230°C. Spectra were recorded using a DSQ mass spectrometer (Thermo), scanning from m/z 20 to 250 at 2 scans s<sup>-1</sup>.

The main peak observed on the GC chromatogram was at 12.22 min, all other peaks were less than 20% of the intensity of this peak. The mass spectrum (Supplementary Fig. 1) was consistent with that of 2-methyl-1,7-dioxaspiro[5.5]undecane, a 170Da spiroacetal with two 6-membered rings and one methyl group attached to an alpha-carbon.

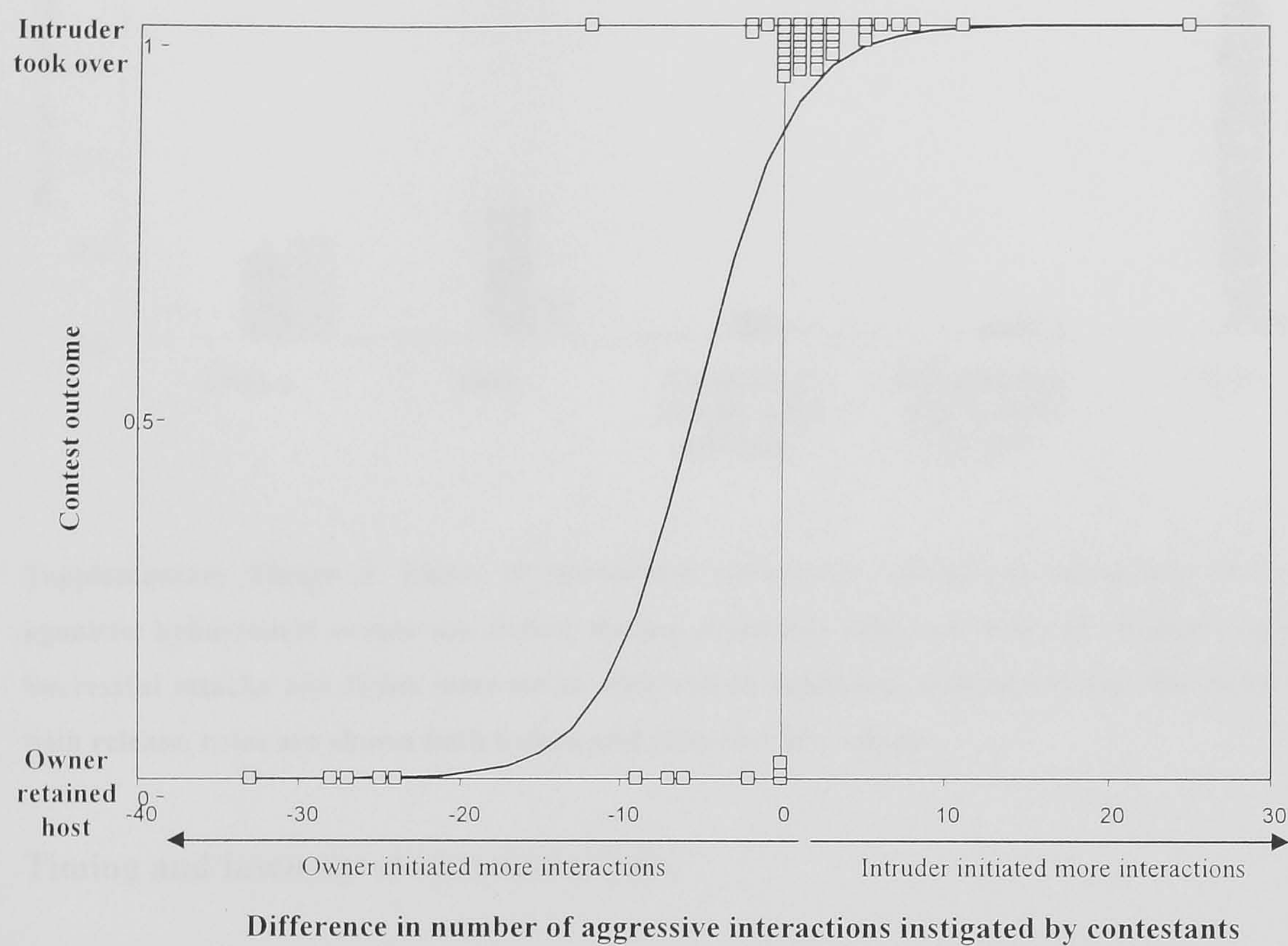


Supplementary Figure 1: GC-MS spectra of the main peak observed in the analysis of *G. legneri* headspace.



Contest outcome in relation to instigation of agonistic interactions

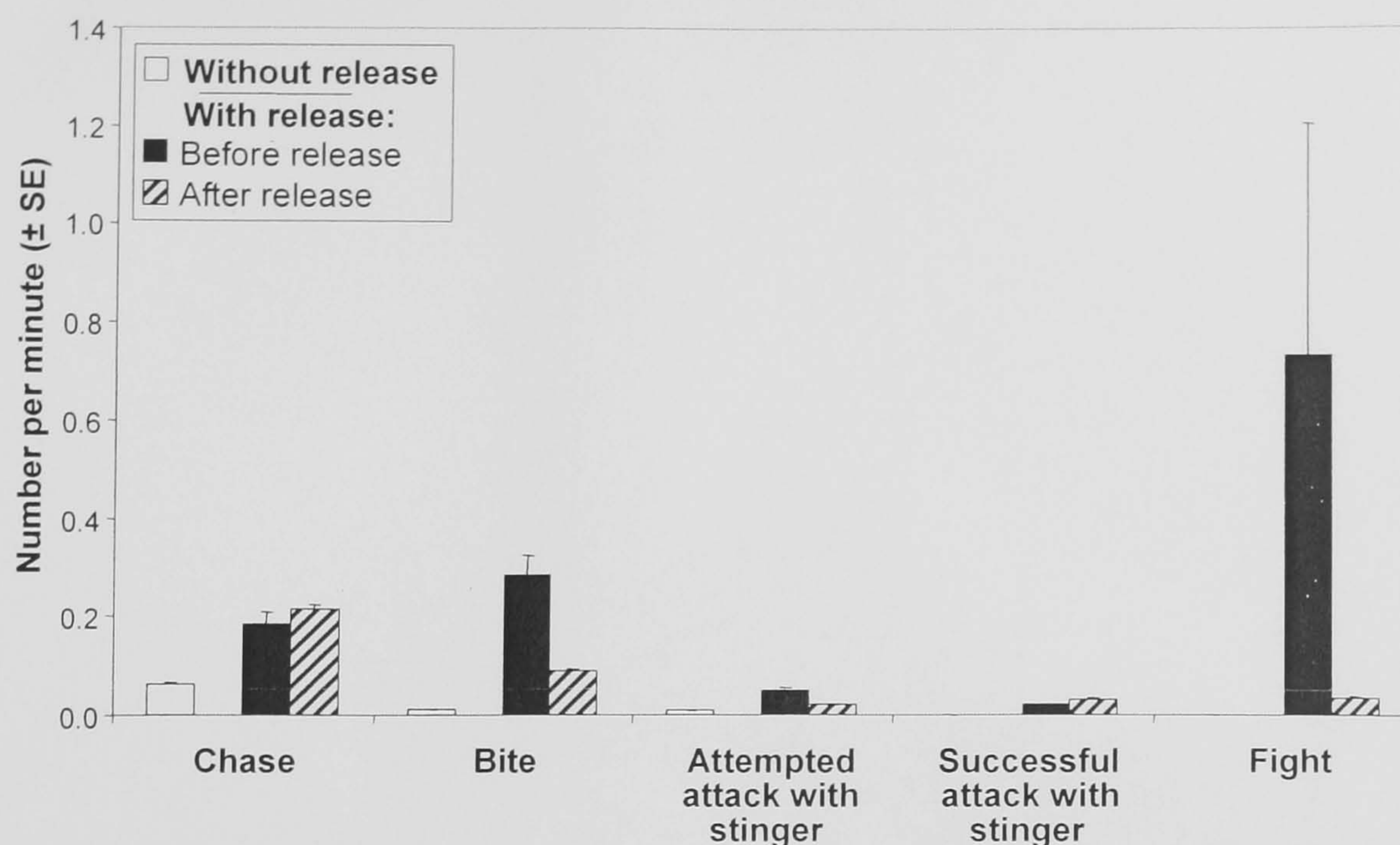
Contests outcome depended strongly on which female instigated the most aggressive interactions (chases, bites, attacks with stinger, attempted attacks and fights) within a replicate (Supplementary Fig. 2).



Supplementary Figure 2. Contest outcome in relation to the difference in number of aggressive interactions instigated by competing females (number instigated by intruder - number instigated by owner). The fitted curve shows the probability of intruder take-over as estimated by logistic regression. Data points are vertically displaced from their binary positions to show numbers of observations.



### Effect of chemical release on subsequent behaviour



Supplementary Figure 3. Effect of spiroacetal release on subsequent behaviour. Rates of agonistic behavioural events are shown during replicates with and without chemical release. Successful attacks and fights were never observed in replicates without release. For replicates with release, rates are shown both before and after the first release.

### Timing and intensity of chemical release

Chemical releases during contests typically had short half-peak widths (mean $\pm$ SD, 40 $\pm$ 25s) and short times to reach peak intensity (12 $\pm$ 7s). Given the 25ml min<sup>-1</sup> sampling flow rate and ~4.7cm<sup>3</sup> chamber volume, clearance of spiroacetal was relatively rapid, supporting the assertion that emissions were single, short-duration events. The gas phase concentration of the spiroacetal released during contests (range 0.2-18.2 mg m<sup>-3</sup>) was generally lower than during experimental stressing with a paintbrush (mean $\pm$ SEM, 78.8 $\pm$ 13.3 mg m<sup>-3</sup>) or squashing (mean $\pm$ SEM, 98.3 $\pm$ 31.2 mg m<sup>-3</sup>): such comparisons are, however, likely to be compromised because females emitting during contests were usually retreating from the vicinity of the APCI-MS intake.