The Potential of *Sclerodermus brevicornis* as a Native Biocontrol Agent of Invasive Wood-boring Beetles in European Agro-forest Ecosystems

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

December 2019

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Abstract

This thesis presents a series of experiments aimed to develop the potential of the parasitoid wasp *Sclerodermus brevicornis* (Hymenoptera: Bethylidae) as an agent of biological control of invasive wood boring beetles. This development includes relatively straightforward considerations, such as finding suitable alternative hosts for its efficient mass rearing and, because *Sclerodermus* are quasi-social, it also includes gaining an understanding of the reproductive behaviours that make up its unusual life-history, with particular focus on the importance of interactions between kin. The first chapter introduces the economic impact of invasive wood-boring long-horned beetles on forests and biological pest control, especially using parasitoids, including bethylids. Next, the underlying principles of the experiments performed are presented. The first experimental work (Chapter 2) deals with the efficiency of rearing *S. brevicornis* on a new factitious (alternative) host. The suitability of rice moth (*Corcyra cephalonica*) larvae is explored and it is concluded that this species is suitable to mass-rear *S. brevicornis* efficiently, even though it is a lepidopteran and the natural hosts of *Sclerodermus* are coleopterans. The second set of experiments (Chapters 3 and 4) explores effects of kinship between *Sclerodermus* co-foundresses. The potentially interacting roles of host size, foundress number and relatedness as evolutionary influences on cooperative reproduction were evaluated. Kinship effects were found consistently, notably in the timing of host attack. Individual females appear to be reluctant to attack large and dangerous hosts unless the benefits of their success will be shared among their kin. It is concluded that while cooperative reproduction in *Sclerodermus* can be selected for by direct fitness benefits, it will be subject to modification by inclusive fitness

considerations. On the basis that quasi-sociality probably evolved from a less complex social system, and with the aspiration of generating insights into transitions between 'levels' of sociality, the third set of experiments (Chapters 5 and 6) explored host size, foundress number and kinship effects in two members of the bethylid genus *Goniozus*: these are naturally sub-social, exhibiting maternal care but not cooperative reproduction. Effects of kinship were found in both species, most notably on the sex ratio of offspring broods. In *G. nephantidis* sex ratios of multi-foundress groups responded to foundress number when females were non-siblings but not when they were siblings. In *G. legneri*, sex ratios were less female biased when mothers had mated with a male from a different strain. Both these effects are in the direction predicted by sex ratio theory that considers variation in relatedness within local mating population structures. Although the focus of these studies is on understanding the reproductive biology of *Sclerodermus*, along with *Goniozus*, to explore the origins and maintenance of quasi-sociality, advances in this area have potential to enhance the deployment of these parasitoids in biological pest control. They can feed into the design of mass-rearing and release programmes for *Sclerodermus brevicornis*, and thus contribute to tackling wood-boring long-horned beetles that are invasive in European agroforestry.

Acknowledgements

All experimental work were conducted under the direct supervision of Dr Ian Hardy and in collaboration with Dr Daniela whose entomological laboratory at the University of Milan, Italy was used for the second, third and fourth experimental chapters of this thesis, whereas the last two experimental chapters were conducted at the ecology laboratory of the University of Nottingham, The United Kingdom.

This PhD project was made possible by The Merit Scholarship Programme sponsored by The Islamic Development Bank with collaboration of The University of Nottingham, UK.

I wish to express my sincere thanks and appreciation to my supervisor, Dr Ian Hardy who convincingly encouraged me to be professional and become stronger even when the road got tough. I would also like to express all my thanks to my second supervisors: Prof Karl and Dr Daniela Lupi for their assistance and guidance during this period. I further express my appreciation and thanks to Dr Helen West for her considerable efforts to help me as my internal assessor. I also wish to express my gratitude to Emma Hooley for her excellent administration. Special thanks to Dr Costanza Jucker (The University of Milan) for the persistent help. Many thanks to Laura Holt for the lab assistance.

I am deeply grateful to many friends in my support: Abdulhamid Al-Riyami, Yusuf Barre Abdullahi Gedi, Fu'ad Mire Hassan, Abdulwahab Musa, Tarik Al-Mandhari, Saeed Ahmad, Mohamed Saeed, Edna Can, Shadia Kareem and my helpful colleagues in the Department of Food, Environmental and Nutritional Science (DeFENS), University of Milan, Italy.

I wish to acknowledge the persistent support and encouragement of my siblings: my brother Ahmed-Rashid Abdi and his family; my brother Ahmed-Fowzi Abdi and his family; and my sister Hodan Abdi and her family.

I would have never achieved this goal without the sacrifice and great love of my family: my wife Istanbul Mohamed Muse; my sons: Fowzan, Nabil and Akram; and my late daughter Maryam.

Above all, all praise and thanks be to ALLAH for giving me the strength, ability, knowledge and opportunity to proceed successfully. Without his blessings, dreams would not have come true and this achievement would not have been possible.

To the soul of my parents who taught me that "Hard and smart work pays off"

To the soul of my beloved daughter Maryam

To my beloved wife Istanbul and my children Fowzan, Nabil and Akram

To my brothers Ahmed-Rashid and Ahmed-Fowzi and their families

To my sister Hodan and her family.

Chapter 1: General Introduction

1.1 Invasive wood-boring long-horned beetles in European agroforestry ecosystems

It is widely held that forests are key components of many world ecosystems and that they are of both economic and long-term environmental importance. This thesis examines the reproductive biologies of parasitoid wasps that are natural enemies of pests of natural forest and agri-forest systems. I first introduce the topics of forests and forest pests before introducing approaches to the control of these pests and the fundamental biology of the parasitoids studied.

1.1.1 Definition of forests

The definition of forest according to the Clean Development Mechanism (CDM) of the Kyoto Protocol is an area that contains more than half of one hectare with a minimum of 10-30% “tree” crown cover, with “tree” defined as a plant that is capable of growing to be more than 2-5 m tall (UNFCCC, 2002). Based on the Global Forest Resource Assessment, a forest is alternatively defined as a land area spanning more than a half hectare covered with trees taller than five meters and also with more than 10% of canopy cover [or covered with trees able to reach these aforementioned thresholds *in situ* (FAO, 2006)]. Hence, agricultural or urban lands even with some tree cover are excluded as forests by this definition, but in contrast, forest lands that were naturally disturbed or harvested are included. It is important to account for the differences in forest definitions, and for land-use considerations, it is necessary to differentiate between the Food and Agriculture Organisation (FAO) definition for a forest and the other definition of a forest that focuses on the presence or absence of trees.

Failing to account for these definition differences might have profound implications for any large-scale overview. For example, contrasting conclusions were drawn by two independent studies during a same period: Hansen *et al.* (2010) reported a six percent loss of forest cover in the United States between 2000 and 2005, whereas Smith *et al.* (2009), using the FAO definition, pointed out a net gain of forest land during the same period. The contradictory conclusions are due to the different definitions adopted.

1.1.2 Forest classification

Forests are classified in different ways according to the biome in which they exist in combination with the longevity of the dominant species' leaves (i.e., evergreen or deciduous), or whether the forest is predominantly composed of broadleaved, coniferous or mixed trees. The United Nations Environmental Programme – World Conservation Monitoring Centre (UNEP-WCMC) categorised world forests into twenty-six major types which can be clustered into six major groups: temperate needle-leaf, temperate broad-leaf and mixed, tropical moist, tropical dry, sparse trees and parkland, and forest plantations. This achieved a harmonised global forest classification, rather than national or regionally based definitions. On the other hand, the World Wide Fund for Nature classified forests into six types based on their location and the climate of their environment: this classification includes Tropical, Sub-tropical, Mediterranean, Temperate, Coniferous and Montane forests.

1.1.3 Distribution of forests

Forests are widely distributed across the world. Forests in Asia (including Asian Russia), represent 31% of the global total forest area, whereas forests in South

America rank second with 21%, followed by 17% each in Africa, in North America, while the percentage in Europe is 9%, and 5% in Oceania (FAO, 2010). There are, however, no forests in Antarctica. Five percent of global forests are plantations generally exploited for commercial purpose (Robbins, 2007).

1.1.4 Importance of forests

Globally, forests cover around 4.03 billion hectares, which is approximately 30% of the total land area of the earth (FAO, 2010). Beer *et al.* (2010) reported that forests account for three quarters of terrestrial gross primary production. Forests also account for 80% of the total plant biomass of the Earth (Kindermann *et al.*, 2008) and they contain more carbon in soils and biomass than there is in the atmosphere (Pan *et al.*, 2011). Forests provide habitat for the majority of the species living on earth (Lawesson *et al.*, 1998; Diaz, 2006; McAlpine *et al.*, 2006; Bohn & Huth, 2017). Moreover, forests provide essential ecosystem services and valuable goods to humanity, such as food, fibre, timber, medicine, water, and climate moderation (Byron & Arnold, 1999; Jackson *et al.*, 2005; McKinley *et al.*, 2011). In addition, forests play a crucial role in livelihood of world's poor communities, and provide them with a reliable sources of energy and shelter (Secretariat, 2010). Good forest management is considered as an effective means of enhancing and maintaining high carbon sequestration rates. In turn, this will mitigate climate change problems, and provide further ecosystem services (Ruiz-Peinado *et al*., 2017). It should be taken into the account that the deadwood component of some forests contains a high quantity of carbon which is always encouraged for biodiversity policies but, in turn, this

would result in greater soil carbon sequestration (Magnússon *et al.*, 2016). Forests are also important for recreational purposes (Mayer & Hoppe, 1984).

1.1.5 Gain and loss of forests

The delivery of significant ecosystem services, including, biodiversity richness, carbon storage, climate regulation, and water supplies is affected by changes in forest cover (Foley *et al.*, 2005). By using earth observation satellite data, Hansen *et al.* (2013) reported a global forest loss (2.3 million square kilometres) and gain (0.8 million square kilometres) between 2000 and 2012.

The greatest loss was experienced by the tropical domain due to factors including deforestation. The subtropical forests were adversely affected by extensive forestry land uses (Drummond & Loveland, 2010). However, it was reported that the rate of Brazilian rainforest clearing was reduced and that, consequently, decreased the annual forest loss. The ratio of loss to gain was relatively low in the temperate climatic domain which also has a forestry dominant change dynamic. Given slower regrowth dynamics, after forest loss mostly caused by fire, boreal forests ratio of loss to gain was reported high (Hansen *et al.*, 2013).

Reforestation and afforestation activities in many parts of the world, particularly in East Asian countries, are considered as the common factors contributing to the gain of forests (Fang *et al.*, 2001). Furthermore, forest conditions in many regions were improved by adopting good forest management practices. For instance, the European forest standing biomass was increased by ~40% between 1950 and 1990, and the forest area remained unchanged due to the improved forest management (Kauppi *et al.*, 1992; Nabuurs *et al.*, 2003). On the other hand, the most common causes for forest losses are land-use

activities, primarily for agricultural expansion and unsustainable timber extraction (Ramankutty & Foley, 1999; Gibbs, 2000), as well as replacement of natural forest with highly managed forests, such as timber plantations in North America and oil-palm plantations in Southeast Asia (Turner II *et al.*, 1990).

1.1.6 Forest pests

Apart from other abiotic limiting factors of forests distribution, such as geographic and climatic (Woodward, 1987; Woodward *et al.*, 2004; Seidl *et al.*, 2017), forests also suffer from the damage and loss caused by biotic factors including pathogens and insect pests (Seidl *et al.*, 2017) which are indirectly introduced through land-use activities (Nepstad *et al.*, 1999). Recently, pests and plant diseases were reported to be having adverse impacts on ecosystem services directly through the destruction of service providers, or indirectly, as an environmental consequence of their management (Cheatham *et al.*, 2009; MacLeod *et al.*, 2010). Annually, pests, pathogens, and other biotic forcing factors impact on more than 20.4 million forested hectares in the United States alone, causing an annual economic loss of about two billion US dollars (Dale *et al.*, 2001). Gibbs and Wainhouse (1986) discussed in detail the spread of forest pests and diseases in northern-hemisphere. They noted that a common reason for this destructive spread is due to the actions of human beings who introduce insects or pathogens from one region to another, often disturbing any co-evolutionary and population dynamics between the host and its locally present natural enemies.

A substantial literature shows a global spread and establishment of pests and diseases. Examples of pathological pests include the chestnut blight epidemic in the eastern United States of America during the summer of 1904 caused by

the fungus *Endothia parasitica* noticed on the American chestnut and later in 1938 on the European chestnut in Italy (Kuhlman, 1978; Elliston, 1982). Other pathological examples include white pine blister rust (Boyce, 1938) and many others. Among the most damaging of all forest insects and one of the most imported insects from Europe to North America is the Gypsy moth, *Lymantria dispar* (L.), (Doane & McManus, 1981). This insect pest is the most important defoliator of deciduous forests of the eastern United States (Mcmanus & Csóka, 2007). Another entomological forest pest is balsam woolly aphid *Adelges piceae*, considered as a serious pest of true firs and has caused great loss of timber (McGugan & Coppel, 1962; Coulson & Witter, 1984).

China is currently classified as the country with the most rapidly increasing forest resources in the world. However, China's forests are infested by serious forest pest problems. Currently, there are more than eight thousand species of potential forest pests including insects, pathogens, rodents and weeds. Among those, 300 species are of economic and ecological significance and around half of that three-hundred are classified as serious pests, including 86 insect species (Ji *et al.*, 2011). In 2005, Pimentel *et al.* (2005) reported that the annual economic loss in the United States forests that is solely attributed to forest pests was estimated about US$ 2 billion whereas in China, Ji *et al.* (2011) reported that the total area infested by forest pests in the late 1990s was about 7 million hectares with an economic loss of about US$ 1.03 billion (RMB 7.2 billion). The infested area increased during the period between 2001 and 2005 to about 10 million hectares, and then dramatically to 12 million hectares in 2007, incurring direct economic losses estimated about US$ 3.37 billion (RMB 23.6 billion).

Focusing on forest insect pests, trees, like agricultural and horticultural crops, are susceptible to insect damage. They are attacked in the nursery by leaf eaters; root grubs and termites in the field during the early establishment; and during the later stage perpetually by defoliators, gall formers, sap-suckers, stem, pod or fruit borers, and seed eaters (Rao *et al.*, 2000). With regard to the tropics, Rathore *et al.* (1995) showed that about twenty agro-forestry trees in Sub-Saharan Africa are infested by a compilation of insects, and some species of those trees, such as eucalypts (*Eucalyptus* spp.) host as many as 160 insects across different parts of tropics. Similarly, Vir and Verma (1996) reported that seventeen leguminous trees used in dryland agroforestry in India were infested by insects. The Net Primary Production (NPP) of the temperate forests in Northern America is negatively impacted by wood borers and invasive pests, compared to defoliators (Flower & Gonzalez-Meler, 2015). Mountain pine beetle (*Dendroctonus ponderosae*), a bark-boring beetle which is native to western North America, greatly reduces density of pine stem, and changes species composition and structure after long periods following disturbances (Dordel *et al.*, 2008). The Emerald ash-borer beetle (*Agrilus planipennis*) is another example of an insect pest which is native to the temperate north-eastern Asia (Valenta *et al.*, 2017). In the late 1990s, these beetles were accidently introduced into the United States (Siegert *et al.*, 2014), and have, so far, caused the death of millions of ash trees (Prasad *et al.*, 2010).

Boreal forests were, and still are, of great economic importance for Northern Hemisphere countries, and have played a strategic role in the historical development of those countries. These forests cover approximately 30% of the Earth’s forested land, and also account for 43% of the total amount of carbon

sequestered in their close canopy forests worldwide (Brown, 1996). However, these forests are intensely infested by several insect pests. For instance, the spruce budworm (*Choristoneura fumiferana)* is a defoliator that causes considerably more damage than any other insect in North America's boreal forests (Volney & Fleming, 2000). Jack pine budworm (*Choristoneura pinus*) is another defoliator that feeds on Jack pine trees, whereas the forest tent caterpillar (*Malacosoma disstria*) is a native defoliator of a range of tree species with a wide distribution in North America. However, the latter is a chronic pest in the prairie/boreal forest ecotone of central Canada (Ives & Wong, 1988).

Generally, MacLean (1985) summarised a number of studies on damage caused by spruce budworm, and pointed out in his conclusion that the loss of stand volume over an outbreak cycle ranged from 18% to 81% depending on the intensity of outbreak and host species infested. On the other hand, losses caused by a single jack pine budworm outbreak were lower (10%) in younger stands, compared to the loss in older stands which is 18% (Volney, 1998). A damage percentage ranged between 2 and 28% of the basal area stands was caused by the forest caterpillar (Hildahl & Reeks, 1960), and similarly, Mattson and Addy (1975) from simulation models suggested that about quarter of standing volume loss in young aspen tree stands denotes a typical outbreak.

1.1.7 Invasive wood boring long horn beetles in Europe

1.1.7.1 Definition of "Biological Invasion"

Among the most serious challenges currently facing forests are biological invasions. Much of the World's biota, after millions of years of evolution, has formed largely separated co-evolving ecological communities. Now many ecological barriers are being broken down by globalisation activities that result

in the frequent movement and exchange of species among and within continents. Invasion is defined as the process by which a living organism moving from its native habitat, or donor region, ultimately becomes a pest in a 'recipient' habitat (Vermeij, 1996). This process occurs gradually and in three discrete stages: arrival, establishment and finally spread, which ultimately debilitates ecological and economic systems (Dobson & May, 1986). Some invasive species significantly affect ecosystem processes and prevent coevolution with native biota (Wilcove *et al.*, 1998; Simberloff, 2000; Assessment, 2005). A substantial literature shows that many species that spread from their geographical origin to invade new habitats are insects (Pellizzari *et al.*, 2005; Pimentel *et al.*, 2005; Roques *et al.*, 2009; Jucker & Lupi, 2011).

1.1.7.2 Invasion pathways

Forest trees are vulnerable and exposed to invasion risks through the growing international flow of goods and people which, consequently, enable the access of exotic pests already occurring in many parts of the world (Zúbrik *et al.*, 2006). Developed countries are the main importers of wood products that increases the rate of wood continental flow (Laarman & Sedjo, 1992). For instance, the increasing incidence of new invasive tree pest and disease introductions into the United Kingdom and generally elsewhere has been attributed to globalization activities, increased wood products and live plant trade and transportation, human movement and climate change (Liebhold *et al.*, 2012; Potter & Urquhart, 2017).

1.1.7.3 Impacts of pest invasions

Not every invading organism ultimately becomes a pest. For instance, in the early 1970s, the European strain of the *Scleroderris* fungus, *Gremmeniella abietina*, was discovered in the north-eastern U.S.A. (Setliff *et al.*, 1975). The considerable concern was the spread of this greatly alarming, highly damaging fungus across the coniferous region of North America. However, due to climatic limitations, the fungus did not spread well beyond the area of discovery (O'Brien, 1984).

On the other hand, invasive forest pests, similar to those of native pests, influence forest resources ecologically and economically by substantially affecting tree growth, reproduction and mortality. The best example of this is the long-term effect of the chestnut blight fungus which has resulted in the elimination of American chestnut (Kuhlman, 1978). Also, invasive forest pests can affect forest resource use for timber, wildlife, recreation, etc. (Kendall & Arno, 1990). Additionally, impacts on endangered species or endangered ecosystems is another important aspect of invading forest pests (Hain & Arthur, 1985; Witter & Ragenovich, 1986). It is important to mention that biological invasions by insects, plants and fungi generally cause substantial ecosystem disturbances that influence both economic and socioeconomic factors (Zúbrik *et al.*, 2006).

Forest carbon storage over the long term can be adversely influenced by invasive species by modifying availability of nutrients, species composition, and primary productivity (Albani *et al.*, 2010; Peltzer *et al.*, 2010), taking into account that forest fragmentation (Murphy, 2005), more extreme climatic events (Diez *et al.*, 2012; Kasson & Livingston, 2012) and climate change (Bradley *et al.*,

2010) are exacerbating factors for the effects of invasive pests. Moreover, invasive pests have large adverse economic impacts on both ecosystem services and forest products (Holmes *et al.*, 2009). For example, an annual economic damage of approximately 2.1 billion dollars is incurred in the U.S.A. by just three invasive pests, namely hemlock woolly adelgid, *Adelges tsugae*, emerald ash borer, *Agrilus planipennis*, and gypsy moth, *Lymantria dispar dispar* (Aukema *et al.*, 2011). In Europe, the highest estimate of the total monetary impact of invasive species was approximately 10 billion Euros annually (Hulme *et al.*, 2009).

1.1.7.4 Examples of invasive pests across different parts of the world

A number of studies show that introductions of invasive pests have profound consequences on human wellbeing and ecosystem services (Boyd *et al.*, 2013; Freer-Smith & Webber, 2017). Their outbreaks have attracted media attention and intense public interest. Among the best examples are Dutch elm disease in the United Kingdom in the 1970s (Tomlinson & Potter, 2010); the outbreak of the Asian long-horned beetle, *Anoplophora glabripennis*, in New York in 1990s (Haack *et al.*, 1997); and the recent outbreak ash dieback, *Hymenoscyphus fraxineus*, in the United Kingdom (Heuch, 2014).

Brazilian forests have been invaded by a variety of insect pests. For instance, the introduced forest pests, such as the wood wasp, *Sirex noctilio* Fabricius, an important *Pinus* spp. insect pest (Iede & Zanetti, 2007) affected an area of four hundred thousand hectares, and also caused losses of around US$ 9 million annually (Schuhli *et al.*, 2016). Another introduced quarantine forest pest is the polyphagous bostrichid beetle, *Sinoxylon unidentatum* which intensely infests a broad range of forest trees, such as teak plantations, tiger wood, balsam trees

(Lunz *et al.*, 2010), mahogany and rubber trees (Peres Filho *et al.*, 2006). Moreover, Brazilian forests have also been infested by other exotic insect pests, such as the Australian longicorn beetle, an important borer pest of eucalyptus (Silva *et al.*, 1968; Wilcken *et al.*, 2002). Schuhli *et al.* (2016) reviewed in detail the topic of the introduced forest pests in Brazil. Valenta *et al.* (2015) reported that European ash species are threatened by the invasive wood-boring beetle, emerald ash borer *(Agrilus planipennis)*, native to East Asia and spreading currently westwards from European Russia.

1.1.7.5 Invasion of wood-boring long-horned beetles to the European agro-forestry systems

Wood-boring forest pests encompass many species and families belonging to the insect order Coleoptera whose larvae or adults feed on and destroy wood, i.e., xylophagy. Mostly, wood-boring beetles belong to one of the following families: Cerambycidae (long-horned beetles), Curculionidae (bark beetles) or Buprestidae (metallic-coloured wood-boring beetles). The family Cerambycidae contains wood-boring long-horned beetles which is considered as a cosmopolitan family of beetles (Gomes Gonçalves, 2017). The main pathway of transport and introduction of invasive pests is the rapidly expanding international trade (Hulme, 2009), and a number of introduced insects have become serious forest pests (Haack *et al.*, 1997; Haack, 2006; Hérard *et al.*, 2006; Roques *et al.*, 2009).

In this thesis, I will focus on four wood-boring long-horned beetles that have invaded European agroforestry ecosystems, namely Japanese Sawyer Beetle (JSB), Yellow Long-horned Beetle (YLB), Asian Long-horned Beetle (ALB), and Citrus Long-horned Beetle (CLB). Wood-boring beetles spread as their

immature stages live inside wooden materials imported for building or packaging purposes, or in wood for the furniture industry and also in timber imported for pulp (Cocquempot, 2006; Haack, 2006; Liebhold *et al.*, 2006; Liebhold & Tobin, 2008; Gandhi & Herms, 2010; Marini *et al.*, 2011; Lupi *et al.*, 2013; Rassati *et al.*, 2014; Rassati *et al.*, 2015).

1.1.7.5.1 Japanese sawyer beetles

The Japanese sawyer beetle (JSB), *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), is considered as a major coniferous forest pest, particularly pine trees (*Pinus* spp.). It is also an important vector of the Pine wilt disease causal agent, the exotic Pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner et Buhrer), (Nematoda: Aphelenchoididae) in eastern Asia (Kobayashi *et al.*, 1984). Tragic timber losses in pine forests were caused by this nematode in China and Japan during the last century (Mamiya, 1988; Ning *et al.*, 2004). Pine wilt disease is considered as one of the most destructive forest diseases in Japan, Korea, China, Laos and Vietnam (Makihara, 2004), and a serious threat to conifer trees worldwide (Vicente *et al.*, 2012). One of the highly impacted areas in eastern Asia by this pest is the mainland of China (Wan *et al.*, 2009). Mamiya (1976) reported that an average of 15,000 nematodes can be carried by this beetle, and moreover, 75% of Japanese sawyer beetles from the field may contain pinewood nematodes, which shows that the beetle is an efficient pine wilt disease vector. The flying capacity for the long-horned beetles of genus *Monochamus* has enabled pinewood nematodes to rapidly and easily spread after its introduction to Asia (Yi *et al.*, 1989; Futai, 2008), Portugal (Mota *et al.*, 1999; Vieira *et al.*, 2007), and Portuguese island of Madeira (Fonseca *et*

al., 2012), as well as several outbreaks of pine wilt disease in Spain (Abelleira *et al.*, 2011).

In May, 2013, Ostoja-Starzewski (2014) reported that a single live adult of *Monochamus alternatus* was detected in the United Kingdom for the first time in a warehouse facility, but unfortunately, its geographical origin was unknown. Two months later, in July, 2013, a member of the public found a live adult emerged from a dining chair that was recently purchased, taking into account that the United Kingdom alone imported approximately 84,000 dining chairs of the type found to be infested during the 2012-13 financial year. Ostoja-Starzewski (2014) showed that this trade is considered as an efficient pathway with potential of widespread introductions of Japanese sawyer beetle and other wood-boring pests.

1.1.7.5.2 Asian long-horned beetles

The Asian long-horned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae) is one of the wood-boring forest pests, native to East Asia (Lingafelter & Hoebeke, 2002). The literature shows that this highly polyphagous species has invaded North America and Europe and caused major economic losses (Nowak *et al.*, 2001; Faccoli & Gatto, 2015). This pest attacks recently cut logs, as well as both young and stressed forest trees (Peng & Liu, 1992; Gao, 1993; He & Huang, 1993). The host range of this pest is not only confined to *Ulmus* spp.*, Populus* spp.*, Salix* spp. and *Acer* spp. in China (Wang, 2004), but also 34 other tree species, belonging to several genera, were reported to be infested (Lingafelter & Hoebeke, 2002; Haack *et al.*, 2010).

In 2012, an outbreak of this forest pest at Paddock, Kent (United Kingdom) necessitated financially and environmentally costly eradication action (Leather,

2014; Straw *et al.*, 2015). The website of the Department for Environment Food and Rural Affairs (DEFRA, UK) states that if this pest becomes established, it can threaten horticulture and the wider environment and also it can infest a very wide range of broadleaved trees (DEFRA website). This pest can be unintentionally imported in wood packaging material (Haack *et al.*, 2010; Eyre & Haack, 2017). The pathway of introduction to Europe and North America is via wooden pallet movement from China where this pest is very common. Ayberk *et al.* (2014) collected adults of Asian longhorn beetle from Istanbul province, a case that was considered as the first record of this pest in Turkish fauna. This pest is currently distributed in Asia, mainly in China, Korea (Lingafelter & Hoebeke, 2002; Williams *et al.*, 2004) and Japan (Kim *et al.*, 2018). It is also found in U.S.A. (Haack *et al.*, 1996; Poland *et al.*, 1998; Haack, 2003) and Canada (Hopkin *et al.*, 2004). Moreover, it was detected in some European countries, such as Austria, Czech Republic, France, Germany, Italy, Netherlands, the United Kingdom and the European part of Turkey, particularly the European part of Istanbul (Tomiczek *et al.*, 2002; Cocquempot & Hérard, 2003; Benker *et al.*, 2004; Maspero *et al.*, 2007; Hérard *et al.*, 2009; Hugel & Brua, 2009; Faccoli *et al.*, 2011; Forster & Wermelinger, 2012; Ayberk *et al.*, 2014; Faccoli *et al.*, 2015).

1.1.7.5.3 Citrus long-horned beetles

The Citrus long-horned beetle (CLB), *Anoplophora chinensis* (Coleoptera: Cerambycidae), is a native of eastern Asia, and widely distributed in China, Japan and Korea, as it is also found in south eastern Asian countries, such as Vietnam, Taiwan and the Philippines (Lingafelter & Hoebeke, 2002). CLB is highly polyphagous, infesting a wide range of host trees in several families,

including mainly deciduous broadleaf trees and two conifers (Lingafelter & Hoebeke, 2002). This pest is considered as a major citrus pest in Japan (Adachi, 1988, 1989; Adachi & Korenaga, 1989; Adachi, 1990, 1994) and it is rarely found in cut wood.

Haack *et al.* (2010) in his historical review of data from 1980 to 2008 reported 74 interceptions of citrus long-horned beetles worldwide. Similarly, some other CLB interceptions were reported in Europe, including United Kingdom, the Netherlands, Guernsey and Italy (Eyre & Haack, 2017). Fewer interceptions were reported from Northern America (Haack *et al.*, 2010). As of December, 2017, the European countries where CLB were intercepted are Croatia, Denmark, France, Germany, Guernsey, Italy, Lithuania, the Netherlands, Switzerland, UK and the European part of Turkey (Herard & Maspero, 2019).

1.1.7.5.4 Yellow long-horned beetles

The yellow longhorn beetle (YLB), *Psacothea hilaris* (Coleoptera: Cerambycidae) is also a polyphagous species. This pest includes 13 morphologically distinct subspecies (Kusama & Takakuwa, 1984); *P. hilaris hilaris* is the subspecies that is found in Europe. The host range of this pest is mostly restricted to trees of the family Moraceae, mainly the two genera of *Morus* and *Ficus*. It is considered a serious pest of sericulture (silk production) in its native area (Iba, 1993). Almost, no sericulture is practiced in Europe but *Morus* trees are common in southern Europe, usually grown for ornamental purposes. In addition, *Ficus* species, such as *Ficus carica* are also grown around the Mediterranean basin for fig production and ornamental purposes (Sabbatini Peverieri *et al.*, 2012). The pest has been intercepted in different European countries, including the United Kingdom in 1997 at Derbyshire, East

Midlands (EPPO, 2008/201). It was also detected in northern Italy (Lombardy region) in September, 2005, and one month later another YLB was found in Bergamo province of the same region (Jucker *et al.*, 2006). Then, in 2007, the species was detected again in the province of Como (Lombardy region). In 2012, YLB was detected in Bavaria (Lupi *et al.*, 2013).

1.1.8 Management of wood-boring long-horned beetles

In China, cultural or mechanical control of Asian long-horned beetles and citrus long-horned beetles were adopted by planting trap trees (Feng *et al.*, 1999; Ying, 2012). Plant breeders devoted considerable efforts to breeding cultivars resistant to borers, such as *Populus deltoides* "Lux (I-69/55)" which is a resistant conifer cultivar to Asian long-horned beetles (Jun & Zhangyi, 1994; Qin *et al.*, 1996; Jia & Li, 2008). For application of biological control method of these pests, the efficacy of a number of entomopathogens (Hajek & Tobin, 2010) and other biological control agents were evaluated. The later method is considered environmentally safer compared to the conventional methods that rely upon chemicals.

In the case of the Asian long-horned beetle for instance, mortality increased whereas fecundity decreased when *Beauveria brongniartii* (Saccardo) Petch was used; this is a fungus native to North America (Dubois *et al.*, 2004). Pests were also exposed to other pathogens, including *Metarhizium brunneum* (Ugine *et al.*, 2013); and also to entomopathogenic nematodes (Fallon *et al.*, 2004). Parasitoids and predators were also used in the biological control of forest pests. *Dastarcus helophoroides* (Fairmaire), a parasitoid with broad host range was utilised to control cerambycid long-horned beetles, such as *Anoplophora glabripennis* and *Monochamus alternatus* (Miura *et al.*, 2003; Li *et al.*, 2007b;

Wei *et al.*, 2009a). Parasitoid species of the bethylid genus *Sclerodermus*, which the core part of this thesis is about, were also used in China to combat forest insect pests (Cheng *et al.*, 2003; Zhao *et al.*, 2008; Lai *et al.*, 2012; Yang *et al.*, 2012a; Zhang *et al.*, 2012). The predatory beetle, *Rhizophagus grandis* was reared and then released to control some forest insect pests, such as red turpentine beetle (Zhao *et al.*, 2008; Zhao *et al.*, 2009; Wei *et al.*, 2010). Chemical control, the conventional method to control pests, was also applied to the native and introduced pests. In China, for example, Cypermethrin, a pyrethroid insecticide, was used to control Asian long-horned beetles (Liu *et al.*, 1999) as well as neonicotinoid insecticides, such as Clothianidin and Thiomethoxam (Wang *et al.*, 2005).

To illustrate the appropriate management strategy for invasive forest pests, the current phase of the invasion should be taken into account. Each phase (arrival, establishment, spread and persistence) has its own suitable strategy. By the first phase, i.e., the pest arrival phase, prevention is the best strategy to apply by excluding any potentially hazardous invading pest from the region. Inspection and quarantine are two useful methods of excluding unwanted species. During the establishment phase, when the invading pest is restricted to a small geographical area, eradication is the best strategy by applying population suppression tactics that ultimately result in the extinction of the invasive pest. The high cost of this process (i.e., eradication process) is compensated by the monetary gain of avoiding the persistent loss caused by the eradicated pest (Knipling, 1978). If populations of the invasive pests are detected very early by extensive monitoring, then it is appropriate to set up a less expensive eradication programme. The principle of eradication is always

under considerable controversy. Some scientists argued that this process is the effective way to manage invasive pests during specific phases (Eden, 1978; Knipling, 1978), whereas others have shown their considerable concern about adverse ecological effects caused by eradication programmes (DeBach, 1964; Dahlsten, 1986; Perkins, 1989).

If the prevention of pest arrival and eradication prior to establishment are not achieved, then the invasive pest will proceed to develop to the spread phase. The first action to be taken in this phase is to exclude invasive pests via effective inspection and quarantine to minimize the spread (Weber, 1930; Rohwer, 1979). The early detection and eradication along the expanding front of the infested area can contribute to slow down the rate of expansion (Liebhold *et al.*, 1992). If the invasive pest exceeds the point, where an eradication programme is not useful, appropriate management strategies should be set up. Among these strategies, should be a biological control programme developed after exploring the range of the invasive pest species to discover its natural enemies (DeBach, 1964; DeBach *et al.*, 1971; Laing & Hamai, 1976; Crawley, 1988).

Biological control of forest native or invasive pests was successfully applied in China through utilisation of biocontrol agents including parasitoids, such as the genus *Sclerodermus* (Hymenoptera: Bethylidae) to control cerambycid or buprestid wood-boring beetles (Lai *et al.*, 2012; Zhang *et al.*, 2012; Yang *et al.*, 2013), although in Europe, long-horned beetle pest control measures have not yet included utilizing parasitoid wasps. Recently in China, while a considerable literature has grown up around the genus *Sclerodermus*, work by Lupi *et al.* (2017) is the only European study to evaluate the reproductive performance of a *Sclerodermus* native to Europe in the context of biocontrol. This study has

shown that this European *Sclerodermus* spp. has the potential to be mass-reared efficiently and effectively released to biologically control the wood-boring long-horned beetles invading the continent. Realizing this potential is of current importance and working towards that, ultimately via successful biocontrol applications, by gaining a deeper understanding of the fundamental biology of these parasitoids, is one of the key aims of this thesis.

1.2 Biological control and biocontrol agents

This section discusses the biological control of invasive wood-boring long-horned beetles, particularly by utilising parasitoids. Firstly, the need for biological control and risks associated with it will briefly be discussed and then I will present some of the existing literature that shows the significant role of parasitoids as biological control agents of invasive and native insect pests of forests worldwide.

1.2.1 Need for biological control

Recently, the human global population has increased (Cohen, 1995) from a few million around 10,000 years ago, to a current estimated population of seven billion (Roberts, 2011) which, consequently, increased the demand for enhanced food production (Zeigler & Mohanty, 2010; Ronald, 2011). In order to achieve that, the “Green Revolution” was started in the late 1960s, and agricultural production and productivity were improved and increased through adopting modern strategies, such as development of high-yielding varieties of cereal crops (Gaud, 1968), although it seems that probably there is currently need for a second phase to that revolution (Zeigler & Mohanty, 2010; Lucas, 2011). It is necessary to improve both of production and protection of

agricultural products for global food security (Benton *et al.*, 2011; Gregory & George, 2011; Ronald, 2011). During pre- and post-harvest periods, high proportions of produce are lost due to agricultural pests, including insects and pathogens, which in turn necessitate the adoption of methods of pest control. Whilst chemical control might achieve better results, associated toxicity has an adverse impact on human health as well as on the environment in general (Pimentel *et al.*, 1980). This control method can also kill non-target species, including natural enemies, the absence of which could eventually lead to a resurgence of pest problems. Another reason for finding an alternative method of chemical control is the toxic residual effect in food that raises a considerable concern for food safety. Finally, reliance upon pesticides for pest control can lead to development of pest resistance to pesticides (Hajek & Eilenberg, 2018). Due to all abovementioned adverse effects associated with chemical control, research efforts have been directed to look for alternative control methods such as biological control. Biological control is the deliberate use of natural enemies, such as pathogens, parasitoids and predators to reduce the undesirable impacts of pests and diseases through regulating the densities of their population and maintaining them below the economic damage threshold level within the released environment (DeBach & Rosen, 1991; Eilenberg *et al.*, 2001). Regarding the pathogens, the bacteria *Bacillus thuringiensis* is the most sold bio-pesticide and most successful pathogen (Kharbade *et al.*, 1998; Hansen & Salamitou, 2000; Lacey *et al.*, 2001). Pathogens also include other micro-organisms, such as fungi (Ihara *et al.*, 2003; Akbar *et al.*, 2004; Gindin *et al.*, 2006), nematodes and viruses (Lacey *et al.*, 2001; Hajek & Eilenberg, 2018). On the other hand, parasitoids belonging to order Hymenoptera seem to be the

most effective natural enemies, and most of the successful biological control programmes were conducted by utilising parasitoids of this order (Greathead, 1986; Murphy & Moore, 1990; DeBach & Rosen, 1991; Wajnberg & Hassan, 1994; Mason & Huber, 2001; Jervis, 2005). For instance, *Bracon hebetor* and *Cotesia glomerata* are hymenopteran parasitoids utilised for biological control of pests. With respect to predators, coccinelid beetles (Order Coleoptera) are considered the most successful predators used as biocontrol agents of arthropod pests in a range of agroecosystems (Caltagirone & Doutt, 1989; Grill *et al.*, 1997). *Coccinella septempunctata* and *Rodolia cardinalis* are examples of frequently used predators.

The biological control of pests attracted wide attention after the successful control of cottony cushion scale (*Icerya purchase*) infestation by utilising parasitoids of vedalia beetle which was introduced to California from Australia. This biological control programme saved the Californian citrus industry in the late 19th century (Caltagirone & Doutt, 1989). Generally, the successful biological control method can be considered a relatively cheaper method with potentially minimal adverse environmental effects and also with few or no considerable concerns to cause health risks to humans.

1.2.2 Risks associated with biological control programmes

With regard to the risks associated with biological control, caution must be applied, especially in classical biological control, when foreign natural enemies are deliberately imported from another region, because this might have adverse or negative impacts on the non-target species (Stiling & Simberloff, 2000; Follet & Duan, 2012) which ultimately have complex consequences on the food web linkages and ecosystem in general (Simberloff, 1992; McEvoy, 1996; Thomas

& Willis, 1998; Memmott, 2000; Henneman & Memmott, 2001; Louda *et al.*, 2003; McCoy & Frank, 2010). Basic certain conditions should be available in the imported organism that will be utilised as a biocontrol agent, such as relatively short life cycle as compared to the pest as well as high efficiency in reproduction and easy to mass-rear (Huffaker & Messenger, 1976; DeBach & Rosen, 1991). On the other hand, another significant issue that should be raised if some natural enemies pass the evaluation tests successfully is whether to release single or simultaneously multiple species to handle pests (Smith, 1929; Watt, 1965; Myers *et al.*, 1989; Denoth *et al.*, 2002; Batchelor *et al.*, 2005). Several studies were conducted to compare single and multiple species releases (Denoth *et al.*, 2002; Matsumoto *et al.*, 2003; Snyder *et al.*, 2008), but still seems to be a controversial topic with uncertainty of whether expanding the biodiversity of natural enemies will result in the strength or weakness in the biological control method. Some researchers supported single release (Myers *et al.*, 1989; Wen & Brower, 1995; Ferguson & Stiling, 1996; Rosenheim *et al.*, 1999; Snyder & Ives, 2001; Denoth *et al.*, 2002; Pedersen & Mills, 2004; Batchelor *et al.*, 2005; Batchelor *et al.*, 2006) whereas other researchers were in favour of multiple releases (Kindlmann & Růžička, 1992; Bográn *et al.*, 2002; Wilby *et al.*, 2005; Snyder *et al.*, 2006).

Having discussed briefly the need for, and risks associated with biological control, I now discuss its role as an integral part of successful forest insect pest management programmes. Regarding pathogens, for instance, many species of entomopathogenic organisms are employed as biocontrol agents of forest insect pests, such as *Entomophaga maimaiga* fungus to control gypsy moth (Elkinton *et al.*, 1991; Hajek & Roberts, 1991; Weseloh & Andreadis, 1992;

Hajek, 1997; Hajek & Webb, 1999) and *Deladenus siricidicola* nematode to control monterey pine woodwasp, *Sirex noctilio* (Bedding, 1993). Overall, microbial pesticides play an important role in integrated forest insect pest management (Evans, 1997; Van Frankenhuyzen *et al.*, 2000). On the other hand, predators are utilised to biologically combat forest entomological pests. Among those predators, for example, are click beetle predators, such as *Cryptalaus berus* larva, that have great potential as predatory natural enemies in the biological control of Japanese sawyer beetle, *M. alternatus* (Zhang & Yang, 2006; Zhang *et al.*, 2008). In terms of parasitoids, they are widely used as successful biocontrol agents of forest insect pests. This is, for example, evident in the case of *Chouioia cunea* Yang which is a gregarious pupal endo-parasitoids used effectively to control fall webworm, *Hyphantria cunea*, an invasive forest insect pest (Yang, 1989). Similarly, the parasitoid beetle, *Dastarcus helophoroides*, is effectively utilised for the biological control of the larvae of Japanese sawyer beetle, *M. alternatus* (Huang *et al.*, 2003; Lei *et al.*, 2003; Wang *et al.*, 2004; Zhang & Yang, 2006; Li *et al.*, 2007a).

1.2.3 Parasitoids as biocontrol agents against forest insect pests

This section has thus far focused on the importance of biological control as an integral component of pest management programmes in general and forest insect pests in particular as well as associated risks that should be taken into account when applying biological control programmes. I now outline the role of parasitoids as biocontrol agents deployed against forest insect pests. In particular, I present the use of parasitoid species belonging to the bethylid genus *Sclerodermus* as biocontrol agents of invasive and native forest insect pests.

Taxonomically, parasitoids belong to different insect orders, including Hymenoptera, Lepidoptera, Diptera and Coleoptera (Clausen, 1940), but the vast majority are classified under the order of Hymenoptera that contains more than 65,000 described species (Gordh *et al.*, 1999). Parasitoids are considered the most widely utilised biological control agents, with many cases of success reported (Sampaio *et al.*, 2009). They lay their eggs in or on the body of their hosts that are ultimately killed, and as such they are of great ecological importance as natural regulating factors for populations of other organisms. Worldwide, mass-rearing programmes of biocontrol agents including parasitoids have proven to be a successful, economically and environmentally sound alternative pest control method. Van Lenteren (2012) listed at least 230 invertebrate natural enemies reared during the year 2010 for pest management programmes worldwide, and among these, around 52% of reared arthropods were represented by hymenopteran parasitoids. Parasitoids are commercially mass-reared using either a short-term system with high daily parasitoid production, or a long-term procedure with lower per day production (Smith, 1996). The challenge faced by parasitoid mass-rearing processes is the need to match the appropriate timings of each production, release date and suitable susceptible phase of the target pest (Knoll *et al.*, 2017). Another rearing-related challenge that can pose problems is the transportation of parasitoids from rearing facilities to the sites of application (Sithanantham *et al.*, 2013) as well as shipping conditions and vibration during transportation (Smith, 1996). On the other hand, parasitoids are often more susceptible to chemical pesticides than predators, and adult parasitoids were observed to be more susceptible to pesticides than the hosts they attack. However, the immature parasitoids,

particularly if they are protected within the body of their hosts, may show more tolerance to pesticide exposure than the adults, but they die with the death of their host (Hoffmann & Frodsham, 1993).

In addition to quantifying the biological and/or technical parameters, it is also important to consider the cost/benefit analyses of biological control programmes, as an indicator of their economic impact success (Valente *et al.*, 2018). This has been seen, for example, in the case of the biological control programme of *Gonipterus platensis* (Coleoptera: Curculionidae), an invasive pest of *Eucalyptus* which was accidently introduced from Australia to other parts of the world (Mapondera *et al.*, 2012; Hurley *et al.*, 2016). In Portugal, the wood losses due to this pest added up to 648 million Euros during the last two decades. The biological control of this pest by using the egg-parasitoid, *Anaphes nitens* (Hymenoptera: Mymaridae) resulted in positive benefit-cost ratio by showing an increase of losses 3.7 to 11 times in the absence of this parasitoid (Valente *et al.*, 2018).

Existing literature recognises the significant role of parasitoids as biological control agents of invasive and native insect pests of forests worldwide. A worldwide example of the importance of parasitoid utilisation as biocontrol agents is the case of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* which invaded chestnuts (*Castanea* spp.) across different continents, such as Asia, Europe and North America (Brussino *et al.*, 2002; Rieske, 2007; Graziosi & Santi, 2008). Many parasitoid species were explored worldwide from the native and invaded areas of the pest to regulate its population. Among those countries are Japan (Yasumatsu & Kamijo, 1979; Kamijo, 1981), the United States of America (Rieske, 2014), Europe (Aebi *et al.*, 2006; Schönrogge *et al.*,

2006; Aebi *et al.*, 2007; Quacchia *et al.*, 2008; Askew *et al.*, 2013) and China (Bailey *et al.*, 2009; Zhi-yong *et al.*, 2009).

In China, a number of parasitoids were discovered or introduced from foreign countries to control forest insect pests. In an investigation of biological control agents of fall webworm, *Hyphantria cunea*, which is an invasive forest pest, twenty-six parasitoid species, mostly hymenopterans, were associated with this pest (Yang, 1989; Yang & Wei, 2003; Yang & Baur, 2004; Yang *et al.*, 2014). For instance, the gregarious pupal endo-parasitoid, *Chouioia cunea* was the most recommended and effectively utilised to control this dangerous forest pest (Yang, 1989). Other examples in China include the parasitoid beetle *Dastarcus helophoroides* to control larvae and pupae of Japanese sawyer beetle, *M. alternatus* (Huang *et al.*, 2003; Lei *et al.*, 2003; Wang *et al.*, 2004; Zhang & Yang, 2006; Li *et al.*, 2007a) and *Dastarcus helophoroides* for the control of oak longhorn beetle larvae and pupae (Wei *et al.*, 2008a; Wei *et al.*, 2008b; Wei *et al.*, 2009a; Wei *et al.*, 2009b).

In the United States, a classical biological control programme was begun to control emerald ash borer, *Agrilus planipennis*, which is a destructive invasive pest of ash trees introduced from the Asian continent during the 1990s (Haack *et al.*, 2002; Cappaert *et al.*, 2005; Siegert *et al.*, 2014). In 2007, three parasitoids of this pest native to China were released, namely *Tetrastichus planipennisi*, an endoparasitoid of late-instar larvae (Liu *et al.*, 2003; Yang *et al.*, 2006); *Spathius agrili*, an ectoparasitoid of late-instar larvae (Liu *et al.*, 2003; Yang *et al.*, 2005); and *Oobius agrili*, an egg parasitoid (Zhang *et al.*, 2005). Later in 2015, a fourth parasitoid *Spathius galinae*, an ectoparasitoid of late-

instar larvae (Belokobylskij *et al.*, 2012; Duan *et al.*, 2012) introduced from Russia was also approved for release.

In Canada, MacQuarrie *et al.* (2016) reported that during the period between the 1890s and 2000s, parasitoids dominated the overall number of natural enemies released in biological control programmes in Canadian forests. They also reported that among parasitoids, particularly, species belonging to the order Hymenoptera dominated.

1.2.3.1 Parasitoid species of genus Sclerodermus (Hymenoptera: Bethylidae) as biocontrol agents against forest insect pests

Bethylid parasitoid wasps are widely used as biocontrol agents for some wood-boring long-horned beetles (Wang, 2005; Hong *et al.*, 2008; Shimazu, 2008; Li *et al.*, 2010; Wang *et al.*, 2010; Tang *et al.*, 2012; Wei *et al.*, 2013; Wei *et al.*, 2014; Yang *et al.*, 2014). Among those are parasitoid wasp species of the genus *Sclerodermus* which are effectively and successfully utilised for biological control programmes of forest insect pests in China (Yang *et al.*, 2014; Wang *et al.*, 2019).

Worldwide, the bethylid genus *Sclerodermus* comprises at least 81 described species (Gordh, 1990; Lanes & Azevedo, 2008) of which 17 species were found in Mediterranean areas (Andrè *et al.*, 1904; Kieffer, 1914) associated with long-horned beetles classified as invasive exotic pests (Hérard *et al.*, 2007; Lupi *et al.*, 2014). Members of this genus permanently paralyse their hosts, i.e., idiobionts and then lay their eggs onto them, i.e., ectoparasitoids (Li & Sun, 2011; Hu *et al.*, 2012). Since their morphological adaptations enable them to effectively search for their hosts which are the larvae of wood-boring beetles, females of this genus are selected to successfully attack their hosts that are

usually found in enclosed spaces (Kühne & Becker, 1974; Evans, 1978; Yang *et al.*, 2012a; Baena & Zuzarte, 2013; Jiang *et al.*, 2015).

With respect to host species and sizes, *Sclerodermus* females attack different host species that range from small to large sizes (Mendel, 1986; Hsu & Wu, 1989; Yang *et al.*, 2014), with individuals tending to effectively handle relatively small hosts (Yang *et al.*, 2014). A group of females of genus *Sclerodermus* can cooperate, and consequently, supress larger hosts (Tang *et al.*, 2014), and after oviposition, each individual does not exclusively brood-guard their own offspring (Hu *et al.*, 2012; Wu *et al.*, 2014), but multiple females may tend a group of offspring of a single paralysed host until the emergence of adult offspring (Bridwell, 1920; Kühne & Becker, 1974; Casale, 1991; Hu *et al.*, 2012; Yang *et al.*, 2012a; Tang *et al.*, 2014). *Sclerodermus* individuals show cooperative reproductive behaviour without division of labour, and as such may be socially classified as quasi-social hymenopteran parasitoids, a topic that will be discussed in the next section (Tang *et al.*, 2014). Several wasp-host studies reported that females of *Sclerodermus* species efficiently suppress their hosts, wood-borers (Gao *et al.*, 2016a; Wang *et al.*, 2016; Wei *et al.*, 2016; Wei *et al.*, 2017). The sex ratio for parasitoid wasps belonging to this genus is strongly female-biased (Bridwell, 1920; Kühne & Becker, 1974; Mamaev, 1979; Zhang & Tian, 1985; Liu *et al.*, 2011; Hu *et al.*, 2012; Yang *et al.*, 2012a; Tang *et al.*, 2014; Wei *et al.*, 2014; Kapranas *et al.*, 2016; Lupi *et al.*, 2017). Wing dimorphism (alate or apterous) is common in both sexes of adult *Sclerodermus* wasps (Bridwell, 1920; He *et al.*, 2004; Yang *et al.*, 2012a), although apterous males in this genus are uncommon (Zhang & Tian, 1985; Wang *et al.*, 2015; Lupi *et al.*, 2017; Wei *et al.*, 2017).

In China, the use of bethylid parasitoid wasps of the genus *Sclerodermus* as biocontrol agents has formed an integral component of successful management programmes of forest insect pests (Chen & Cheng, 2000; Du *et al.*, 2006; Tang *et al.*, 2012; Yang *et al.*, 2014; Jiang *et al.*, 2015; Wang *et al.*, 2019). For instance, parasitoid species of the genus *Sclerodermus* including *S. guani* and *S. sichuanensis* showed promise for successfully controlling the wood-borer larvae of Japanese sawyer beetle, *M. alternatus* larvae (Lai *et al.*, 2012; Zhang *et al.*, 2012; Zhang, 2012; Yuanliang *et al.*, 2013; Zhan *et al.*, 2014). *S. sichuanensis* is also another example from this genus that is efficiently used as a biocontrol agent against the wood-boring long-horned beetle, *Saperda balsamifera* larvae in China (Da, 2015). Females of *S. pupariae*, are also used as biocontrol agents of the emerald ash borer, *Agrillus planipennis* which is a destructive forest pest (Yang *et al.*, 2012a; Gao *et al.*, 2016a). The young larvae of oak long-horned beetles, *Massicus raddei* are also controlled in China by using females of *S. pupariae* (Zhao *et al.*, 2008; Yang *et al.*, 2012a).

In Europe, approximately one century ago, *Sclerodermus brevicornis* (the *Sclerodermus* species studied in this thesis) which is native to Europe, was reported to be associated with *Oxypleurus nodieri*, a wood-boring long-horned beetle (Kieffer, 1914). Recently in 2011, Lupi *et al.* (2014) recorded this species in association with yellow long-horned beetle, *Psacothea hilaris hilaris* in northern Italy. The potential of this species to suppress the populations of exotic and invasive wood-boring long-horned beetles was further studied by Lupi *et al.* (2017). The coleopteran invasive pest species that were used as factitious hosts for *S. brevicornis* by Lupi *et al.* (2017) included the citrus long-horned beetle, *Anoplophora chinensis* and the Asian long-horned beetle, *Anoplophora*

glabripennis, both introduced from the far-east of Asia and are classified as quarantine pests in Italy since they were detected in 2000 and in 2007, respectively (Colombo & Limonta, 2001; Maspero *et al.*, 2007; Favaro *et al.*, 2015). The third invasive pest included in that study was the notifiable pest that is not included among quarantine pests in Italy, the yellow long-horned beetle, *Psacothea hilaris hilaris* which was detected in southern Europe in 2005 and considered a potential threat for Mediterranean fig and mulberry trees (Jucker *et al.*, 2006; Lupi *et al.*, 2013; Lupi *et al.*, 2015b). *S. brevicornis* successfully attacked all three factitious host species but the offspring failed to develop to maturity on the small host sizes. Adult offspring produced from hosts presented to *S. brevicornis* foundresses ranged between 2 to 373, with a mean of 86 adult offspring, whereas the sex ratio was strongly female-biased (approximately only 9% of adult offspring produced were males). It has been shown that the Asian species of genus *Sclerodermus* are mass-reared and released to become an integral part of effective management of forest pests in China (Chen & Cheng, 2000; Du *et al.*, 2006; Tang *et al.*, 2012; Yang *et al.*, 2014; Jiang *et al.*, 2015), therefore, Lupi *et al.* (2017) suggested that similar management measures might be employed within Europe through focusing on the European species, *S. brevicornis*, which has potential to be efficiently mass-reared and successfully utilised as biocontrol agents of invasive long-horned beetles.

As discussed above, parasitoid wasps in the genus *Sclerodermus* are used effectively as biocontrol agents against destructive invasive wood-boring beetles in China. In addition, it was also mentioned that parasitoid wasps of *S. brevicornis*, a promising native European species, have potential to be utilised as biocontrol agents against invasive wood-boring long-horned beetles within

Europe. However, there has been little attempt to enhance efficiency via an understanding of the evolutionary forces that shape the reproductive behaviour of those parasitoids, although these are increasingly identified. For instance, Tang *et al.* (2014) attributed the unusual quasi-sociality and the strongly female sex ratios exhibited by *S. harmandi* to the direct fitness benefits of cooperative host suppression and subsequent brood care.

1.3 Sociality in parasitoids, especially bethylids

Having briefly introduced the concept that *Sclerodermus* are social (or more specifically, quasi-social) parasitoids in the previous section, this section will summarize sociality in insects, sociality in parasitoids in general and in bethylids in particular. Sub-sociality and quasi-sociality exhibited by species of the genera *Goniozus* and *Sclerodermus*, respectively will be discussed. I also introduce the importance of kin selection in social interactions, in particular the establishment and maintenance of cooperative reproductive behaviours exhibited by insects in general and parasitoids in particular.

1.3.1 Introduction

The remarkable success of some insects, notably the termites and ants is attributed to their social life-style (Wilson, 1971), and they are considered an important component of biological diversity of earth. For instance, Wilson noted that approximately about 75% of the insect biomass in the world is made up of the eusocial insects, including termites, ants and some bees and wasps. Social insects succeeded ecologically in both temperate and tropical ecosystems. In terms of energy consumption, social insects collectively consume more biomass and energy than vertebrates. On the other hand, termites and ants play

an important role in nutrient cycling in tropical and temperate ecosystems. In addition, Hölldobler and Wilson (1990) reported that termites and ants turnover an amount of soil which exceeds the cycling by earthworms in tropical and temperate habitats. Moreover, ants living in the Brazilian amazon region release a substantial amount of atmospheric formic acid that consequently acidifies natural rainwater that, in turn, contributes to the weathering process of limestone-based rock formations.

With regard to reasons behind the evolution of social interactions, Davies *et al.* (2012) listed three ecological drivers that could lead to a high enough benefit/cost ratio to select for cooperation and eusociality worthwhile. The first driver is the life insurance benefits if foundresses die before the end of brood care (Gadagkar, 1991; Field *et al*., 2000), whereas the second deriver is represented by the benefits of fortress defence by having valuable or defendable source, such as food (Foster, 1990; Tóth & Duffy, 2005; Duffy & Macdonald, 2010). Finally, several researchers considered food distribution as a major ecological factor driving the evolution of cooperation (Jarvis *et al.*, 1994; Faulkes *et al.*, 1997).

1.3.2 Classification of level of sociality

The broad definition of insect social behaviour includes all insects that interact with other individuals of their species. However, regarding degrees of insect social behaviour, the increasing complexity of insect sociality hierarchy includes asociality, subsociality, quasisociality, semisociality and eusociality (Wilson, 1971, 1975; Gullan & Cranston, 1994; Choe & Crespi, 1997; Costa, 2006, 2018) (Table 1.1). This social classification is subject to change as theory and

understanding of the subject progresses (Costa & Fitzgerald, 2005; Costa, 2018).

Starting from the top of the hierarchy, insects with overlapping generations, reproductive labour division with sterile and fertile castes, and cooperation among colony members are commonly classed as eusocial, including eusocial hymenoptera (ants, social bees and some wasps) and the termites as classical examples (Wilson, 1971, 1975; Queller & Strassmann, 2003; Zablotny, 2009), as well as several other orders among which eusociality has been observed (Aoki, 1977; Crespi, 1992; Kent & Simpson, 1992). If members of the same generation co-occur in a colony and show cooperative brood care with reproductive division of labour, then they are considered as semisocial, and the primitive eusocial Hymenoptera, for example, are listed under this category (Wilson, 1971, 1975; Michener, 1990). In addition, quasisocial insects do not have reproductive castes and each individual contributes to reproduction with cooperative care of broods. This social behaviour has been observed in some hymenopteran species, notably *Sclerodermus* (Bridwell, 1920; Roberts & Dodson, 1967; Mamaev, 1979; Casale, 1991; Tang *et al.*, 2014). Moreover, insects are socially termed “communal” if members of the same generation co-occur in a colony, but without providing any care for their broods. Examples of this social behaviour can be found in larval stages of some lepidopteran insects, such as tent-making caterpillars, some of sulphur butterflies, silk moths and nymphalid butterflies (Zablotny, 2009). With respect to subsociality, each female provides maternal care only for its offspring, and hence no cooperative brood care is exhibited. Among subsocial insects are many species belonging to a variety of orders, such as Coleoptera (Brandmayr, 1992), Dermaptera

(Rankin *et al.*, 1996), Hemiptera (Tallamy & Schaeffer, 1991), Lepidoptera (Costa & Pierce, 1991), Hymenoptera (Linksvayer, 2010; Rehan & Richards, 2010) and others. Finally, asocial or solitary insects, by contrast, do not exhibit any of the abovementioned characteristics (Wilson, 1971; Gullan & Cranston, 1994). On the other hand, all levels of sociality other than eusociality are collectively termed as “presocial”, whereas the term “parasocial” is alternatively used in place of social levels of communal, quasisocial and semisocial (Wilson, 1971, 1975).

Table 1.1 Social organization levels in insects, after Michener and Wilson

	Parental care	*Alloparental care*	*Reproductive division of labour*	*Overlapping adult generations*
Presocial				
Subsocial	+	-	-	-
Parasocial				
Communal	+	-	-	-
Quasisocial	+	+	-	-
Semisocial	+	+	+	-
Eusocial	+	+	+	+

Eusocial species dominate in terms of population sizes, although presocial species are much more common (Nowak *et al.*, 2010). Despite the abovementioned diversity of sociality hierarchies exhibited by insects, social evolution research has historically and intensively focused on ‘eusociality’ which is a small subset of social forms (Costa, 2018). In recent decades, several researchers attempted to address this imbalance through their synthesis and reviews of social diversity among the other non-eusocial insects (Choe & Crespi, 1997; Costa, 2006; Royle *et al.*, 2012; Costa, 2016; Rubenstein & Abbot, 2017).

1.3.3 Sociality in parasitoids

Many hymenopterans exhibit highly advanced social behaviour (Hamilton, 1964a; Bourke & Franks, 1995; Hughes *et al.*, 2008; Boomsma, 2013) but parasitoids, mostly belonging to the infraorder of *Parasitica*, are typically socially solitary and only a few species show social traits. One example is the polyembryonic wasp *Copidosoma floridanum* which has clonal development and 'reproductive' and 'soldier' castes in the immature stages (Giron *et al.*, 2004; Dunn *et al.*, 2014).

Bethylid wasps, parasitoids of mostly lepidopteran and coleopteran larvae, belong to the same major hymenopteran taxonomic group (*Aculeata*) as the eusocial Hymenoptera (Peters *et al.*, 2017) but are not eusocial. Some are socially solitary, such as females of bethylid wasp species *Laelius pedatus*, which does not brood guard after oviposition (Mayhew, 1998), whereas others including *Goniozus* wasps exhibit subsocial behaviour in the form of brood guarding (maternal care) (Hardy & Blackburn, 1991; Mayhew & Hardy, 1998; Hardy *et al.*, 1999; Goubault *et al.*, 2007; Bentley *et al.*, 2009; Venkatesan *et al.*, 2009a; Hardy *et al.*, 2013). They provide maternal care for their broods for a period of time immediately after oviposition (Doutt, 1973; Hardy & Blackburn, 1991; Goubault *et al.*, 2007; Bentley *et al.*, 2009; Hardy *et al.*, 2013), and females protect offspring from any intruders and other conspecifics until they develop into later stages (Hardy & Blackburn, 1991; Mayhew & Hardy, 1998; Venkatesan *et al.*, 2009a). Species in the bethylid genus *Sclerodermus* have evolved quasisociality in which multiple foundresses handle hosts in groups and exhibit a cooperative reproductive behaviour (Bridwell, 1920; Tang *et al.*, 2014; Lupi *et al.*, 2017).

Both the subsociality and quasisociality exhibited by parasitoids can largely be explained in terms of direct fitness benefits to individual females: either selfish defense of own offspring (Hardy *et al.*, 2013) or mutually beneficial host exploitation (Tang *et al.*, 2014), respectively. However, these behaviours may be modified by inclusive fitness considerations (Lizé *et al.* (2012) and see below).

1.3.3.1 Quasisociality in the genus Sclerodermus

Sclerodermus females search actively for host larvae, which they paralyse by stinging (Xu *et al.*, 2002; Shao-chuan, 2007; Yao & Yang, 2008; Liu *et al.*, 2011). A host may be suppressed by several females (Bridwell, 1920; Kühne & Becker, 1974; Tang *et al.*, 2014; Lupi *et al.*, 2017). The females (termed foundresses) then oviposit onto the host's integument and subsequently tend, cooperatively, their collective broods of offspring as these feed on the host, until and beyond their pupation (Hu *et al.*, 2012; Wu *et al.*, 2014). Quasisocial behaviour in *Sclerodermus* has been explained in terms of the direct fitness benefits to cooperation accrued to individuals within groups of females that attack hosts: females acting alone may not be able to suppress and successfully reproduce on a host, especially if the host is large (Tang *et al.*, 2014), a 'collective action benefit' (Shen *et al.*, 2017). This abovementioned explanation for quasi-sociality does not, however, preclude that kin selection effects, based on the genetic relatedness between interacting individuals (inclusive fitness Hamilton (1964a)), may also influence the occurrence and success of cooperative reproduction and thus the evolution of quasi-sociality. Hence, one of the main aims of this thesis is explore the role of kinship effects in this unusual cooperative reproductive behaviour.

1.3.3.2 Subsociality in the genus Goniozus

Wasps belonging to the genus *Goniozus* are classified as subsocial as they exhibit brood guarding behaviour which is common among bethylid wasps (Hardy *et al.*, 1999). They provide maternal care for their broods for a period of time immediately after oviposition (Hardy & Blackburn, 1991; Goubault *et al.*, 2007; Bentley *et al.*, 2009; Hardy *et al.*, 2013), and females protect offspring from any intruders and other conspecifics until they develop into later stages (Hardy & Blackburn, 1991; Mayhew & Hardy, 1998; Venkatesan *et al.*, 2009a).

1.3.4 Social interactions

Hamilton (1964a) classified social behaviour interactions among individuals into four categories based on the loss or the gain of offspring through an individual's performance of the behaviour: mutualism, altruism, selfishness and spite (Table 1.2). Mutualism occurs when both actor and recipient gain fitness, whereas altruism is a behaviour in which the altruist (the actor) loses fitness and the beneficiary (the recipient) gains. Selfishness is defined as the social action in which the actor gains and the recipient loses. Spite is a social interaction behaviour which causes harm to both the actor and recipient.

Table 1.2 Social behaviour classification according to the effects of the fitness of the actor and the recipient

Effect on actor	Effect on recipient	
	+	-
+	Mutual benefit	Selfishness
-	Altruism	Spite

After Hamilton (1964a), Trivers (1985) and West *et al.* (2007a).

1.3.5 Role of mutual benefit and altruism in sociality

Having seen the above-shown social interaction classifications, it could be reasonably accepted that mutual benefit behaviours pose no evolutionary difficulty since the interacting individuals benefit more than they would on their own. On the other hand, since altruism incurs a cost, the altruistic behaviour is less likely to evolve in general. In the case of closely related individuals, the likely major driver and explanation for acting altruistically is the kin selection in which altruists gain inclusive fitness benefits by helping relatives. The colony or the group may gain many advantages from altruism, although it can be detrimental to the altruistic individual. For instance, the group defensive strategies are utilised by most social insects when the colony is under attack during which altruists (defenders) are usually vulnerable to the risk, but for the sake of their inclusive fitness.

However, the altruistic social interaction between unrelated individuals occurs rarely and is considered a wide research topic in which “Game Theory” is often applied. There are two evolutionary forces behind the evolution of altruism between unrelated individuals, and the first one is reciprocal altruism which is favoured when individuals interact repeatedly as long as the reciprocal benefit received by the altruist outweighs the initial cost. The indirect reciprocation is the second solution for how altruistic behaviour can evolve between unrelated individuals. This type of reciprocation often occurs when the altruist tends to be repaid by individuals other than the recipients they help.

Sociobiological studies focus on understanding how cooperative or altruistic behaviours among individuals increase their genetic legacy, either by direct or inclusive fitness. The major focus has been on social insects (Bourke & Franks,

1995; Robinson, 2002; Strassmann & Queller, 2007), with a considerable attention also being given to the cooperative mammals and birds (Cockburn, 1998; Clutton-Brock *et al.*, 2001; Cornwallis *et al.*, 2010). By applying the theory of kin selection, remarkable progress towards understanding insights of cooperation exhibited by these organisms was achieved during the past few decades (Queller, 1992; Frank, 1998; West *et al.*, 2007a). This is what makes mutualistic and altruistic social interaction behaviours play a significant role in the evolution of the two abovementioned behaviours (Lehmann & Keller, 2006), unlike selfishness and spite, which are not typically considered as promoters of cooperative behaviour.

1.3.6 Kin selection

Understanding the major evolutionary forces and mechanisms involved behind the evolution and maintenance of cooperation is considered a significant central problem in the field of biology. Specifically, it was unclear the reasons behind the altruistic behaviour exhibited by altruists (Darwin, 1859). Several theoretical constructs have surfaced as potential explanations in light of Darwin's puzzle, including kin selection (Hamilton, 1964a), direct benefits (West-Eberhard, 1975; Brown, 1983; Connor, 1995), reciprocal altruism (Trivers, 1971) and group selection (Wilson, 1971; Wilson & Wilson, 2007). These possible explanations were reviewed by (Queller, 1985; Dugatkin, 2002; Sachs *et al.*, 2004; Noë, 2006; Nowak, 2006; West *et al.*, 2007a; West *et al.*, 2007b; Clutton-Brock, 2009). Particularly, the concept that cooperative reproductive behaviour may spread among individuals via kin selection is currently a central paradigm in evolutionary biology (Silk, 2002; Foster *et al.*, 2006; West *et al.*, 2007b; West *et al.*, 2007a; Abbot *et al.*, 2011; Herbers, 2013).

Several studies on social behaviour evolution have confirmed the significance of the genetic relationship among colony mates, as well as the importance of kin selection and reproductive altruism (Hamilton, 1963; Wilson, 1971; Hamilton, 1972; Lin & Michener, 1972; Alexander, 1974; Michener, 1974; West-Eberhard, 1975; Wilson, 1975; Evans, 1977; West-Eberhard, 1978a, 1978b; Lehmann & Keller, 2006; West *et al.*, 2007a; Abbot *et al.*, 2011; Bourke, 2011; Gardner *et al.*, 2011; West & Gardner, 2013).

Historically, Charles Darwin was the first to discuss kin selection in his famous book, "*On the Origin of Species*" (Darwin, 1859). In 1930s, R. A. Fisher and J. S. B. Haldane provided the first formal (mathematical) treatments (Fisher, 1930; Haldane, 1932). In the first half of 1960s, W. D. Hamilton refined the concept which was mathematically improved later by George Price (Hamilton, 1964a), while the term "kin selection" is attributed to John Maynard Smith who coined it during the same decade. As outlined by Hamilton (1964a), kin selection altruism could be favoured through two mechanisms: firstly, kin recognition which is the ability of an individual to recognise or positively discriminate its relatives on the basis of kinship. Secondly, viscous populations which often occurs among populations with a low dispersal rate and this, consequently, makes kin selection possible in the absence of kin recognition of relatives.

Kin selection is considered an essential natural selection form that is used for explanation of eusocial character evolution. Hamilton (1964a) published the inclusive fitness model ($C/B < r$) illustrating the economic basis for self-sacrifice as a result of altruistic behaviour. This rule denotes that altruistic behaviour evolves when the benefit, B (in number of offspring equivalents) received by the related recipient (r) exceeds the cost, C (in number of offspring equivalents). In

other words, ($rB > C$) denotes that genes are increased in frequency by kin selection when recipient's genetic relatedness to the altruist multiplied by the benefit to the recipient outweighs the reproductive cost to the altruist. This theory predicts that social behaviours between relatives will evolve if these behaviours increase the inclusive fitness of individuals, either indirectly through increasing the reproductive success of relatives, or directly through their own reproductive success. An example for what Hamilton's rule depicts is provided by the haplo-diploid eusocial Hymenoptera. The diploid sisters of social hymenoptera should tend to interact altruistically because a gene in one sister has 75% chance of also being shared in the other sister by common descent. With this higher relatedness (0.75), the benefit for an altruistic behaviour does not have to be high to promote the spread of altruism and evolution of social behaviour throughout such a population (Zablotny, 2009). Altruistic behaviour and passing on the shared genes is more likely engaged among closely related individuals than distantly related or non-related individuals. Relatives also resolve or minimise competition over resources via their capacity to recognise their conspecifics (Hamilton, 1964a; Smith, 1964; Lizé *et al.*, 2012; Herbers, 2013).

Generally, the evolution of intraspecific cooperation and altruism is studied within kin selection framework when the socially interacting individuals are related (Hamilton, 1964a; Grafen, 1984; Taylor, 1992; Frank, 1998; West *et al.*, 2002). There is a considerable number of studies showing that kin discrimination has indeed been observed in a phylogenetically wide range of living organisms, including plants (Dudley & File, 2007), social amoebae (Mehdiabadi *et al.*, 2006), Gram-negative bacteria (Alteri *et al.*, 2013;

Rendueles *et al.*, 2015), mammals (Silk, 2002), birds (Komdeur & Hatchwell, 1999), amphibians (Blaustein & Waldman, 1992) and social insects (Keller, 1997; Queller & Strassmann, 2003).

There are two proposed mechanisms for discriminating kin: discrimination by association (Porter, 1988; Halpin, 1991), or by phenotypic matching (Beecher, 1982; Holmes & Sherman, 1983; Lacy & Sherman, 1983; Porter, 1988; Halpin, 1991). With regard to the first mechanism, if there are social cues, such as persistent affiliative associations, discrimination most probably occurs through learning processes (Sherman *et al.*, 1997; Komdeur & Hatchwell, 1999). The learned discrimination of kin in some societies has been conveyed through association (Todrank *et al.*, 1998; Hatchwell *et al.*, 2001; Russell & Hatchwell, 2001), or through spatial distribution of kin (Komdeur & Hatchwell, 1999). The phenotype matching mechanism (Hauber & Sherman, 2001; Tang-Martinez, 2001) can occur through matching of vocalizations (Price, 1999), odour cues (Sun & Müller-Schwarze, 1997; Heth *et al.*, 1998; Mateo, 2003) and even genetic-based recognition system (Blaustein & O'Hara, 1981).

Kin recognition was reported to play a significant role in the evolution of plant reproductive strategies. Torices *et al.* (2018) showed empirically that individuals of insect-pollinated herbs, *Moricandia moricandioides*, produced larger floral displays when growing with kin in order to attract pollinators. Moreover, seed germination time is a significant trait for plant competitive ability (Seiwa, 2000; Afonso *et al.*, 2014). This time has been observed to vary as a consequence of the neighbouring seed relatedness. Yamawo and Mukai (2017) reported that seeds of *Plantago asiatica* (Plantaginaceae) adjusted emergence timing in response to the presence of *Trifolium repens* seed and relatedness of

neighbouring *P. asiatica* seed (sibling or non-sibling seed). *P. asiatica* seeds emerged faster when *T. repens* seed is encountered in the presence of a sibling seed than in the presence of a non-sibling.

A number of studies have shown that unicellular prokaryotes, such as bacteria have the capacity for kin discrimination (Rendueles *et al.*, 2015; Lyons *et al.*, 2016; Lyons & Kolter, 2017). For instance, Lyons and Kolter (2017) reported a different method of kin discrimination presented by the bacterial species *Bacillus subtilis* through exclusion of non-kin species to protect effectively public goods. Several studies have been conducted to test kin discrimination or recognition ability in unicellular eukaryotes (Mehdiabadi *et al.*, 2006; Ostrowski *et al.*, 2008; Reece *et al.*, 2008; Chaine *et al.*, 2010; Kalla *et al.*, 2011; Espinosa & Paz-Y-Miño-C, 2012; Nkhoma *et al.*, 2012) and possible ability for kin recognition, and genetic-based mechanisms of conspecific detection have been observed in some taxa (Queller *et al.*, 2003; Smukalla *et al.*, 2008; Benabentos *et al.*, 2009; Hirose *et al.*, 2011). Espinosa and Paz-y-Mino-C (2014) have discussed studies conducted on unicellular eukaryotes, protists in which the evolutionary and ecological importance of a potential ability for kin discrimination is highlighted. This study summarised and presented as an example that kinship effects were observed in five major protist lineages, including Mycetozoa (Queller *et al.*, 2003; Mehdiabadi *et al.*, 2006; Ostrowski *et al.*, 2008), Dikarya (Smukalla *et al.*, 2008), Apicomplexa (Nkhoma *et al.*, 2012), Ciliophora (Chaine *et al.*, 2010), and Archamoebae (Espinosa & Paz-Y-Miño-C, 2012).

Kin selection effects were also evident among avian societies (Komdeur & Hatchwell, 1999), such that a considerable number of bird species exhibited a

preferential allocation of help to the closest kin (Glen & Perrins, 1988; Emlen, 1991; Halpin, 1991; Emlen, 1997).

Indeed, kin discrimination which operates via familiarity and phenotypic matching is widely documented for mammals (Tang-Martinez, 2001). For instance, familiarity-based kin discrimination which occurs through learning process (Kareem & Barnard, 1982) was observed in Belding's ground squirrels (Holmes & Sherman, 1983; Mateo, 2003) and house mice, *Mus musculus domesticus* (König, 1994), whereas kin discrimination via phenotypic matching was observed in rodents (Yamazaki *et al.*, 2000; Mateo, 2003), carnivores (Wahaj *et al.*, 2004) and primates (Wedekind & Furi, 1997; Charpentier *et al.*, 2008; MacDonald *et al.*, 2008). Kin in mammals may also be recognised based on visual (Parr & De Waal, 1999) or vocal cues (Holekamp *et al.*, 1999), or using both ways (Rendall *et al.*, 1996; Kazem & Widdig, 2013).

Generally, studies of kinship effects presented by non-social arthropods have received less attention (some specific examples are found in Jasieński *et al.* (1988); Faraji *et al.* (2000); Schausberger and Croft (2001); Magalhães *et al.* (2005); Schausberger (2007)). Nevertheless, Fellowes (1998) acknowledged that relatedness among non-social arthropods could have an effect on some behavioural elements, such as sex allocation under local mate competition (Frank, 1985; Brun *et al.*, 1995), resource exploitation (Barnard, 1991), cannibalism (Osawa, 1992), inbreeding avoidance (Latter & Robertson, 1962; Sharp, 1984), super-parasitism (Godfray, 1994) and aggregation when vulnerable to predation risk (Young *et al.*, 1994). Roeder *et al.* (1996) found that spider mites are able to discriminate between kin and non-kin. They evaluated the effect of degrees of relatedness on sex allocation decisions, and they found

that inbred sisters produce offspring with strongly female-biased sex ratio, and generally, the offspring were highly female-biased when produced by sister females.

With regard to insects, kin recognition in eusocial species such as termites, ants, some bees and wasps operates at the level of colony through the variation of cuticular odour (Lenoir *et al.*, 1999; Van Zweden & d'Ettorre, 2010), although within-colony kinship effect disappears or is at least hard to perceive (Arnold *et al.*, 1996; Tarpy *et al.*, 2004; Nehring *et al.*, 2011; Boomsma & d'Ettorre, 2013; Helanterä *et al.*, 2013; Leadbeater *et al.*, 2014). Additionally, kinship effects were observed to mediate predation risk faced by groups of mixed-size siblings. Since prey size matters to predation risk, small prey (e.g., larvae) is less likely to be targeted, particularly when near larger prey which might behave altruistically when the smaller larvae are kin (sibling). de Bruijn *et al.* (2014) evaluated the existence of kinship-dependent survival in groups of sibling or non-sibling larvae of western flower thrips presented to predatory mites. They found that the survival of small larvae in sibling groups was significantly higher than in non-sibling groups.

Among the parasitoid Hymenoptera, it has been shown that aggression during resource competition, whether between larvae or between adult females, can be reduced when competitors are (actual or perceived) close relatives (Giron *et al.*, 2004; Giron & Strand, 2004; Lizé *et al.*, 2012; Dunn *et al.*, 2014). Consistent with kin selection theory which predicts that individuals show less aggression and more altruism towards relatives (Hamilton, 1963; Hamilton, 1964a), Lizé *et al.* (2012) showed that while the outcomes of dyadic contest behaviour between *Goniozus* females can largely be explained in term of direct fitness benefits,

furthermore, it has been shown that contestants are less aggressive towards siblings than towards non-siblings, suggesting that inclusive fitness considerations moderate competition. Other studies reported that soldiers of *Copidosoma floridanum* aggressively attack non-relative clone-mates after recognizing its kin (Giron *et al.*, 2004). Tang *et al.* (2014) showed that quasi-sociality exhibited by *Sclerodermus* spp. foundresses is in favour of direct fitness benefit.

However, some studies have empirically shown that kin selection does not play a role in shaping of some behaviours, such as sex allocation. Optimality theory of sex allocation in structured populations predicts that mothers produce adult offspring with higher female-biased sex ratios when laying eggs with relatives than with non-relatives (Hamilton, 1967). Contrary to the prediction of the theory, for instance, Shuker *et al.* (2004a) showed experimentally that females of *Nasonia vitripennis* did not adjust the sex ratios of adult offspring produced according to the relatedness of their oviposition partners, or to the environmental cues that could indicate or signal relatedness.

Finally, there is relatively small body of literature that criticized the theory of kin selection. Alonso (1998) was the first to raise an objection, followed by another study conducted by Alonso and Schuck-Paim (2002) who argued that kin selection theory was used to explain behaviours which are not altruistic. They highlighted that alternative theories to explain biological altruism and sex ratio conflicts were neglected in favour of kin selection theory. Famous sociobiologists, such as E. O. Wilson, Bert Hölldobler and Martin Nowak raised the debate again after the above-mentioned criticism was mostly ignored (Wilson & Hölldobler, 2005; Wilson, 2008; Nowak *et al.*, 2010). Nowak *et al.*

(2010) argued that inclusive fitness theory works only in a limited domain, and described it as an unnecessary detour which contributes no remarkable insight or additional information. On the other hand, *Nature* published in 2011 an article (Abbot *et al.*, 2011) that was co-authored by more than one hundred researchers as a strong rebuttal to Nowak *et al.* (2010).

So far, this section has attempted to provide a brief summary of the literature relating to the principles of social behaviours exhibited by interacting individuals and their hierarchies and social interactions among living organisms. It has been particularly focused on the role of kin selection in altruism and cooperation existence among interacting individuals. Mostly, insects were selected as examples throughout this section. As such, this thesis is concerned with the fundamentals of behavioural ecology and sociobiology, just as much as it is concerned with the potential for biological pest control. The section that follows moves on to present the substantial role that theoretical and empirical sociobiological and behavioural-ecological studies can play in the enhancement of the efficiency of parasitoids which, in turn, contribute to the optimal deployment of parasitoids in field applications against pests.

1.4 From behavioural ecology to biological control

Behavioural ecology is branch of science that studies the fitness consequences of behaviour. This branch of science has attained the attention of biologists mainly since the mid of 1960s. Different animals, such as mammals, birds, fish and also insects were used to develop the principles of this science. Parasitoids are of value as experimental materials for behavioural and population studies because they are abundant, easy to rear and to handle; moreover, they are

used as biocontrol agents to regulate the population of many insect pests (Waage & Hassell, 1982; Godfray, 1994).

Parasitoids are obliged to locate hosts in order to reproduce and as a result they evolved a wide range of strategies to find and attack hosts. This made natural selection operate strongly on parasitoids, and consequently, they are considered ideal models for testing evolutionary related hypotheses. Parasitoids, utilised as model organisms, played an essential role in understanding, developing and testing behavioural-ecological theories, such as foraging behaviour, clutch size and sex allocation. Since the reproduction of parasitoids is linked to the reduction of the host population (i.e., insect pests), they can be successfully utilised on a large scale to implement biological control programmes against agricultural and agro-forestry insect pests. Behavioural ecological studies of parasitoids are conducted to uncover the combination of behavioural attributes that could contribute to enhance parasitoid biocontrol efficiency. Successful biological control research programmes are designed on the basis of suitable frameworks initially derived from behavioural ecology theories (Luck, 1990; Waage, 1990). Among those research programmes for instance, are improvement of mass rearing efficacy of biocontrol agents, assessment of failure and success of released natural enemies, and development of suitable natural enemy selection criteria.

A thorough understanding of the ecology and behaviour of natural enemies is required to contribute to constructing a reliable and predictable framework of biological control by using parasitoids (Lewis *et al.*, 1990; Luck, 1990). A considerable literature has been published on the behavioural ecology of parasitoids. These studies stressed the need to utilize a scientific approach

(Van Lenteren, 1980; Mackauer *et al.*, 1990) and provided a means to improve pest control efficacy in biological control programmes. For example, behavioural ecology studies aimed to increase female-biased sex ratios have enhanced the efficiency of mass-released parasitoids, because it is females that locate and attack suitable hosts (Luck, 1990; Hardy & Goubault, 2007; Ode & Hardy, 2008). Having given this example, kin selection theory predicts that females of haplodiploid wasps are expected to produce adult offspring with strongly female-biased sex ratios if they mate with their siblings. This biased sex ratio prediction is attributed to inbreeding in haplodipoids that results in foundresses being relatively more related to their daughters than to their sons. Some studies reported that females do not adjust their sex ratio behaviour according to relatedness of the male with which they mate (Reece *et al.*, 2004; Shuker *et al.*, 2004a), but I found (*see results in chapter five*) that sibling foundresses of *Goniozus nephantidis* produce adult offspring with a highly female-biased sex ratio. This kinship effect on parasitoid sex allocation strategies enables mass rearing programmes to be able to enhance the efficiency of their productivity by producing parasitoid wasps with a strongly female-biased sex ratio, noting, as abovementioned, that females are those who suppress pests.

Clutch size decisions are influenced by host size (Charnov *et al.*, 1981; West, 2009). Therefore, it is predicted that sibling foundresses lay larger clutch sizes on larger hosts. In addition, large clutch sizes usually tend to produce adult offspring with highly female-biased sex ratios (Charnov, 1982; Hardy *et al.*, 1992), and with bigger size (Hardy *et al.*, 1992). Thus, mass rearing parasitoids on the basis of this behavioural ecological perspective will enhance biocontrol

programmes as a result of releasing efficient and bigger females in the field with a better ability to locate and suppress hosts (Kazmer & Luck, 1995; West *et al.*, 1996; Ellers *et al.*, 1998).

1.5 Summary of thesis structure

The experimental work conducted during the course of this project can be grouped into three parts. The first deals with the efficiencies of rearing *Sclerodermus brevicornis* on a new factitious (alternative) host, whereas the second and third parts focus mainly on exploring kinship effects in quasi-social and sub-social bethylids, respectively.

1.5.1 Part one: A moth as a factitious host for a beetle parasitoid

Chapter 2. An alternative host for *Sclerodermus brevicornis* was explored to promote the efficiency of mass-rearing and releasing programs of the adult females of this parasitoid. Larvae of rice moth were used as candidate factitious hosts and effect of pre-paralysis treatment and foundress number variation were explored. This showed that rice moth larvae are suitable and identified conditions that can be used to rear *S. brevicornis* efficiently.

1.5.2 Part two: Kinship among quasisocial parasitoids

Chapter 3. The role of co-foundress relatedness and number in the cooperative reproductive behaviour exhibited by *S. brevicornis* females was explored. The time lag to (small, standard sized) host paralysis is shorter when co-foundress relatedness was greater. Hamilton's rule was used to suggest (using data from other *Sclerodermus* species) how co-foundress relatedness and the greater risks and greater benefits of attacking larger hosts could combine to determine the limits to the size of a host that a female is selected to attack to provide

essential reproductive resources to co-foundresses as well as to herself. It was predicted that the time to host attack would, in consequence, be affected by an interaction between host size and relatedness.

Chapter 4. Co-foundress relatedness and host size were varied for the two-foundress case, to test the prediction generated in Chapter 3, and this was supported. Hamilton's rule, using data from *S. brevicornis* again suggested how both host size and kinship are likely to influence host attack decisions by individual females and, in consequence, the stability of cooperative reproductive behaviour.

1.5.3 Part three: Kinship among subsocial parasitoids

Chapter 5. Having established that kinship effects operate among quasi-social bethylids in Chapters 3 and 4, this chapter further explored kinship among females of the naturally sub-social *Goniozus nephantidis.* The relatedness and number of co-foundresses confined with a host was varied to discover whether prolonged female-female contact would elicit behavioural and reproductive responses that might reveal incipient higher-level sociality. It was found that close female-female relatedness was associated with more female biased sex ratios, similar to those produced by single foundresses, while non-kin females produced only mildly female biased sex ratios.

Chapter 6. Having found a sex ratio effect of female-female kinship in Chapter 5, this chapter explored the effect of mother-mate relatedness on the sexual composition of adult offspring produced by *Goniozus legneri*. Relatedness was varied by providing virgin females with a mate which was either a sibling, a non-sibling from the same strain, from a strain with geographically similar origin or

from a strain with geographically distant origin. It was found that sex ratios are more biased when a female had mated with a male from a different strain.

Overall, this thesis contributes to the potential for controlling invasive forest pests by providing recommendations for mass-rearing their native natural enemy, *Sclerodermus brevicornis*, a quasi-social parasitoid, and by exploring previously unknown aspects of *Sclerodermus* reproductive behaviour. The key finding that kinship amongst foundress groups affects host suppression indicates that there will often be tensions within the observed cooperative behaviour, especially when inter-foundress relatedness is low. Kin effects are also found in sub-social parasitoids. Close kin do not appear to cooperate in host suppression but do appear to alter their sex allocation behaviour. Mothers also appear to adjust sex allocation according to their kinship with their mates. The effects of kinship found in these studies will generally enhance the performance of bethylids as agents of biological control as close relatedness leads to earlier host attack and the recruitment of greater number of female parasitoids into subsequent generations.

Part one: A moth as a factitious host for a beetle parasitoid

Chapter 2: Performance of *Sclerodermus brevicornis*, a parasitoid of invasive longhorn beetles, when reared on rice moth larvae

2.1 Abstract

Biological control efficiency can be improved by developing an effective mass-rearing system to produce large numbers of high-quality parasitoids. This study explores alternative rearing hosts for *Sclerodermus brevicornis* (Hymenoptera: Bethylidae), a potential biological control agent for the suppression of exotic and invasive wood-boring long-horned beetle populations in the European agroforestry ecosystems. We trial larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as hosts for *S. brevicornis*. We quantify the probability and timing of host attack and parasitism as well as reproductive success, offspring production and the characteristics of adult offspring. As *S. brevicornis* is a quasi-gregarious species (multiple females, communally produce offspring broods), we also explore the effects of varying the number of females to which individual hosts are presented, with the aim of determining the optimal female-to-host ratio. As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we trial the use of adult females of another species of the bethylid wasp, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to presentation to *S. brevicornis*. We identify the conditions within our experiment that maximise offspring production per host and offspring production per adult female parasitoid. We find that *C. cephalonica* is suitable as a factious host and, as it is considerably more straightforward to laboratory-rear than cerambycid species, it is a strong candidate for adoption by future *S. brevicornis* mass-rearing and release programmes.

2.2 Introduction

Members of the genus *Sclerodermus* Latreille, 1809 (Hymenoptera: Bethylidae) are small-sized (1.5 – 6 mm) parasitoid wasps (Evans, 1978). There are approximately 81 species worldwide (Gordh & Móczár, 1990; Lanes & Azevedo, 2008). Species of this genus are idiobiont ectoparasitoids (Li & Sun, 2011; Hu *et al.*, 2012), typically of coleopteran larvae. Morphological characteristics of *Sclerodermus* enable them to find and attack their hosts in enclosed spaces, such as feeding tunnels (Kühne & Becker, 1974; Evans, 1978; Yang *et al.*, 2012a; Baena & Zuzarte, 2013; Jiang *et al.*, 2015). Some species are of considerable economic importance as they are used in biological control programmes (Yang *et al.*, 2014) while others may be medically detrimental (Evans, 1978; Papini, 2014; Yang *et al.*, 2014; Skvarla, 2018).

In China, *Sclerodermus* species have formed an integral part of effective and successful forest pest management programs (Chen & Cheng, 2000; Yang, 2004; Kaishu, 2006; Tang *et al.*, 2012; Yang *et al.*, 2014; Jiang *et al.*, 2015). Among these pests are the pine sawyer beetle, *Monochamus alternatus* (Coleoptera: Cerambycidae), a vector of pine wood nematode, *Bursaphelenchus xylophilus* (Lai *et al.*, 2012; Yang *et al.*, 2012b; Zhang *et al.*, 2012; Yang *et al.*, 2013; Yang *et al.*, 2014); the oak long-horned beetle, *Massicus raddei* (Coleoptera: Cerambycidae) (Wu *et al.*, 2008; Wang *et al.*, 2010; Tang *et al.*, 2012; Yang *et al.*, 2012a); and the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) (Wu *et al.*, 2008; Yang *et al.*, 2012a). Several Asian species of long-horned beetles have now invaded Europe and some of the 17 recorded species of *Sclerodermus* (Andre, 1881; Kieffer, 1914), have now been reported as associated with these invasive pests (Hérard *et al.*,

2007; Lupi *et al.*, 2014). One of these European natives, *Sclerodermus brevicornis* (Kieffer), was first reported as associated with the long-horned beetle, *Oxypleurus nodieri* Mulsant (Coleoptera: Cerambycidae) approximately one century ago (Kieffer, 1914) and much more recently, in 2011, was reported in association with the invasive Asian longhorn beetle, *Psacothea hilaris hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Lupi *et al.* (2014). It was subsequently shown that *S. brevicornis* can be laboratory reared on *P. hilaris hilaris* and two other invasive longhorn beetles species and that it has potential for deployment in biological control programs (Lupi *et al.*, 2017).

While rearing *S. brevicornis* on longhorn beetles is entirely possible, the process of rearing the hosts is time-consuming and physically demanding (Lupi *et al.*, 2013; Lupi *et al.*, 2015b; Lupi *et al.*, 2017). Moreover, *S. brevicornis* foundresses are best left undisturbed for at least four days to successfully paralyse their host, a process that increases the overall duration of parasitoid rearing. The success of parasitoids used in biological control programs is often determined by finding suitable alternative hosts used for their mass-rearing (Lemos *et al.*, 2003; Ramalho & Dias, 2003; Pratissoli *et al.*, 2004). Such hosts should ideally have low production costs and no adverse effects on the reproductive and behavioural performance of the parasitoids (Pereira, 2006). Therefore, attempts have been made to find suitable a factitious host for *S. brevicornis*. Thus far, it has been found that female *S. brevicornis* do not successfully parasitize pupae of the mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae), or late-instar larvae of greater wax moth, *Galleria mellonella* L. (Lepidoptera: Galleridae) (D.L. unpublished data).

Here we evaluate the potential use of larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), as hosts for *S. brevicornis*. Besides being a pest insect, the rice moth is commonly used as a factitious host for rearing around 75 species of natural enemies (Chaudhuri & Senapati, 2017), including parasitoids and predators (Hardy & Blackburn, 1991; Jalali & Singh, 1992; Sahayaraj, 2002; Ballal *et al.*, 2003; Jalali *et al.*, 2003; Lenin & Rajan, 2016; Subandi *et al.*, 2017) and indeed it is common to produce biocontrol agents on *C. cephalonica* rather than on their natural hosts (Kumar & Murthy, 2000). We explore the performance of *S. brevicornis* when presented with *C. cephalonica* larvae by quantifying the probability and timing of host attack and parasitism as well as reproductive success, offspring production and characteristics. As *S. brevicornis* is a quasi-gregarious species (multiple females, termed 'foundresses' attack single hosts and produce offspring broods on them communally), we also explore the effects of varying the number of females to which individual hosts are presented, with the aim of determining the optimal female-to-host ratio (Wei *et al.*, 2017). As time to host attack can be a limiting factor in *S. brevicornis* rearing protocols, we trial the use of adult females of another species of the bethylid wasp, *Goniozus legneri* Gordh, to paralyse *C. cephalonica* larvae prior to their presentation to *Sclerodermus* females. *Goniozus legneri* is a gregarious ecto-parasitoid present in several agroecosystems and easy to rear in the laboratory (Legner & Silveira-Guido, 1983; Legner & Gordh, 1992; Steffan *et al.*, 2001; Zaviezo *et al.*, 2007); it is also known to typically attack and paralyse *C. cephalonica* larvae within minutes of presentation (I.H. pers. obs.).

2.3 Materials and Methods

2.3.1 Host and parasitoid cultures

A stock culture *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) was set up in Plexiglas cages (length: 36cm × width: 26cm × height: 25cm) where the adults could fly and mate. To obtain eggs, females were captured and positioned into a small container to allow oviposition. Eggs were then collected and transferred into Petri dishes (15 cm diameter × 2 cm deep) containing a diet used for rearing Pyralid moths following Limonta *et al.* (2009). Petri dishes were kept closed until used for experiments or until pupation to obtain new adults for the stock culture.

Sclerodermus brevicornis was maintained on the larvae of *Psacothea hilaris hilaris* (Coleoptera: Cerambycidae). Two different systems for rearing *Sclerodermus* on *P. h. hilaris* were set up according to the instar (pre-imaginal or adult) following the protocol in (Favaro *et al.*, 2017). To obtain new offspring of *S. brevicornis*, six to eight newly emerged females were presented with individual late instar larvae of the long horned beetle in plastic Petri dishes (5 cm diameter × 4 cm deep) on a bed (ca. 1.5 mm) of cork oak granules, following Lupi *et al.* (2017).

A culture of *Goniozus legneri* (Hymenoptera: Bethylidae) was reared on *Corcyra cephalonica*. Individual female parasitoids were presented with single host larvae in glass vials of 10mL with a base of 1.4 mm. The *G. legneri* was obtained from the University of Nottingham, UK, in 2016, and is the strain referred to as ‘U’ in Khidr *et al.* (2013).

All cultures and experiments were carried out in a climate room at 26.0°C ±0.5°C, photoperiod 16:8 (L:D), and 70 ±5% R.H. in a laboratory at the University of Milan, Italy.

2.3.2 Experiments

All the experiments were carried out using *Corcyra cephalonica* as a host, selecting larvae ranging from 0.030-0.040g. One of two procedures were adopted before *C. cephalonica* were presented to *S. brevicornis*: either *S. brevicornis* females were presented with unparalysed hosts or hosts were first presented to, and paralysed by, a female *G. legneri* before being removed from the *G. legneri* female and present to *S. brevicornis.* Then, in each replicate, a host was placed into a plastic vial (10cm long × 1.3cm diameter) and either 1, 2, 3, 4, 5 or 6 adult *S. brevicornis* females (foundresses) were added into each vial. The vials were closed with a gauze and cotton wool. To give adequate overall statistical power (Smith *et al.*, 2011), we set up 92, 224, 111, 54, 50 and 54 replicates for foundress numbers of 1, 2, 3, 4, 5 and 6, respectively, giving a total number of 585 experimental units. We ensured that a balanced range of pre-paralysed or unparalysed hosts were represented within the replicates of the first four foundress groups. Five- and six-foundress groups were not presented with pre-paralysed hosts.

Vials were observed daily to record host paralysis (occurrence and timing), egg laying (occurrence and time of first oviposition to begin a clutch), pupation (timing of first within a brood), emergence of adult offspring (timing, number and sex) and whether offspring were alate or apterous (*S. brevicornis* is wing dimorphic in both sexes (Evans, 1978)). Observations were made while attempting not to disturb the wasps and in consequence some offspring may

have been overlooked at earlier developmental stages. If a host was oviposited on, it was considered to have been successfully parasitized. Parasitism rate was defined as the proportion of replicates in which oviposition occurred out of total number of replicates in which hosts were paralysed. The production of at least one adult offspring in a replicate was defined as reproductive success.

2.3.3 Statistical analyses

Data were analysed using Generalized Linear Modelling approaches (Aitkin et al., 1989; Crawley, 1993; Faraway, 2006). All analyses were carried out using the statistical software package *GenStat* (version 17, VSN International, Hemel Hempstead, UK).

The explanatory variables 'foundress number' and the 'use of *G. legneri'* (as a pre-host-paralysing agent) were fitted as factors. For some analyses foundress number was initially treated as a factor with six levels but, because there was only variation in *G. legneri* use among 1-4 foundress replicates, analyses including consideration of pre-paralysis excluded 5- and 6-foundress replicates and thus fitted foundress number as a factor with four levels. In some analyses we additionally explore results by fitting foundress number as a continuous variable.

The response variables were the parameters recorded during the daily observations and at the end of the experiment. Logistic analyses (GLM) were used to explore binary data on host paralysis, oviposition and foundresses reproductive success; these analyses assumed binomially distributed errors (the reported test statistic is change in deviance, *G*, which is approximately χ^2 distributed, (Crawley, 1993). Other logistic analyses of proportional data with denominators larger than unity (e.g. proportion of alate males or females,

offspring sex ratio, etc.) assumed quasi-binomial error distributions (the reported test statistic is the *F*-ratio, (Crawley, 1993; Wilson & Hardy, 2002). All logistic analyses adopted a logit link function. Log-linear analyses were used to analyse count data (e.g. number of offspring produced) assuming quasi-Poisson error distributions and a log-link function (Crawley, 1993). Data derived from integers (e.g. per-foundress brood size) that were likely to be approximately Poisson-distributed were also analysed using log-linear models (Faraway, 2006; Tang *et al.*, 2014). Following logistic and log-linear analyses, we give the percentage deviance explained (%Dev) as a descriptor analogous to r^2.

Parametric cohort survival analyses with censoring (Aitkin *et al.*, 1989; Crawley, 1993) were used to analyse the influences on times-to-events, such as time to host paralysis, oviposition, larval hatching, spinning cocoons and adult offspring emergence.

2.4 Results

2.4.1 Probability host utilization and developmental success

2.4.1.1 Probability of paralysis

The mean probability of a host (which had not been pre-paralysed by *G. legneri*) being paralysed by *S. brevicornis* was 0.754 (S.E. = +0.021, -0.022, n=411). The probability was greater when there were multiple foundresses (logistic ANOVA: G_5=3.11, P=0.008, %Dev=3.39, Fig. 1). The model was simplified by aggregating foundress number categories to find the minimal adequate model : this showed that there was a no significant difference between the multi-foundress replicates (G_4=0.51, P=0.727, %Dev=0.57, n=350) and that the

probability of paralysis was higher among these than among single foundress replicates (G_1=13.51, P<0.001, %Dev=2.95). Similarly, if foundress number was fitted as a continuous variable, the probability of paralysis increased in a curvilinear relationship with increasing foundress number (logistic regression: G_1=3.15, P=0.076, %Dev=0.69; quadratic term: G_1=7.48, P=0.006, %Dev=1.63, Figure 2.1).

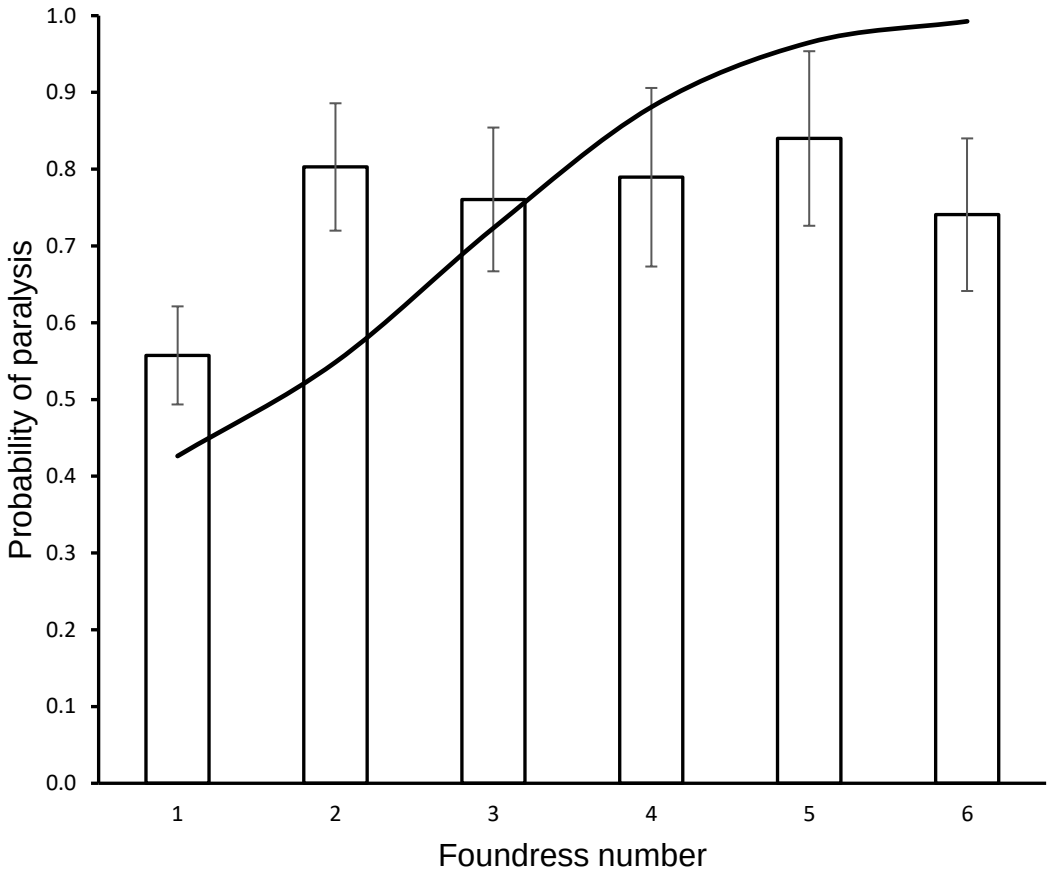


Figure 2.1 Effect of number of foundresses on the probability of paralysis: The mean probability of a host (which had not been pre-paralysed by *G*. *legneri*) being paralysed by *S. brevicornis* was greater when there were multiple foundresses. Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale. The line shows the fitted quadratic logistic regression: probability of paralysis =1/(1+(1/(EXP((0.081×foundress number)+(0.137×foundress number^2)-0.515)))).

2.4.1.2 Probability of oviposition

The overall probability of a presented host being oviposited on by *S. brevicornis* was 0.610 (SE ± 0.020, n=585). There was a significant interaction between foundress number and pre-paralysis treatment: for unparalysed hosts, oviposition probability increased with foundress number but for pre-paralysed hosts, it declined (logistic ANOVA, $G_3 = 6.50$, $P < 0.001$, %Dev = 2.98, n=481). For hosts that were paralysed (whether by *G. legneri* or by *S. brevicornis*), the probability of subsequent oviposition by *S. brevicornis* was 0.7454 (S.E. = +0.019, -0.020). Again, there was a significant interaction between foundress number and pre-paralysis treatment (logistic ANOVA: $G_3 = 6.75$, $P<0.001$, %Dev = 4.21, Figure 2.2).

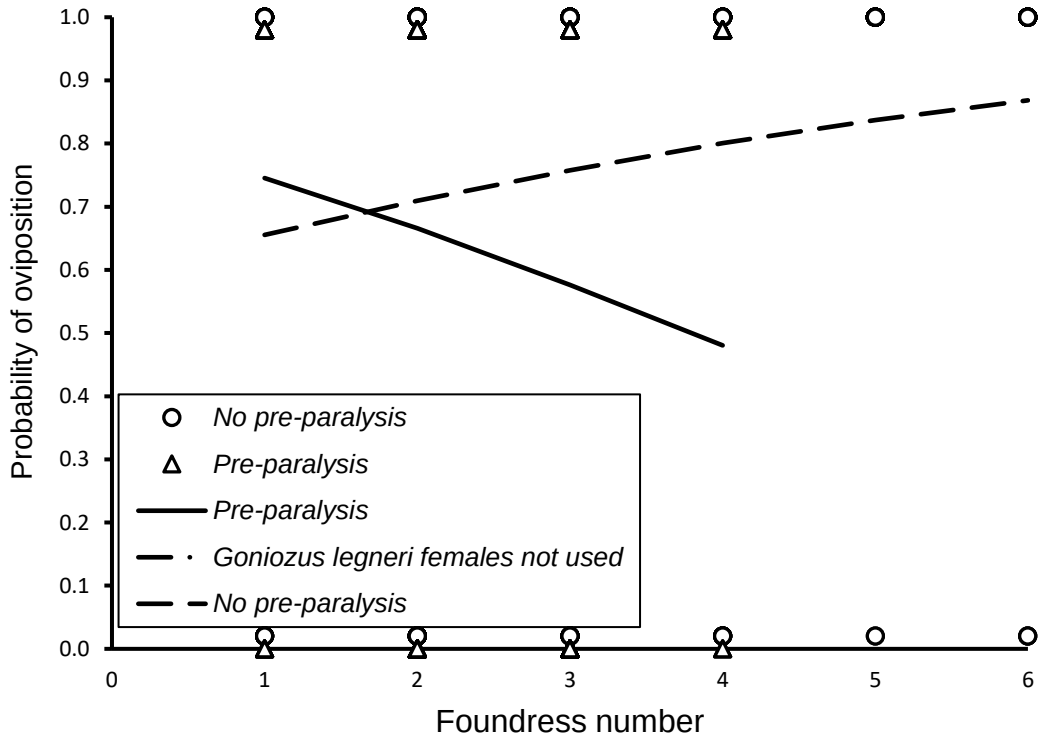


Figure 2.2 The effect of number of foundresses on the probability of oviposition: oviposition probability for previously unparalysed hosts increased with an increase in foundress number, but declined when hosts were pre-paralysed by *G. legneri*. Logistic regression lines fitted separately across the range of foundress numbers evaluated.

2.4.1.3 Probabilities of development towards adulthood

Among the 357 replicates in which eggs were laid, the probability of at least one egg hatching to the larval stage was 0.7956 (S.E. = +0.020, -0.022). This probability was significantly lower among eggs laid by five-foundress groups (logistic analysis: $G_5 = 2.49$, P = 0.029, %Dev = 3.45). The model was simplified by aggregating foundress number categories to find the minimal adequate model (Crawley, 1993): this showed that there was a no significant difference among foundress groups of 1, 2, 3, 4 and 6 ($G_4 = 0.83$, P = 0.506, %Dev = 1.11) and the probability was significantly lower when eggs were laid by five-foundress groups ($G_1 = 9.16$, P = 0.002, %Dev = 2.53). Egg hatching probability was not influenced by the use of *G. legneri* ($G_1 = 2.09$, P = 0.148, %Dev = 0.79; pre-paralysis × foundress number interaction: $G_3 = 0.04$, P = 0.988, %Dev = 0.05).

In replicates with hatched larvae, the probability of some developing to the pupal stage was 0.8416 (S.E. = +0.021, -0.023, n = 284). Larvae produced by four-foundress groups pupated significantly more successfully than those for two or three-foundress groups, whereas pupation success of offspring of other foundress group sizes was intermediate (logistic analysis: $G_5 = 2.79$, P = 0.016, %Dev = 5.62). Pupation probability was unaffected by the use of *G. legneri* ($G_1 = 0.00$, P = 0.947, %Dev = 0.002; pre-paralysis × foundress number interaction: $G_3 = 0.02$, P = 0.997, %Dev = 0.02).

Among replicates in which some offspring reached the pupal stage (n = 239), the probability of at least one adult offspring emerging was 0.983 (S.E. = +0.007, -0.0107); this was unaffected by foundress number ($G_5 = 1.11$, P =

0.355, %Dev = 13.60), the use of *G. legneri* (G_1 = 0.48, P = 0.487, %Dev = 1.24) or their interaction (G_3 = 1.54, P = 0.202, %Dev = 11.88).

2.4.1.4 Overall probability of reproductive success

The overall probability of the successful production of at least one adult offspring from a presented host was 0.4017 (SEM = +0.0204, -0.0201). Success did not differ significantly across all foundress number treatments (logistic ANOVA: G_5 = 1.52, P = 0.180, %Dev = 0.96, n = 585) but there was a positive relationship when foundress number was fitted as a variate (logistic regression: G_1 = 4.52, P = 0.033, %Dev = 0.57, Figure 2.3). Among foundress numbers treatments where pre-paralysis was also varied, there was a significant interaction (pre-paralysis: G_1 = 0.03, P = 0.862, %Dev = 0.005; Interaction: G_3 = 2.72, P = 0.043, %Dev = 1.23, n = 481) such that reproductive success increased with increase of foundress number when *G. legneri* was not utilised but decreased when hosts had been pre-paralysed: this interaction was due to the use of *G. legneri* affecting the probability of oviposition (Figure 2.2). Considering only the hosts that were oviposited on, adult offspring were produced in two thirds of the cases (mean = 0.658, SE = +0.025, -0.026); the probability of post-oviposition success was marginally significantly affected by foundress number (G_5 = 2.23, P = 0.048, %Dev = 2.43) but there was no overall trend as foundress number increased (logistic regression: G_1 = 0.16, P = 0.685, %Dev = 0.04) and the probability was unaffected by the use of *G. legneri* (main effect: G_1 = 0.75, P = 0.385, %Dev = 0.21, n = 280; interaction: G_3 = 0.22, P = 0.884, %Dev = 0.18).

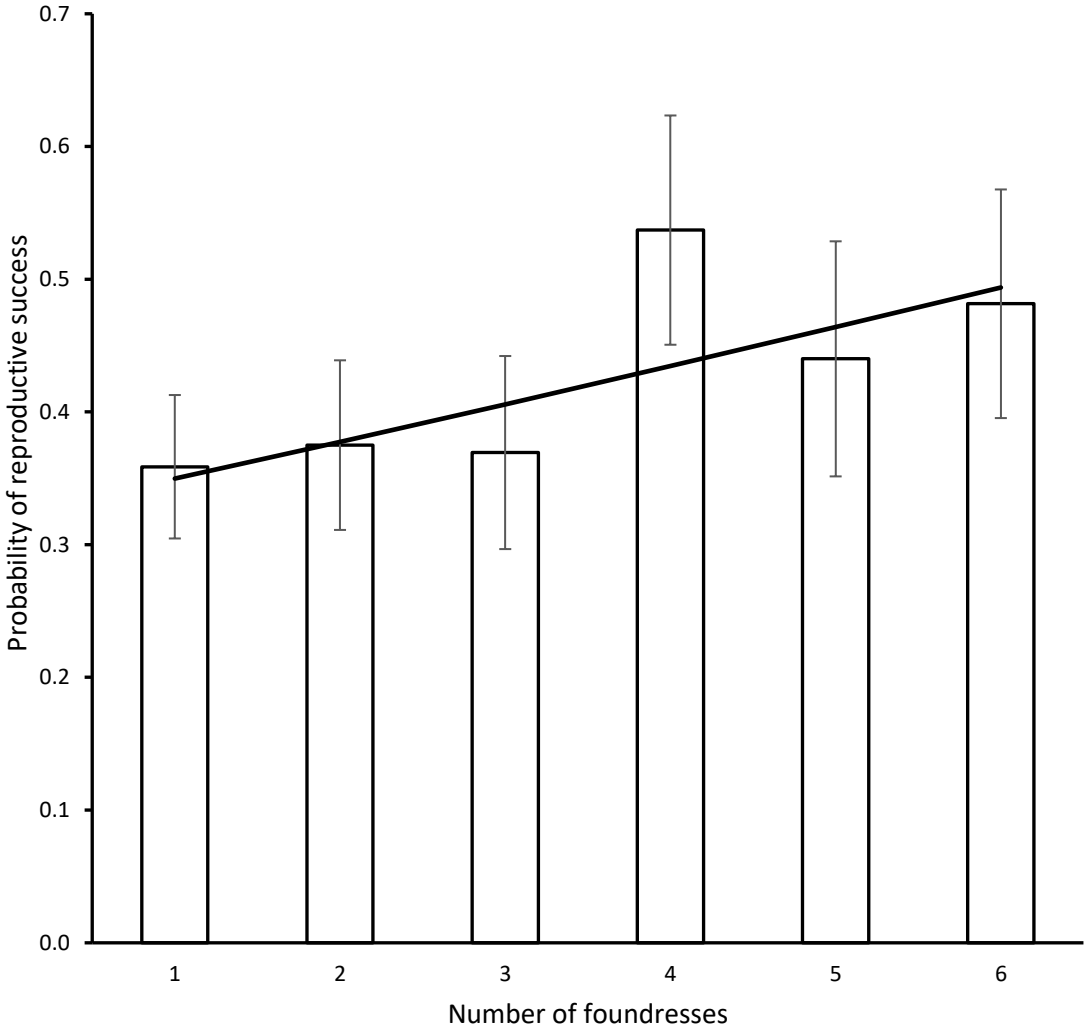


Figure 2.3 The overall probability of reproductive success. The probability of success did not differ significantly between groups (foundress number treated as a factor) but increased significantly when foundress number was fitted as a variate: logistic regression line, probability =1/(1+(1/(EXP((0.119 × foundress number)-0.739)))). Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale.

2.4.2 Timing of host use and offspring development

Descriptive statistics for timings under all experimental treatment combinations are given in Table 2.1.

2.4.2.1 Time to paralysis

Among the 411 replicates in which unparalysed hosts were presented to *S. brevicornis* females, the time to paralyse hosts ranged between 1 and 13 days

(mean=3.95, SE= ±0.12) but not all hosts became paralysed (see above). The time taken to paralysis was affected by foundress number (survival analysis with hosts unparalysed by day 13 treated as censors: G_5= 28, *P*<0.001, %Dev = 4.48). Aggregation of factor levels indicated that timing was not significantly different among multi-foundress treatments (G_4= 3, *P*= 0.558, %Dev = 0.47) but was significantly slower in single-foundress replicates (G_1= 25, *P*<0.001, %Dev = 4.15; Figure 2.4).

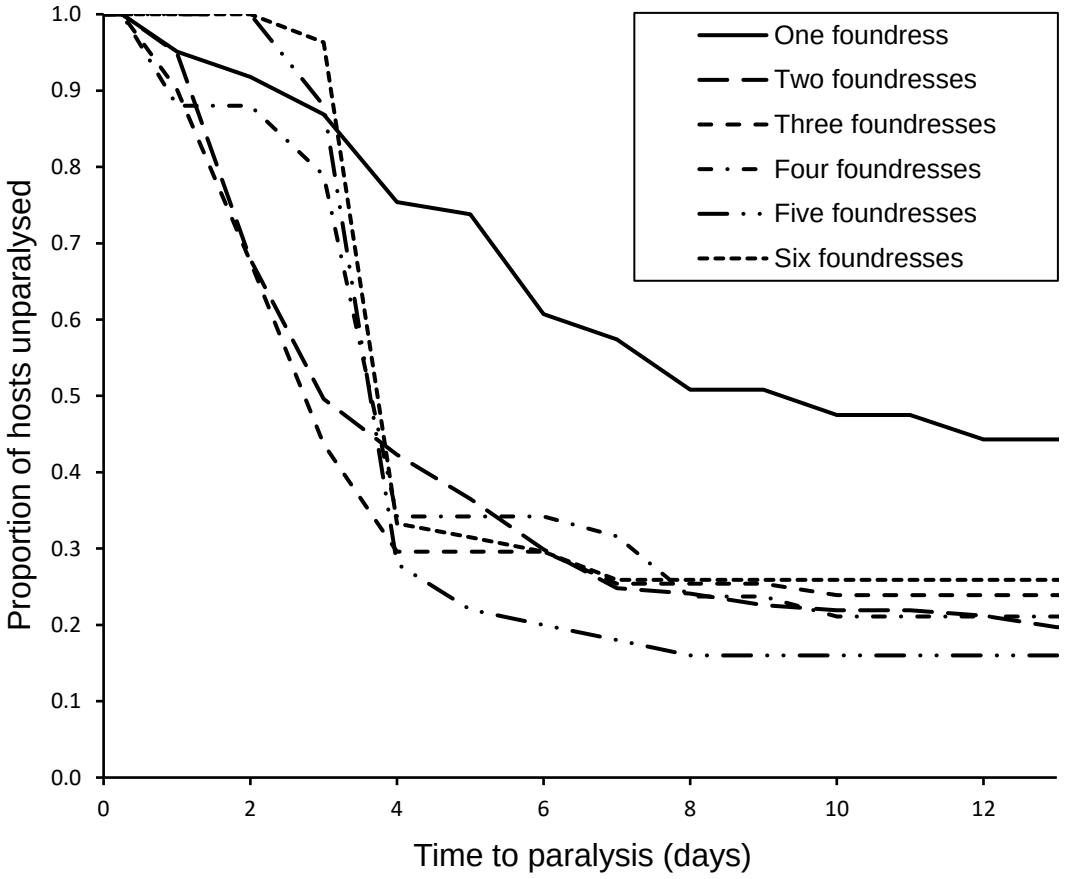


Figure 2.4 The effect of foundress number on time taken to paralyse hosts. Single foundresses took significantly longer to paralyse hosts than did multiple foundresses. Among multiple foundress replicates, the number of foundresses did not affect timing significantly.

2.4.2.2 Time to oviposition

The time from host presentation for eggs to be first observed ranged from 3 to 18 days (mean=7.37, SE= ±0.14) and varied according to the number of foundresses present (G_5 = 35, P<0.001, %Dev = 3.77, n = 582); larger foundress groups typically started ovipositing earlier. There was a significant interaction between foundress number and the use of *G. legneri* (G_3 = 22, P<0.001, %Dev = 3.01, n = 478); groups of three foundresses took longer to lay eggs on hosts that were pre-paralysed compared to other treatment combinations.

Once a host was paralysed, the time until oviposition, ranged from 1 to 17 days (mean=4.22, SE= ±0.15), varied according to foundress number (G_5 = 88, P<0.001, %Dev = 11.59, n = 476), with eggs typically laid earlier by larger foundress groups (Figure 2.5). Times were shorter when *G. legneri* foundresses were not utilised for pre-host-paralysis (G_1 = 18, P<0.001, %Dev = 2.83, n = 394) and, as above, there was a significant interaction between foundress number and the use of *G. legneri* (G_3 = 21, P<0.001, %Dev = 3.3).

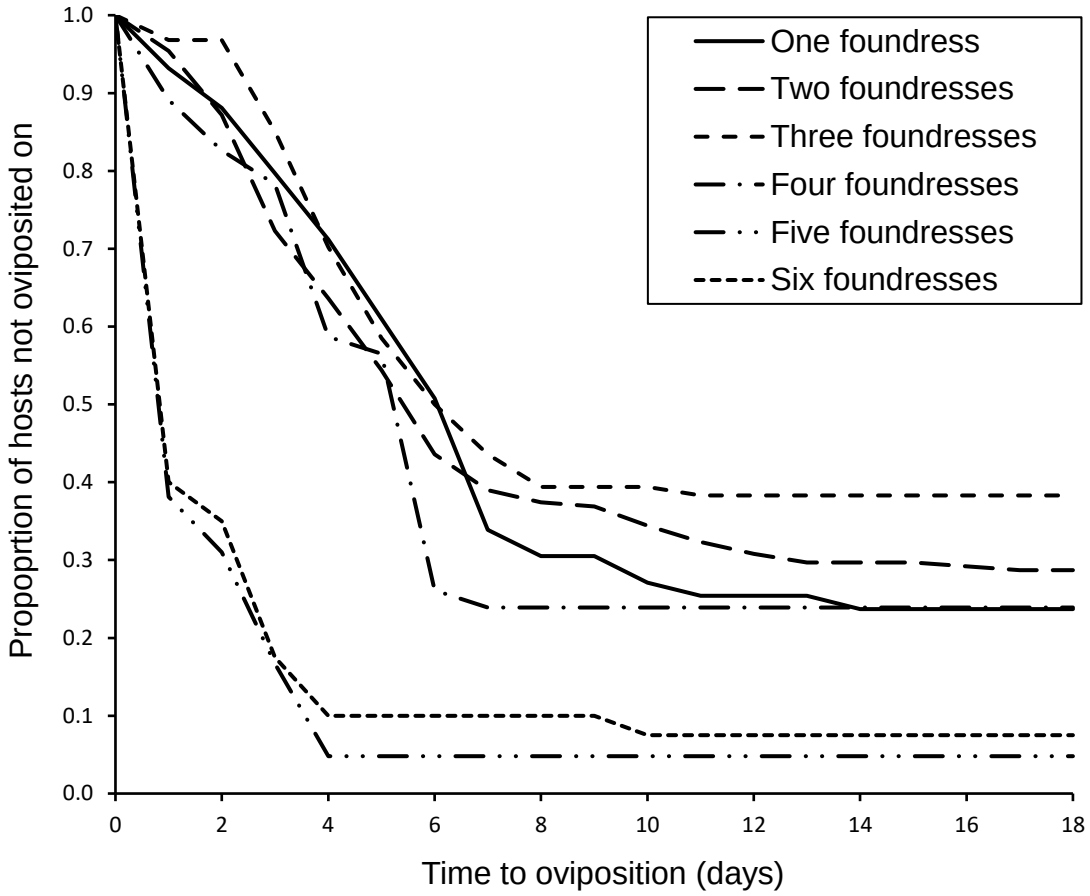


Figure 2.5 Influence of time to egg laying on paralysed hosts. Smaller foundress groups (1-4) took significantly longer to oviposit on hosts than did groups of 5 or 6 foundresses.

2.4.2.3 Time to egg hatching

The time from when at least one egg was first observed on a host to the first observation of larvae on the host ranged from 1 to 12 days (mean = 4.43, SE = ±0.12). This timing varied across foundress number treatments (G_5 = 23, P<0.001, %Dev = 4.33, n = 354); eggs produced by groups of 5-foundresses typically took the longest time to hatch. Eggs hatched marginally significantly earlier when hosts had not been pre-paralysed by *G. legneri* (G_1 = 4, P = 0.046, %Dev = 0.81, n = 277) but there was no significant interaction with foundress number (G_3 = 0.00, P = 1.00, %Dev = 0.17).

2.4.2.4 Time to pupation

Pupae were first observed 2 to 17 days (mean=5.91, SE=±0.16) after the first observation of larvae on a host. Offspring of smaller foundress groups pupated significantly earlier (G_5 = 14, P = 0.0156, %Dev = 3.60, n=282). Time to pupation was unaffected by the use of *G. legneri* (G_1 = 2, P = 0.1573, %Dev = 0.57; interaction with foundress number: G_3 = 2, P = 0.5724, %Dev = 0.72, n=227).

2.4.2.5 Time to adult eclosion

Adult offspring of *S. brevicornis* eclosed from pupae between 6 to 26 days (mean=15.56, SE=±0.223) after the first observation of pupae in the brood. This period differed across foundress number treatments (G_5 = 7, *P* = 0.221, %Dev = 3.99, n = 237). Pupae produced from hosts that were pre-paralysed by *G. legneri* developed to adult offspring within a significantly shorter time (G_1 = 13, *P*<0.001, %Dev = 6.15, n = 189). There was no significant interaction effect between foundress number and the use of *G. legneri* (G_3 = 7, *P* = 0.5724, %Dev = 1.27).

2.4.2.6 Time for egg to adult development

Overall egg-to-adult development ranged between 17 and 40 days (mean = 25.85, SE = ±0.25). Developmental time was significantly shorter when eggs were produced by four-foundress groups (G_5 = 12, *P* = 0.0348, %Dev = 2.22, n=354). This time was significantly not affected by the use of *G. legneri* as a pre-host-paralysing agent (G_1 = 1, *P* = 0.3173, %Dev = 0.18; interaction with foundress number: G_3 = 1, *P* = 0.8013, %Dev = 0.16, n=277)

Table 2.1 Times taken for host use and offspring development (Values are mean number of days ± SEM)

Number of Foundresses	*G. legneri* used to pre-paralyse the host?	Host presentation-to-paralysis	Host presentation-to-oviposition	Paralysis-to-oviposition	Oviposition-to-hatching	Hatching-to-pupation	Pupation-to-eclosion	Total egg-to-adult development
1	Yes	1.70 ± 0.21	9.05 ± 0.58	7.25 ± 0.54	6.35 ± 0.80	7.07 ± 0.81	15.08 ± 0.84	26.92 ± 1.05
	No	5.50 ± 0.50	9.50 ± 0.51	4.27 ± 0.47	5.04 ± 0.59	5.10 ± 0.48	17.00 ± 0.58	25.90 ± 0.80
2	Yes	1.08 ± 0.12	8.60 ± 0.33	7.36 ± 0.31	4.54 ± 0.31	6.06 ± 0.42	13.69 ± 0.46	24.28 ± 0.59
	No	3.76 ± 0.24	7.24 ± 0.29	3.42 ± 0.27	4.67 ± 0.28	6.08 ± 0.37	14.00 ± 0.57	24.50 ± 0.75
3	Yes	0.66 ± 0.14	8.60 ± 0.72	6.00 ± 0.67	4.36 ± 0.53	6.00 ± 1.24	16.22 ± 1.01	26.11 ± 0.79
	No	2.98 ± 0.23	7.36 ± 0.39	4.44 ± 0.36	3.63 ± 0.27	5.09 ± 0.44	17.34 ± 0.51	26.16 ± 0.50
4	Yes	2.01 ± 0.27	7.00 ± 0.72	5.00 ± 0.67	4.60 ± 0.54	8.00 ± 0.72	15.70 ± 0.63	28.30 ± 0.30
	No	4.10 ± 0.40	7.36 ± 0.55	3.68 ± 0.52	4.90 ± 0.48	4.84 ± 0.56	17.00 ± 0.71	26.95 ± 0.71
5	No	4.14 ± 0.15	5.78 ± 0.41	1.68 ± 0.38	4.83 ± 0.28	6.09 ± 0.46	17.00 ± 0.81	27.59 ± 0.84
6	No	4.18 ± 0.12	5.89 ± 0.43	1.87 ± 0.40	4.16 ± 0.29	6.31 ± 0.28	16.00 ± 0.54	26.42 ± 0.45
Overall means		3.01 ± 0.24	7.64 ± 0.49	4.50 ± 0.46	4.71 ± 0.44	6.06 ± 0.58	15.90 ± 0.66	26.31 ± 0.68

Table 2.2 Offspring production according to the combinations of conditions trialled.

Foundress number	Pre-paralysis treatment	Total adult offspring	Females		Males		Per-foundress adult offspring
			Apterous	Alate	Apterous	Alate	
One	Yes	7.194 ± 1.744	5.839 ± 1.601	0.129 ± 0.084	0.032 ± 0.028	1.194 ± 0.282	**7.194 ± 0.854**
	No	4.409 ± 1.243	3.393 ± 1.141	0.001 ± 0.060	**0.067 ± 0.020**	0.590 ± 0.201	4.409 ± 0.609
Two	Yes	5.092 ± 1.041	4.529 ± 0.956	0.001 ± 0.050	0.001 ± 0.017	0.563 ± 0.168	2.546 ± 0.510
	No	5.569 ± 0.830	5.102 ± 0.762	0.015 ± 0.040	0.001 ± 0.013	0.453 ± 0.134	2.785 ± 0.406
Three	Yes	3.450 ± 1.535	3.025 ± 1.409	0.025 ± 0.074	0.025 ± 0.024	0.375 ± 0.248	1.150 ± 0.752
	No	7.423 ± 1.152	6.634 ± 1.058	**0.169 ± 0.055**	0.014 ± 0.018	0.606 ± 0.186	2.474 ± 0.564
Four	Yes	9.812 ± 2.428	8.000 ± 2.228	0.001 ± 0.116	0.001 ± 0.039	**1.813 ± 0.392**	2.453 ± 1.188
	No	6.816 ± 1.575	6.026 ± 1.446	0.158 ± 0.076	0.026 ± 0.025	0.605 ± 0.255	1.704 ± 0.771
Five	No	8.060 ± 1.373	7.500 ± 1.261	0.020 ± 0.066	0.001 ± 0.022	0.540 ± 0.222	1.612 ± 0.672
Six	No	**10.204 ± 1.321**	**9.593 ± 1.213**	0.001 ± 0.063	0.001 ± 0.021	0.611 ± 0.214	1.701 ± 0.647

Note: The numbers in bold fonts represent the maximum offspring production in each column by foundress number and pre-paralysis treatment combination.

2.4.3 Number and characteristics of offspring produced

2.4.3.1 Number of offspring

A mean of 6.34 (SEM = +0.40, -0.38) offspring were produced per host across all 585 replicates. Offspring production did not differ significantly across all foundress number treatments (log-linear ANOVA: $F_{5,579}$ = 1.95, P = 0.084, %Dev = 1.66, n = 585). However, among the treatments where pre-paralysis was also varied, there was a significant interaction between pre-paralysis and foundress number (pre-paralysis: $F_{1,474}$ = 3.84, P = 0.051, %Dev = 0.77; Interaction: $F_{3,476}$ = 3.58, P = 0.014, %Dev = 2.16, n = 481). Overall, a mean of 7.56 (SEM = +0.90, -0.80, n = 174) offspring were produced when hosts were pre-paralysed and 5.84 (SEM = +0.52, -0.48, n = 307) were produced when hosts were not pre-paralysed. The overall mean offspring production per host was at a maximum of 10.20 (SE ± 1.32) when there was six foundresses and the host was not pre-paralysed (Table 2.2) and (Figure 2.8 (a)).

When replicates with no offspring production were excluded, a mean of 15.79 (SEM = +0.63, -0.61) offspring were produced per host. The number of offspring produced was affected by foundress number (log-linear ANOVA: $F_{5,229}$ = 3.31, P = 0.007, %Dev = 6.74, n = 235) in a significantly curvilinear relationship (quadratic log-linear regression: $F_{1,234}$ = 4.07, P = 0.045, %Dev = 1.72, Figure 2.6). Among this restricted set of replicates, production was significantly higher when hosts were pre-paralysed (pre-paralysis: $F_{1,184}$ = 10.32, P = 0.002, %Dev = 5.17, n = 187). There was no significant Interaction between foundress number and the use of *G. legneri* ($F_{3,182}$ = 0.80, P = 0.494, %Dev = 1.20).

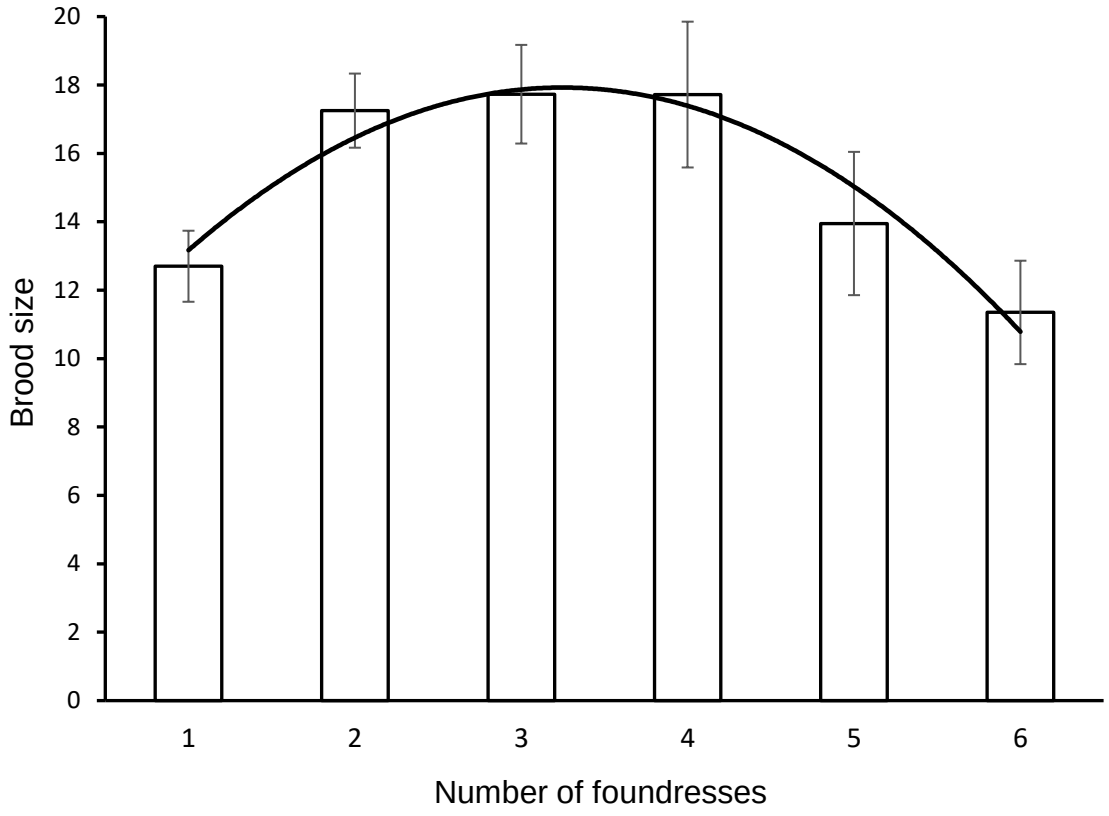


Figure 2.6 The number of adult offspring produced. The mean brood size was larger in intermediate sized foundress groups than in smaller or larger groups. Quadratic log-linear regression: brood size = EXP((-0.942 × foundress number^2) + (6.1177 × foundress number) + 7. 991)). Bars represent standard errors of means and are asymmetrical due to back transformation from the logarithm scale.

The number of offspring produced per foundress per host across all 585 replicates (i.e. including those with complete reproductive failure) had a mean of 2.78 (SEM = +0.18, -0.17) and was typically lower when foundress number was greater (log-linear analysis: $F_{5,579} = 8.67$, $P<0.001$, %Dev = 6.97), or when hosts were not pre-paralysed ($F_{1,477} = 4.97$, $P = 0.026$, %Dev = 0.99, n=481). There was no significant interaction effect between foundress number and pre-paralysis treatment ($F_{3,476} = 2.49$, $P = 0.060$, %Dev = 1.49). The overall mean offspring production per foundress per host was at a maximum of 7.194 (SE ± 0.854) when there was single foundress and the host was pre-paralysed (Table 1.1) and (Figure 2.8b).

Similar patterns were observed among the replicates in which there was some reproductive success: per-foundress offspring produced per host (mean = 6.91, SEM = +0.34, -0.33) declined with foundress number ($F_{5,229}$ = 37.29, P<0.001, %Dev = 44.88, n=235), or when hosts were not pre-paralysed ($F_{1,183}$ = 8.99, P = 0.003, %Dev = 3.45, n = 187, Figure 2.7). There was no interaction between foundress number and *G. legneri* use as pre-paralysis treatment ($F_{3,182}$ = 0.97, P = 0.407, %Dev = 1.12).

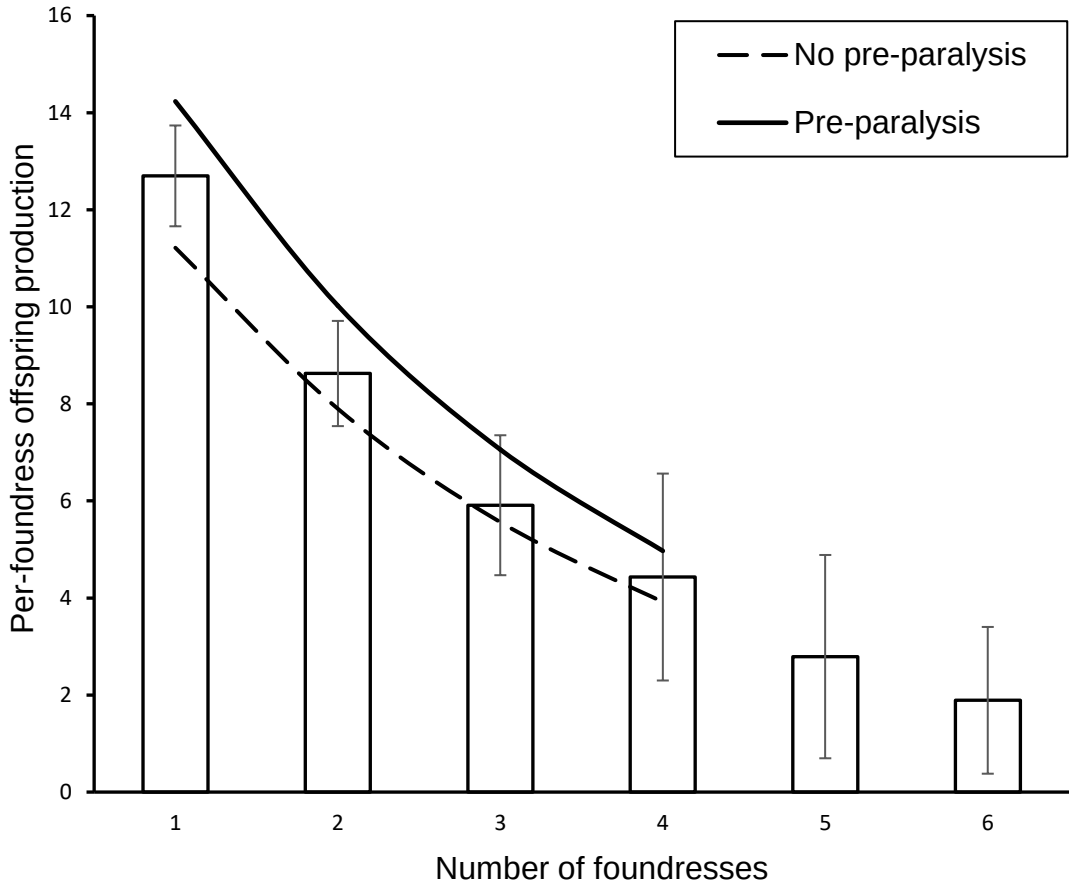


Figure 2.7 Effects of number of foundresses and pre-paralysis on per-foundress offspring production. The number of offspring produced per foundress per host (across replicates in which there was some reproductive success) was typically lower when foundress number was greater. Among foundress numbers 1-4, per-foundress production was also lower when hosts were not pre-paralysed by *G. legneri* females. Bars represent standard errors of means and are asymmetrical due to back transformation from the logarithm scale.

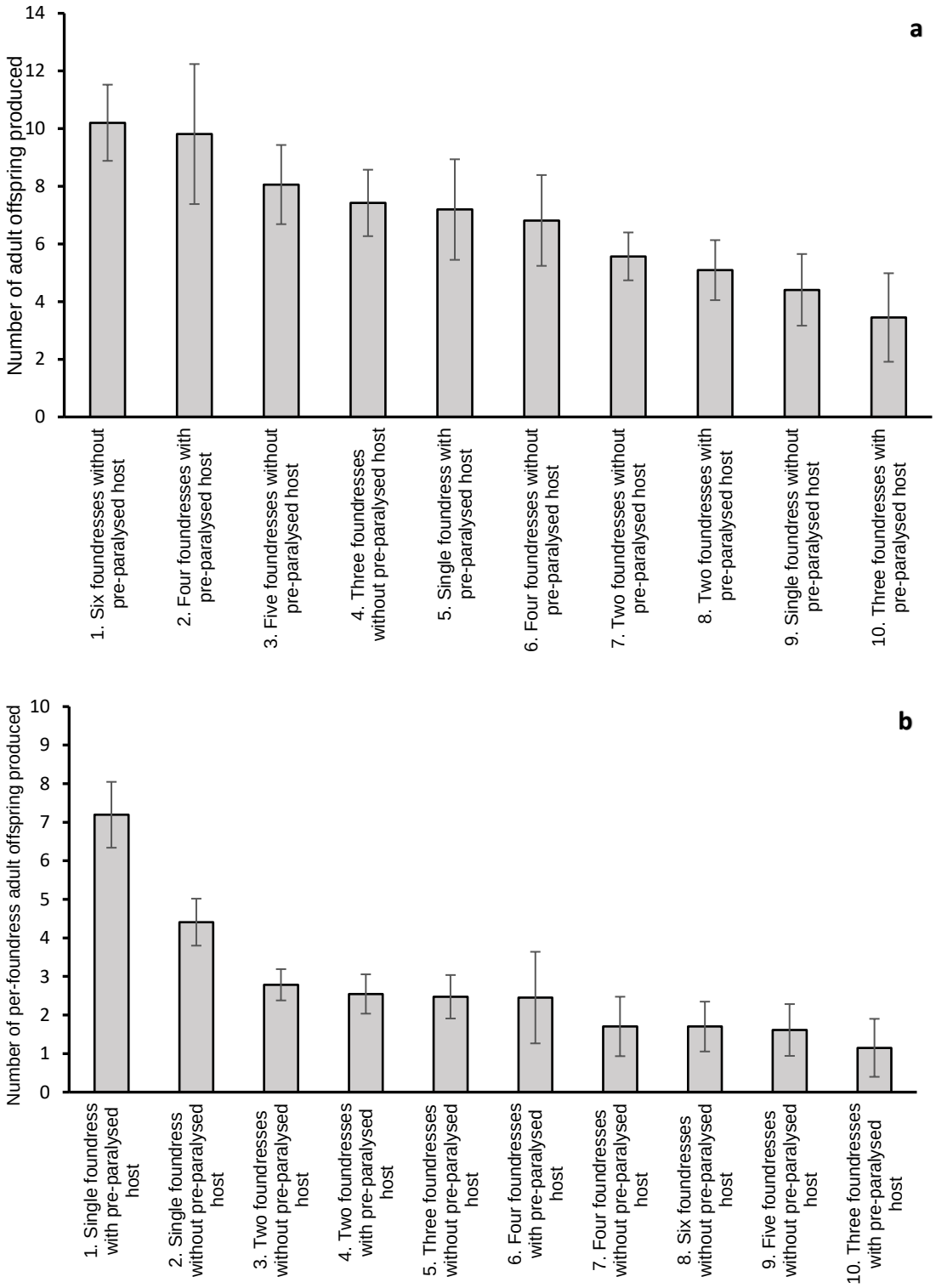


Figure 2.8 Offspring production according to the combinations of conditions trialled. Panel (a) shows the total number of adult offspring produced per host whereas panel (b) shows adult offspring produced per-foundress per host. Bars represent standard errors of means and are asymmetrical due to back transformation from the logarithm scale.

2.4.3.2 Sexual composition of broods

Three of the 235 that produced adult offspring contained male offspring only: a single foundress brood of 13 males, a 2-foundress brood of 15 males and a 3-foundress brood of just one male. As all-male broods typically suggest maternal virginity, and thus a lack of sex allocation control, these replicates were excluded from further sex ratio analysis (following Hardy and Cook (1995) and Kapranas *et al.* (2016)). Two broods with exceptionally high sex ratios were also excluded: a single-foundress replicate of 0.89 (a sex ratio three times higher than any other mixed sex brood, suggesting lack of sex ratio control) and a five foundress replicate of 0.90 (several foundresses may have been virgins). Data remained from 31 single foundress broods, 83 2-foundress broods, 40 3-foundress broods, 29 4-foundress broods, 21 5-foundress broods and 26 6-foundress broods.

Brood sex ratios (the proportion of offspring that were male, m/[m+f]) were very strongly female biased (mean = 0.0838, +SE = 0.0051, -SE = 0.0049). Sex ratios were not significantly influenced by pre-paralysis treatment ($F_{1,175} = 0.98$, P = 0.323, %Dev = 0.40, n = 182) or its interaction with foundress number ($F_{3,177} = 0.56$, P = 0.641, %Dev = 0.69). Sex ratios were typically more female biased in broods produced by larger numbers of foundresses (logistic analysis: $F_{5,226} = 12.44$, P<0.001, %Dev = 21.58, Figure 2.9). Sex ratios were also affected by brood size and by its interaction with foundress number (brood size: $F_{1,221} = 7.77$, P = 0.006, %Dev = 2.42; Interaction: $F_{5,225} = 4.92$, P<0.001, %Dev = 7.64), such that sex ratios declined with an increase of brood size among broods produced by 1, 2, 3 or 5 females and increased among broods produced by 4 or 6 foundresses.

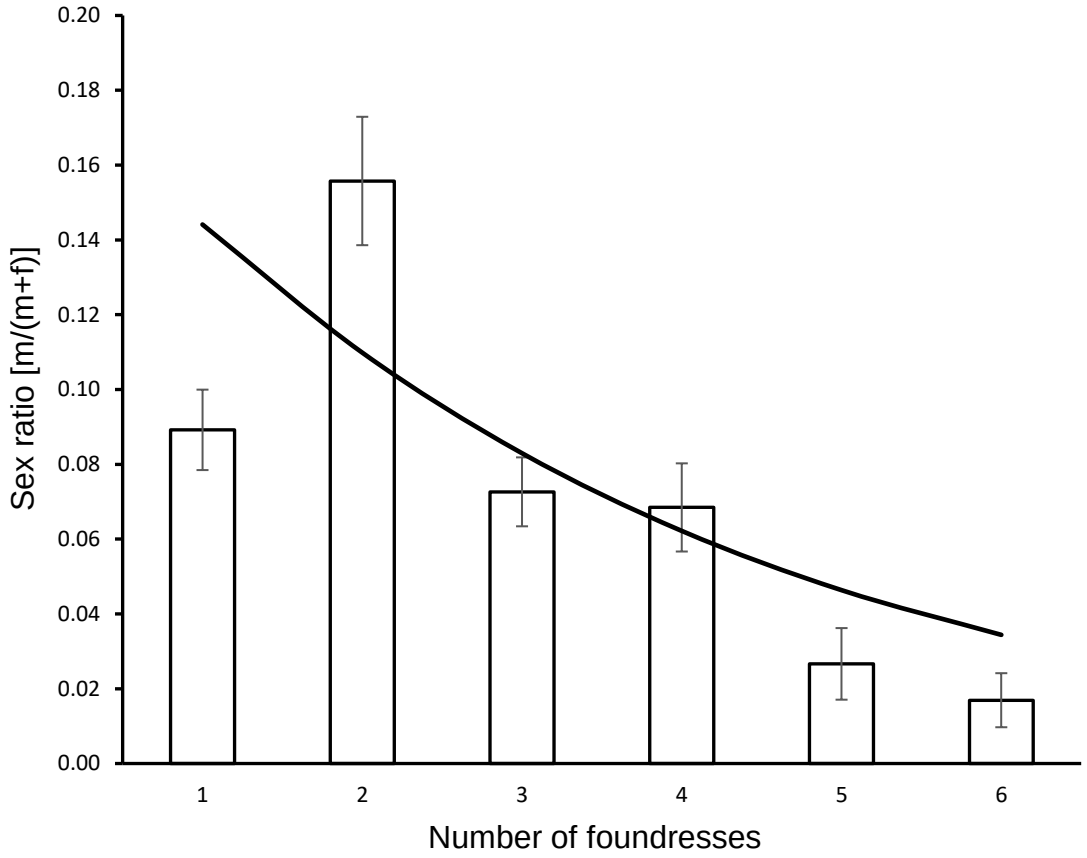


Figure 2.9 Sex ratios in relation to foundress number. Broods produced by larger foundress groups were typically more female biased. Logistic regression: sex ratio = 1/(1+(1/EXP((-0.3106 x foundress number)-1.471))). Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale.

The number of males produced per brood decreased significantly as foundress number increased (log-linear analysis: $F_{5,223}$ = 9.89, P<0.001, %Dev = 15.16) and increased with an increase of brood size ($F_{1,219}$ = 49.78, P<0.001, %Dev = 15.26, Figure 2.10); there was a significant interaction between foundress number and brood size ($F_{5,223}$ = 3.85, P = 0.002, %Dev = 5.90). The mean per-foundress number of males in a brood produced decreased as foundress number increased ($F_{5,223}$ = 18.54, P<0.001, %Dev = 18.43) and increased with brood size ($F_{1,219}$ = 4.88, P<0.001, %Dev = 8.52), with generally greater increases when foundress numbers were greater (Foundress number × brood size interaction: $F_{5,223}$ = 3.32, P = 0.007, %Dev = 3.30).

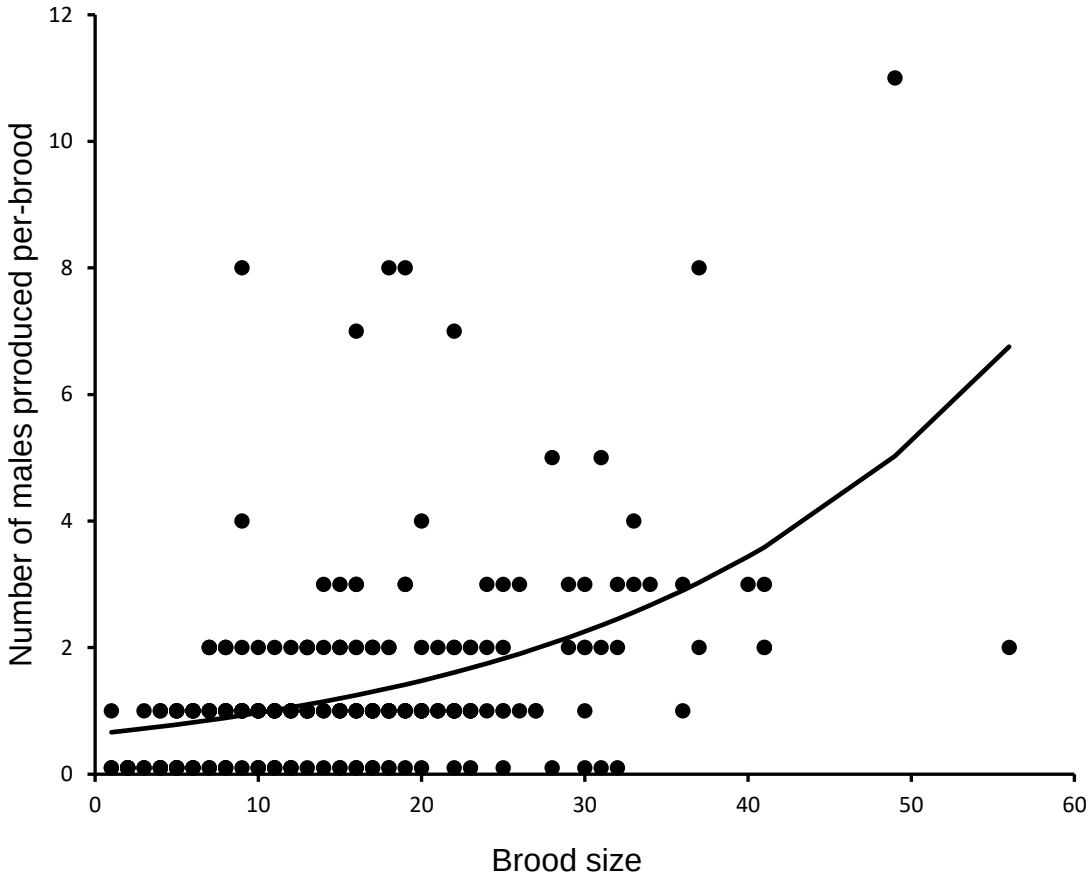


Figure 2.10 Effect of brood size on per-brood male production: The number of males produced per brood increased with an increase of brood size.

The proportion of broods that were all females ('virgin broods', (Kapranas *et al.*, 2016)) generally increased with increasing foundress number (logistic analysis: $G_5 = 2.67$, $P = 0.020$, %Dev = 5.06, Figure 2.11 (a)) and also decreased with an increase of brood size ($G_1 = 22.21$, $P<0.001$, %Dev = 8.41, Figure 2.11 (b)); there was a significant interaction between foundresses numbers and brood size ($G_5 = 3.21$, $P = 0.007$, %Dev = 6.07). Virginity was not significantly affected by the use of *G. legneri* to pre-paralyse the host ($G_1 = 0.04$, $P = 0.845$, %Dev = 0.02, n = 182), nor by its interaction with foundress number ($G_3 = 2.22$, $P = 0.083$, %Dev = 2.91).

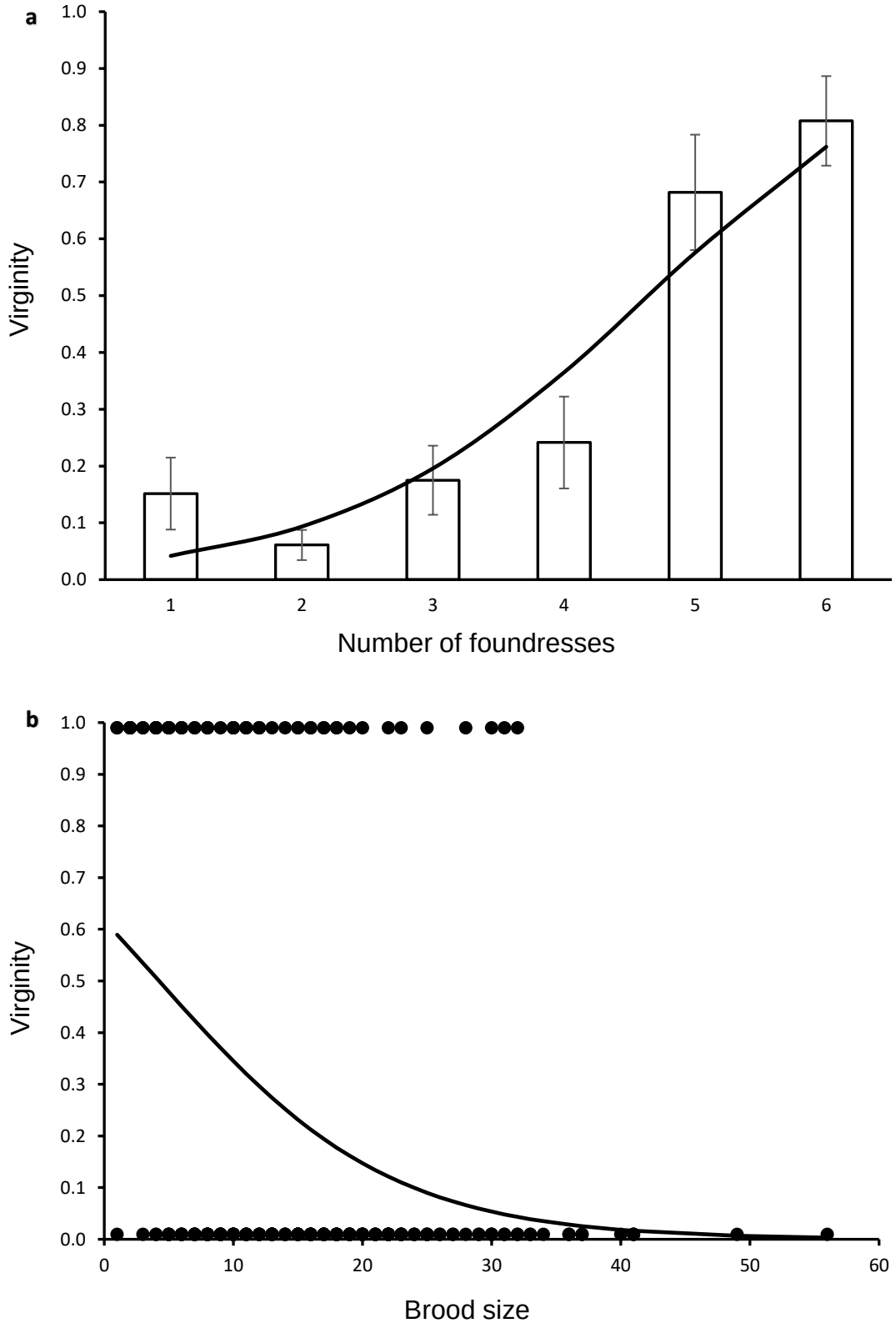


Figure 2.11 The prevalence of virgin broods: Panel (a): The proportion of broods that were all females increased with an increase in foundress number. Panel (b) virginity decreased with an increase of brood size. Lines were fitted by logistic regression.

2.4.3.3 Wing dimorphism

Wing dimorphism occurred in both male and female offspring (Table 2.3). Of the 230 broods containing at least one adult female, there were no instances of exclusively alate (winged) females. In 216 broods, all females were apterous (wingless) while 14 broods contained a mixture of apterous (80.75%) and alate (19.25%) females. Adult males were produced in 170 broods: 164 contained exclusively alate males and six contained a mix of apterous (25.81%) and alate (74.19%) males. Male aptery only occurred when all or most females in a brood were also apterous.

Table 2.3 Wing dimorphism among adult offspring.

Sex	Total offspring	Alate	Apterous
Male	306	298 (97.39%)	8 (2.61%)
Female	3346	46 (1.37%)	3300 (98.63%)

The proportion of females that were alate differed among foundress number treatments ($F_{5,230}$ = 2.41, P = 0.038, %Dev = 4.73, n = 232), such that adult female offspring produced by single foundress groups contained significantly more alate members. This proportion declined as total brood size increased ($F_{1,226}$ = 114.78, P<0.001, %Dev = 5.81) and also as the number of females in a brood increased ($F_{1,231}$ = 10.86, P = 0.001, %Dev = 4.34). There was no significant interaction between foundress number and brood size ($F_{5,225}$ = 2.06, P = 0.072, %Dev = 4.05) or female offspring number ($F_{5,225}$ = 1.87, P = 0.101, %Dev = 3.73).

The proportion of males that were alate was generally high but differed significantly according to foundress number, being lowest when broods were produced by three-foundresses ($F_{5,170}$ = 7.79, P < 0.001, %Dev = 16.60, n = 172). This proportion also declined with an increase in brood size ($F_{1,161}$ = 17.47,

P<0.001, %Dev = 7.44, Figure 2.12) but there was no significant interaction between foundress number and brood size ($F_{5,165}$ = 1.19, P = 0.317, %Dev = 2.53). Alate males were also less common when there were more males in a brood ($F_{1,166}$ = 14.71, P<0.001, %Dev = 5.37) and there was a significant interaction between foundress number and the number of males in a given brood ($F_{5,165}$ = 7.90, P<0.001, %Dev = 14.41).

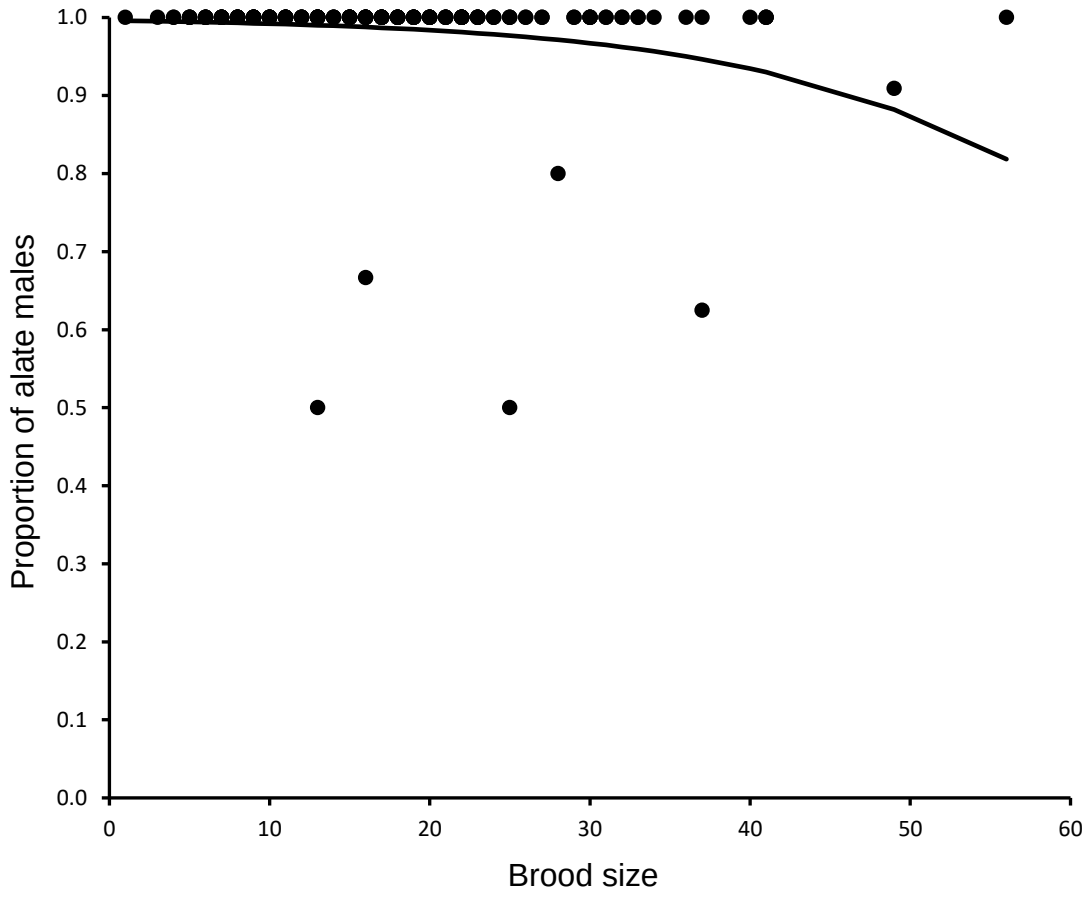


Figure 2.12 The relationship between the proportion of winged males and brood size. Line fitted by logistic regression.

2.5 Discussion

We have shown that larvae of the rice moth, *Corcyra cephalonica,* can be utilized as factitious hosts for *Sclerodermus brevicornis*. This is in contrast to a previous attempt to rear *S. brevicornis* on lepidopteran larvae (*Galleria mellonella,* D.L. unpublished data) and it is noteworthy that *Sclerodermus* species normally develop on coleopterans (Gordh & Móczár, 1990). Around 75% of hosts were attacked and paralysed (similar to estimates obtained from *S. brevicornis* attack on equivalently sized long-horn beetle larvae (Abdi *et al.*, Chapter 4)), and around 60% of all hosts presented had eggs laid onto them (compared to 73% when provided with beetle larvae, (Abdi *et al.*, Chapter 4)). Oviposition was disrupted by the pre-paralysis of hosts using *G. legneri* (possibly due to changes in host odour). However, the probabilities of paralysis and oviposition increased with foundress number, as did the probability of a host yielding at least one parasitoid offspring (as found by Tang *et al.* (2014). Our finding that around half of the presented hosts yielded offspring is consistent with data from *S. brevicornis* reared on beetle larvae (*Psacothea hilaris hilaris*, Lupi *et al.* (2017)) and similar to estimates from other *Sclerodermus* species (Tang *et al.*, 2014).

2.5.1 Timing of offspring production

Lupi *et al.* (2017) reported that the developmental time of *S. brevicornis* on beetle larvae is approximately one month. Our data indicate that overall development times on *C. cephalonica* are similar. The time required to successfully parasitize a host decreased with an increase in foundress numbers, as also found by Tang *et al.* (2014). We also found that *S. brevicornis* foundresses took more time to oviposit on hosts (and for their eggs to eclose)

when hosts were pre-paralysed by *G. legneri* females: we suggest that this this may be related to altered odours when hosts are paralysed. In terms of the timing of parasitoid production, there appear be no major negative effects associated with using *C. cephalonica* as a factitious host.

2.5.2 Numerical offspring production

Across all hosts provided, the numerical production of adult offspring (~6.5 per host) was not affected by the number of foundresses present but, if hosts yielding no offspring were excluded, production was greatest at intermediate numbers of foundresses (peaking at ~18 per host). Our data suggest that to maximise *S. brevicornis* offspring production per foundress per host, hosts should be pre-paralysed and should be presented to a single foundress. Under these conditions around 7.2 offspring per-foundress per host can be recruited into the next generation. This is represented by the parameter *c* in classical discrete-time host-population models: *c* is typically negatively associated with equilibrium host density (Hassell, 2000; Heimpel, 2000), and thus better biocontrol performance. Previous studies on *S. harmandi* (Tang *et al.*, 2014) and *S. pupariae* (Wei *et al.*, 2017) have found that the probability of producing adult offspring from a given host is enhanced when foundress groups were larger than 1. However, the sizes of hosts considered by those studies were substantial (ranging up to 0.64g and 0.24mg, respectively) while the maximum size of *C. cephalonica* hosts studied here was 0.040g: compared to cerambycid larvae, *C. cephalonica* larvae are small hosts.

2.5.3 Offspring properties

Female biased sex ratios are generally a desirable property among parasitoids that are biological control agents because it is females, not males, that attack the target pest (Ode & Hardy, 2008; Li *et al.*, 2010; Boulton *et al.*, 2015). Most adult offspring produced by *S. brevicornis* were females (92%) which accords with prior reports in this species (Lupi *et al.*, 2017) and congeners (Kühne & Becker, 1974; Li & Sun, 2011; Liu *et al.*, 2011; Hu *et al.*, 2012; Tang *et al.*, 2014; Wei *et al.*, 2014; Gao *et al.*, 2016a; Kapranas *et al.*, 2016). It is thought that sex ratio bias in *Sclerodermus* is due to local resource enhancement (LRE), a mutually beneficial female–female interaction that increases the reproductive value of daughters, rather than local mate competition (LMC) which is the more commonly applied explanation of female-biased sex ratios among parasitoids (Tang *et al.*, 2014). Here, we found a slight increase in bias as foundress number increased, while other studies have found the opposite (Tang *et al.*, 2014; Wang *et al.*, 2016). This strongly female-biased sex ratio in *S. brevicornis*, whether caused by LRE or LMC, is considered an asset for biological control programs (Ode & Hardy, 2008). In addition, cooperation among *S. brevicornis* co-foundresses as a result of local resource enhancement (LRE) is likely to increase the range of successfully attackable host sizes (Tang *et al.*, 2014; Kapranas *et al.*, 2016), and in consequence, is likely to improve the biocontrol potential of the parasitoid.

Associated with the highly female-biased sex ratio, some broods contained no adult male offspring at all (virgin broods), which is generally undesirable in rearing programmes because, as a consequence of arrhenotocky, unmated females are constrained to produce only male offspring in the following

generation. As found in prior studies on *S. brevicornis* (Lupi *et al.*, 2017) and in congeners (Kapranas *et al.*, 2016), the prevalence of all-male broods declined strongly with brood size and is likely to be associated with mortality of offspring within developing broods. However, we found that virginity was more prevalent when broods were produced by more foundresses, while Kapranas *et al.* (2016) found the opposite effect of foundress number for *S. harmandi*.

Wing dimorphism was present among the adult offspring of both sexes although the vast majority (*ca.* 99%) of females produced were apterous whereas alate morphs dominated among the males (*ca.* 97%); as observed throughout the genus (Kühne & Becker, 1974; Evans, 1978; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017; Wei *et al.*, 2017). Apterous males were most common when brood sizes were larger and we found that the production of alate females was associated with single-foundress broods. This is consistent with *S. pupariae* in which the proportion of winged female progeny decreases as foundress number increases (Wang *et al.*, 2016). Often wingless forms are confined to a given emergence site whereas winged morphs have greater potential to colonize hosts at greater distances, which is likely to influence the efficacy of mass release programmes.

2.6 Conclusion

Species of *Sclerodermus* are considered among the most effective bethylids used in biocontrol programmes (Yang, 2004; Yang *et al.*, 2014; Jiang *et al.*, 2015). *Sclerodermus brevicornis* is native to Europe and has potential to counter invasion by Asian wood-boring long-horned beetles, especially if mass-reared for subsequent inundative field release (Lupi *et al.*, 2017). However, *S. brevicornis* rearing systems have depended on using larvae of long-horned

beetles that require considerable effort and time to maintain in laboratory cultures release (Lupi *et al.*, 2017). *Corcyra cephalonica* is simple and inexpensive to mass-rear and is a commonly used factitious host for a range of parasitoid species, but has not previously been used for *Sclerodermus*. We conclude that, despite being a relatively small lepidopteran, rather than a large coleopteran, *C. cephalonica* is suitable for the maintenance of laboratory cultures of *S. brevicornis*. Further, the procedure of pre-paralysis is not required; although it can enhance production of offspring per host, it will be labour-intensive in mass-culture facilities and taking human labour into account will likely lead to its exclusion from optimal rearing protocols. While not all *C. cephalonica* hosts presented to *S. brevicornis* females ultimately yield the next generation of parasitoid offspring, it should be possible to obtain outputs of up to around 10 parasitoids per host, the vast majority of which will be mated females. Figure 2.8 summarizes the relative outputs for the combinations of culturing conditions trialled. The development of practicable and efficient mass-rearing systems brings the active deployment of European *Sclerodermus* against invasive long-horned beetles closer to implementation.

Part two: Kinship among quasisocial parasitoids

Chapter 3: Co-foundresses relatedness in a quasi-social parasitoid mediates suppression of dangerous hosts

3.1 Abstract

Explanations for the highest levels of sociality observed across animal taxa typically invoke the concept of inclusive fitness. *Sclerodermus*, a genus of parasitoid hymenopterans, are quasi-social, exhibiting cooperative brood care without generational overlap or apparent division of labour. Foundress females successfully co-exploit hosts that are too large to suppress when acting alone (large hosts often kill attacking females) and the direct fitness benefits of collective action may suffice to explain their cooperation, irrespective of kinship. However, cooperation in animal societies is seldom free of conflicts of interest between social partners, especially when their relatedness, and thus their degree of shared evolutionary interests, is low. Here we screen for effects of varying *Sclerodermus* co-foundress relatedness and number on aspects of cooperative reproduction, between host presentation and offspring maturity. We find that the time taken to paralyse standard-sized hosts is shorter when co-foundress relatedness is higher. This suggests that, while females must access a paralysed host in order to reproduce, individuals are reluctant to take the risk of host attack unless the benefits will be shared with their kin. We use Hamilton's rule and data from prior studies that experimentally varied host size to explore how the greater risks and greater benefits of attacking larger hosts and co-foundress relatedness could combine to determine the size of hosts that a female is selected to attack as a public good. From this, we predict that host size and relatedness will interact to affect the timing of host paralysis; we test this prediction in the accompanying study.

3.2 Introduction

Understanding the evolution and maintenance of complex social systems has been a longstanding and major focus of behavioural and life-history research across a diverse array of animal taxa (Wilson, 1971; Choe & Crespi, 1997; Costa, 2006; Bourke, 2011; Scheiber *et al.*, 2017; Shen *et al.*, 2017; Boomsma & Gawne, 2018; Tanaka *et al.*, 2018). A substantial portion of this sociobiological research has been carried out on eusocial hymenopterans, such as ants, honeybees, bumble bees and vespid wasps, all of which exhibit brood care, reproductive division of labour and overlapping generations (Bourke & Franks, 1995; Crozier & Pamilo, 1996; Boomsma & Gawne, 2018). Conversely, sociality in the parasitoid Hymenoptera ('parasitic wasps') is relatively little explored, chiefly because most species are socially solitary, lacking parental care (Wilson, 1971; Godfray, 1994). However, bethylids in the genus *Sclerodermus* exhibit cooperative brood care, apparently without division of labour, classing them as quasi-social (Li & Sun, 2011; Liu *et al.*, 2011; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017) and are the focus of the current study.

Sclerodermus females search actively for host larvae, which they paralyse by stinging (Xu *et al.*, 2002; Shao-chuan, 2007; Yao & Yang, 2008; Liu *et al.*, 2011). A host may be attacked by several females (Bridwell, 1920; Kühne & Becker, 1974; Tang *et al.*, 2014; Lupi *et al.*, 2017). The females (termed foundresses) then oviposit onto the host's integument and subsequently tend, cooperatively, their collective broods of offspring as these feed on the host, until and beyond their pupation (Hu *et al.*, 2012; Wu *et al.*, 2014). Quasi-social behaviour in *Sclerodermus* has been explained in terms of the direct fitness benefits to

cooperation accrued to individuals within groups of females that attack hosts: females acting alone may not be able to suppress and successfully reproduce on a host, especially if the host is large and dangerous to attack (Kühne & Becker, 1974; Liu *et al.*, 2011; Tang *et al.*, 2014; Wei *et al.*, 2014), a 'collective action benefit' Shen *et al.* (2017). This explanation does not, however, preclude that kin selection effects, based on the genetic relatedness between interacting individuals (inclusive fitness Hamilton (1964a)), may also influence the occurrence and success of cooperative reproduction and thus the evolution of quasi-sociality. Despite ongoing controversy, inclusive fitness is often invoked to understand the evolution of a range of social behaviours, including conflict resolution, cooperation and eusociality (Abbot *et al.*, 2011; Nowak & Allen, 2015; Marshall, 2016; Birch, 2017; Shen *et al.*, 2017). Among the parasitoid Hymenoptera, it has been shown that aggression during resource competition, whether between larvae or between adult females, can be reduced when competitors are (actual or perceived) close relatives (Giron *et al.*, 2004; Giron & Strand, 2004; Lizé *et al.*, 2012; Dunn *et al.*, 2014; Mathiron *et al.*, 2019). Hence, we expected that *Sclerodermus* co-foundresses may adjust their cooperative behaviour according to intra-group relatedness.

Our overarching aim is to explore the importance of kinship effects among parasitoid hymenopterans that have evolved quasi-sociality. Using *Sclerodermus brevicornis* (Kieffer), we evaluate whether relatedness between co-foundresses influences their reproductive performance, in terms of supressing hosts, reproducing on supressed hosts, foundress mortality, and the characteristics of any offspring subsequently produced. We screen for effects of relatedness at numerous life-history stages because conflicts of interest

between co-foundresses may be manifest at any, all or none of these. For instance, foundresses may act as non-attacking free-loaders (Rankin *et al.*, 2007) exploiting the host attack efforts of others, they may have differing interests in terms of their relative contributions to the production and care of offspring groups post-host paralysis, and differing sex ratio optima (Tang *et al.*, 2014). As our investigation is in consequence relatively 'untargeted' (*sensu* Snart *et al.* (2015)) we also test our key conclusions in a more targeted accompanying study (Abdi *et al.*, Chapter 4).

The advantages to an individual foundresses of sharing a host of a given size may depend on the number of co-foundresses with which it is shared (Tang *et al.*, 2014); we thus explore relatedness effects when there are either two or four foundresses, using standardized hosts of relatively small size. While intra-group genetic relatedness is manipulated in our core experiment, we further assess behaviours when co-foundresses have been reared on the same or on different host species and also according to whether foundresses are winged or wingless. Host-of-origin effects may be expected if foundresses use cuticular hydrocarbon cues to assess relatedness (Supplementary Information, Appendix 1) and wing dimorphism effects may be expected if alate females tend to disperse further and co-found broods with non-relatives while apterous females tend to co-found with prior brood-mates (Supplementary Information, Appendix 2).

We find evidence for greater production of offspring per foundress when foundresses are prior brood-mates than when they are non-siblings and also that the time taken to paralyse hosts is shorter when inter-foundress relatedness is higher. We use a simple public goods model (Hamilton's rule) to

illustrate how co-foundress relatedness and the greater risks and benefits of attacking larger hosts could combine to determine the size of host that a female is selected to attack to provide resources shared with co-foundresses. From this, we predict that host size and relatedness will interact to affect the timing of host paralysis. The accompanying study (Abdi *et al.*, Chapter 4) tests this prediction empirically and supports our conclusions.

3.3 Materials and Methods

3.3.1 Insects

Sclerodermus brevicornis (Kieffer) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid wasp. Adult females are typically wingless (apterous) but winged (alate) morphs also occur (Lupi *et al.*, 2017). Males are usually alate but apterous morphs are occasionally produced. *Sclerodermus brevicornis* is a parasitoid of European longhorn beetle larvae (Coleoptera: Cerambycidae) (Kieffer, 1914; Lupi *et al.*, 2017) that can be reared on several non-native, invasive cerambycids, including the Yellow Longhorn Beetle, *Psacothea hilaris hilaris* (Pascoe) (Lupi *et al.*, 2017) and also on larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (Abdi *et al.*, Chapter 2) these two species were used as hosts in this study. Stocks of *P. h. hilaris* were maintained according to Lupi *et al.* (2015a) and *C. cephalonica* were maintained in the same climate chamber, on the standard diet described by Limonta *et al.* (2009). Stocks of *S. brevicornis* were reared on late instar larvae of *P. h. hilaris* and *C. cephalonica*.

All cultures and experiments were maintained in a climate chamber at 25±1°C, and 16H: 8H L:D photoperiod and 60±5% relative humidity. To synchronize parasitoid and host availability, newly emerged *S. brevicornis* adults were

stored in a refrigerator at 5±0.5°C for a maximum of 10 days prior to use in experiments: we have found that such storage does not affect their subsequent performance (D.L. & C.J. pers. obs.).

3.3.2 Experiment

The experiment followed a core 2×2 factorial design, varying foundress number and relatedness, but with additional consideration of wing dimorphism and of the host species on which foundresses had been reared (Table 3.1). There were 20 replicates of each combination of foundress number, relatedness, wing dimorphism, and host species, giving 160 replicates in total (Table 3.1).

In each replicate, a *C. cephalonica* host was placed into a 10 ml glass vial (10cm long × 1.3cm diameter). Hosts were selected to be of near uniform size (mean weight=0.0363mg, range=0.0262 to 0.0534 mg, SD±0.0051: note that the mean head capsule width of *C. cephalonica* hosts in this size range is *ca*. 1.02mm, corresponding to the 'small' host size class of *P. hilaris* (Lupi *et al.*, 2017)). Then either two or four adult female *S. brevicornis* were added into each vial. In some replicates (Table 3.1, rows A & C) co-foundresses had developed in the same brood: as these broods had been produced by several (in most cases sibling) mothers, brood-mates were a mix of siblings and (typically closely related) non-siblings that are likely to recognize each other as familiar (Lizé *et al.*, 2012). In all other replicates, co-foundresses had developed in separate broods and were non-siblings (Table 3.1). In some non-sibling replicates, foundresses had developed on (separate) *C. cephalonica* hosts and in other non-sibling replicates half the foundresses present (one or two) had developed on a *C. cephalonica* host and other half had developed on *P. hilaris* (Table 3.1). In most

replicates all foundresses were apterous but in one set of non-sibling replicates (Table 3.1, row G) the foundresses were alate and had developed on *P. hilaris*. The vials were closed with a gauze and cotton wool, left undisturbed for four days (observing the wasps within this period has previously been found to disrupt their reproductive behaviours, D.L. & C.J. pers. obs.). We then observed each replicate once per day until any offspring matured to the pupal stage (typically 28 days after foundresses were presented with hosts). At each observation, we recorded whether the host had been paralyzed, the number of eggs laid on the host, whether any foundresses had died (until the 12th day in all core replicates, 10th day in host species-of-origin replicates and until 8th day in wing dimorphism replicates) and the developmental stage of any juvenile offspring after eclosion from eggs. We further monitored broods for up to 42 days in total, noting the timing of adult emergence. The numbers, sexes, wing morphologies and size (thorax length) of adult offspring were recorded on their emergence from the pupal cocoons.

3.3.3 Statistical analyses

The explanatory variables foundress number and relatedness (main effects) were fitted as factors, and their interaction was included in initial statistical models. As foundress number in a replicate could change during the experiment, due to foundress mortality, we explored the effects of both initial and current foundress number in some analyses. Effects of host origin and wing morphology, fitted as factors, are reported in the appendices of this chapter. The response variables were the parameters recorded during the daily observations and at the end of the experiment. All statistical tests were two

sided. All analyses were carried out using the statistical software package *GenStat* (version 17, VSN International, Hemel Hempstead, UK).

Binary data on host acceptance and offspring production were explored using logistic analyses (GLM) assuming binomially distributed errors and the reported test statistic is change in deviance, *G*, which is approximately χ^2 distributed (Crawley, 1993). Other logistic analyses of proportional data with denominators larger than unity (e.g. developmental mortality, offspring sex ratio) assumed quasi-binomial error distributions and the reported test statistic is the *F*-ratio (Crawley, 1993; Wilson & Hardy, 2002). Logistic analyses comparing foundress mortality before and after host paralysis employed mixed models (GLMM and Wald χ^2 statistics), with replicate fitted as a random factor. All logistic analyses adopted a logit-link function.

Integer data (e.g. clutch size, number of offspring produced) were explored using log-linear analyses assuming quasi-Poisson error distributions and a log-link function (Crawley, 1993). Data derived from integers (e.g. offspring production per foundress) that were likely to be approximately Poisson-distributed were also analysed using log-linear models (Faraway, 2006; Tang *et al.*, 2014). Following logistic and log-linear analyses, we give the percentage deviance explained (%Dev) as a descriptor analogous to r^2.

Time-to-event data on host paralysis, oviposition, adult offspring emergence and foundress mortality were analysed using parametric cohort survival analyses with censoring (Aitkin *et al.*, 1989; Crawley, 1993). We first compared exponential models (constant hazard function) against Weibull models (time dependent hazard function): in all cases, the Weibull models provided a significantly better description of the data. The influences of the candidate

explanatory factors (see above) were then explored by their inclusion into the Weibull models (Aitkin *et al.*, 1989; Crawley, 1993). In cohort-survival analyses of factors potentially influencing time-to-foundress death, the longevity of each foundress was treated as if it were independent of others in the replicate. Such pseudo-replication is likely to generate Type 1 errors (false significance) but as none of our analyses detected significant effects we adopt these interpretations.

Table 3.1 Experimental design and rationale

Rows A, B, C and D constitute the core 2×2 factorial experiment, designed to evaluate effects of foundress number, foundress relatedness and their interaction, with host species-of-origin and foundress wing morphology held constant. Results of analyses focusing on these replicates are reported in the main body of this chapter and summarized in Table 3.2.

Replicates in further rows were included to provide additional tests. Data from rows B, E and F allow evaluation of effects of host-species-of-origin (fitted as a factor with 3 levels) for the 2-foundress case, as cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and may be used in kinship assessment. Comparison of data from rows B and F served also to check for intrinsic effects of developing on a beetle larva rather than on a moth larva: given that *Sclerodermus* species are not reported to attack lepidopteran hosts in nature, such differences might imply that host-of-origin cannot be regarded as a simple proxy for relatedness. Data from rows B, D, E, and H provide a 2×2 analysis of foundress number and host-of-origin effects within non-sibling groups. Results of analyses focusing on host-of-origin effects are reported in the Supplementary Information, Appendix 1.

Comparison of data from rows F and G provide tests of effects of foundress wing morphology on the reproductive strategies for the 2-foundress case. In nature, alate females may be expected to disperse as individuals and thus to typically reproduce with unrelated co-foundresses, while apterous females may tend to disperse together with their brood-mates. Alate female *Sclerodermus brevicornis* are relatively rare; a shortage of biological material precluded us from including further replicates with winged foundresses. Results of analyses of the effects of wing dimorphism are reported in the Supplementary Information, Appendix 2.

In all replicates, the host provided to the co-foundresses was a *C. cephalonica* larva of standardized size.

Row	Number of foundresses	Foundress relatedness	Host of foundress origin	Foundress morphology	Replicates (total=160)
Core replicates					
A	Two	Brood-mates	*C. cephalonica*	Apterous	20
B	Two	Non-siblings	*C. cephalonica*	Apterous	20
C	Four	Brood-mates	*C. cephalonica*	Apterous	20
D	Four	Non-siblings	*C. cephalonica*	Apterous	20
Additional replicates (Appendices 1 & 2)					
E	Two	Non-siblings	*C. cephalonica* + *P. hilaris*	Apterous	20
F	Two	Non-siblings	*P. hilaris*	Apterous	20
G	Two	Non-siblings	*P. hilaris*	Alate	20
H	Four	Non-siblings	*C. cephalonica* + *P. hilaris*	Apterous	20

3.4 Results

Results of analyses reported in this section are summarized in Table 3.2.

3.4.1 Foundress mortality

Around a quarter (26.67%, 64/240) of foundresses died by the twelfth day after presentation with hosts. Mortality across this 12 day period was not affected by foundress relatedness (logistic ANOVA: G_1=1.17, P=0.282, %Dev=1.51), foundress number (G_1=0.37, P=0.543, %Dev=0.48) or their interaction (G_1=0.30, P=0.584, %Dev=0.39). Within this 12 day period, the timing of foundress mortality (Figure 3.1) was not significantly affected by foundress relatedness, foundress number or their interaction (cohort survival analysis: Relatedness: G_1=2.5, P=0.114, %Dev=0.83; Number: G_1=0.8, P=0.371, %Dev=0.31; Interaction: G_1=0.7, P=0.403, %Dev=0.24). In these analyses, foundresses that did not die by the twelfth day were treated as censors.

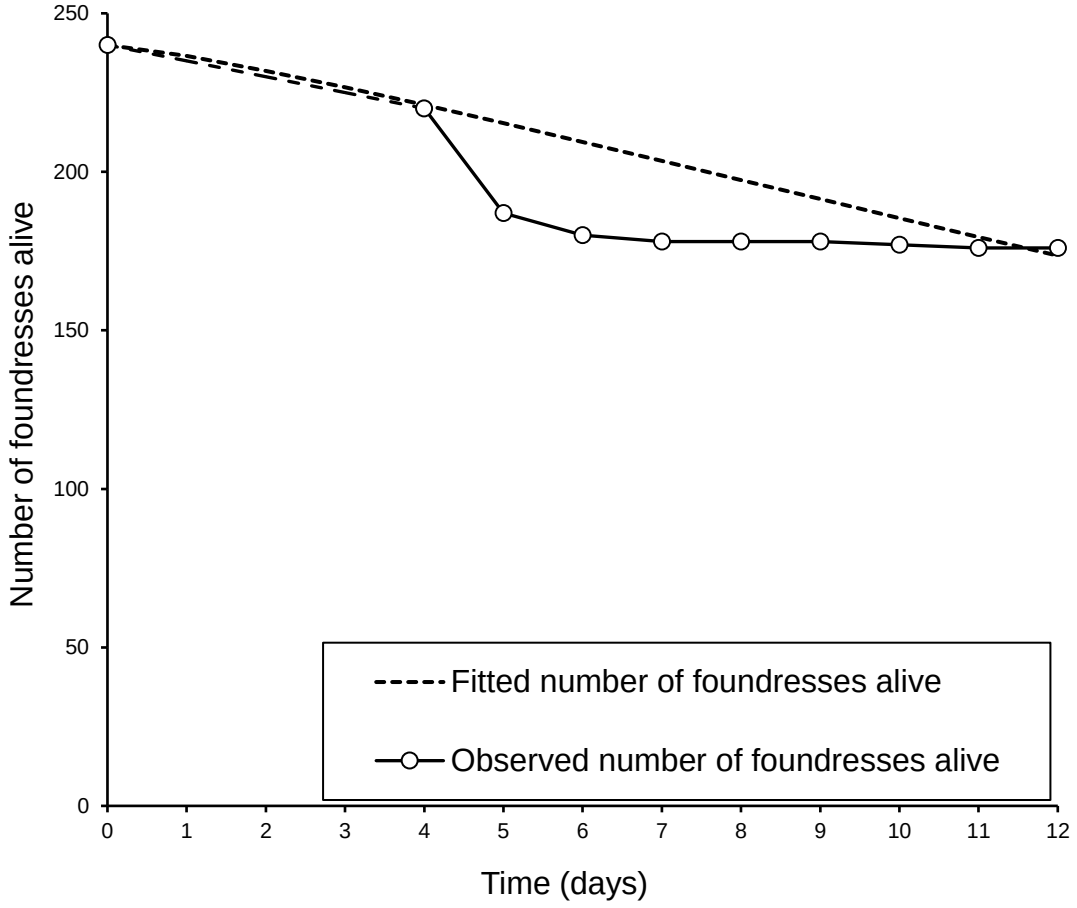


Figure 3.1 Cohort survival of *Sclerodermus* females. The fitted line (short dashes) was estimated using Weibull analysis for time-dependent hazard rates (rate parameter=0.01451, shape parameter=1.25) with foundresses that survived until day 12 treated as censors. To avoid disruption of the females' behaviours, no observations were taken on days 1, 2 and 3, indicated by the longer-dashed line, and the statistical model was fitted assuming no foundresses died during this period.

We re-examined these data by separately assessing the proportion and timing of foundress mortality during the periods before host paralysis and after host paralysis. For all replicates combined, the probability of at least one of the group of foundresses dying was significantly higher during the pre-paralysis period than post-paralysis (GLMM: Wald χ^2=20.08, *d.f.*=1, P<0.001). Similarly across all replicates, the proportion of foundress that died pre-paralysis was significantly higher than the proportion that died post-paralysis (GLMM: Wald χ^2=19.22, *d.f.*=1, P<0.001, Figure 3.2). Analysing the data separately for each

combination of foundress number and relatedness found that proportions of foundresses dying were significantly different before and after paralysis for both of the 2-foundress cases (2-brood-mates: Wald χ^2=17738.16, *d.f.*=1, P<0.001; 2-non-siblings: Wald χ^2=1537597.45, *d.f.*=1, P<0.001); indeed none of these foundresses died post-paralysis, but not for the 4-foundress cases, in which some foundresses died post-paralysis (4-brood-mates: Wald χ^2=19.8, *d.f.*=1, P=0.089; 4-non-siblings: Wald χ^2=1.99, *d.f.*=1, P=0.167).

In terms of the timing of mortality, prior to host paralysis, foundresses in groups of two died marginally non-significantly earlier than those in groups of four (cohort survival analysis: G_1=3.8, P=0.0513, %Dev=1.43). Mortality rate was not significantly affected by relatedness (G_1=2.3, P=0.1294, %Dev=0.86) or by the interaction between foundress relatedness and number (G_1=0.6, P=0.439, %Dev=0.26). In this analysis, foundresses that did not die by the day of host paralysis were treated as censors. After host paralysis, foundresses in groups of four tended to die significantly earlier than foundresses in groups of two (G_1=6.7, P=0.0096, %Dev=9.34), as no post-paralysis mortality occurred in 2-foundress groups. Time to death was not affected by foundress relatedness (G_1=0.2, P=0.6547, %Dev=0.31) or by an interaction between foundress relatedness and number (G_1=0.00, P=1.00, %Dev=0.00). In these analyses, we included all foundresses that were alive at the time of host paralysis and those that did not die by the twelfth day were treated as censors.

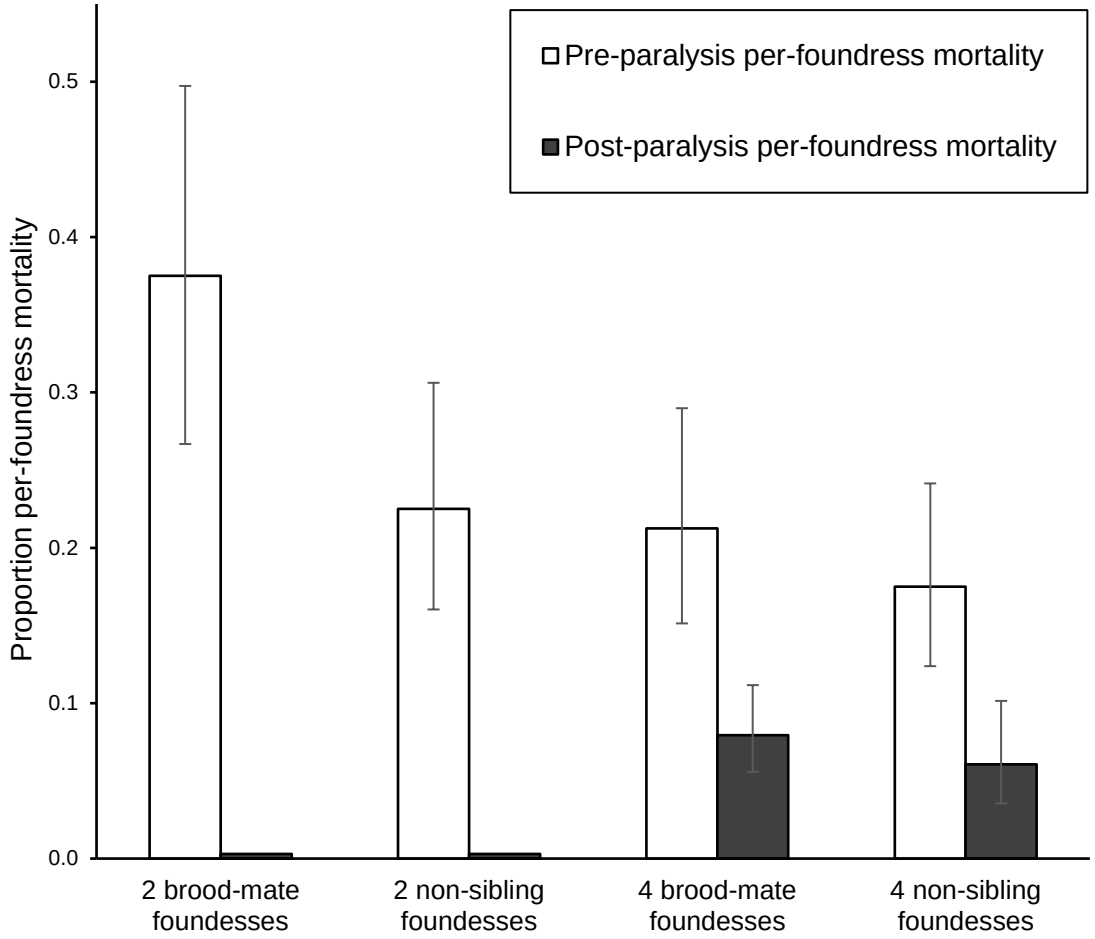


Figure 3.2 Proportion of foundresses dying before and after host paralysis. The overall proportion of per-foundress mortality in the period before host paralysis (white bars) was significantly higher than in the period after paralysis (dotted bars) (see main text). Standard errors of means are asymmetrical due to back transformation from the logit scale.

3.4.2 Paralysis

The percentage of hosts that were paralysed by *S. brevicornis* foundresses was 78.75% (n=80). Paralysed hosts no longer made major movements with their bodies and usually small brown dots were visible at the site of venom injection. This proportion was not affected by co-foundress relatedness (logistic ANOVA: G_1=0.85, P=0.355, %Dev=1.03) but was greater in 4-foundress replicates (97.5%) than in 2-foundress replicates (60%) (G_1=19.57, P<0.001,

%Dev=23.64). There was no significant interaction between foundress relatedness and number (G_1=0.97, P=0.324, %Dev=1.18).

Among the 63 replicates in which host paralysis occurred, the time taken to paralyse the host ranged up to 10 days. The 17 replicates in which there was no paralysis on the twelfth day were treated as censors in the subsequent analysis of influences on the time taken to paralyse hosts. Time-to-paralysis was dependent on both relatedness and foundress number but not on their interaction (cohort survival analysis: Relatedness: G_1=4.0, P=0.0455, %Dev=3.63; Number: G_1=24.0, P<0.0001, %Dev=18.44; Interaction: G_1=0.2, P=0.6547, %Dev=0.29), with hosts being paralysed more rapidly when there were four foundresses present and when foundresses were brood-mates (Figure 3.3): Generally, four-foundress groups paralysed hosts faster than two-foundress groups while the rate of paralysis in both foundress broods was rapid when co-foundresses were brood-mates.

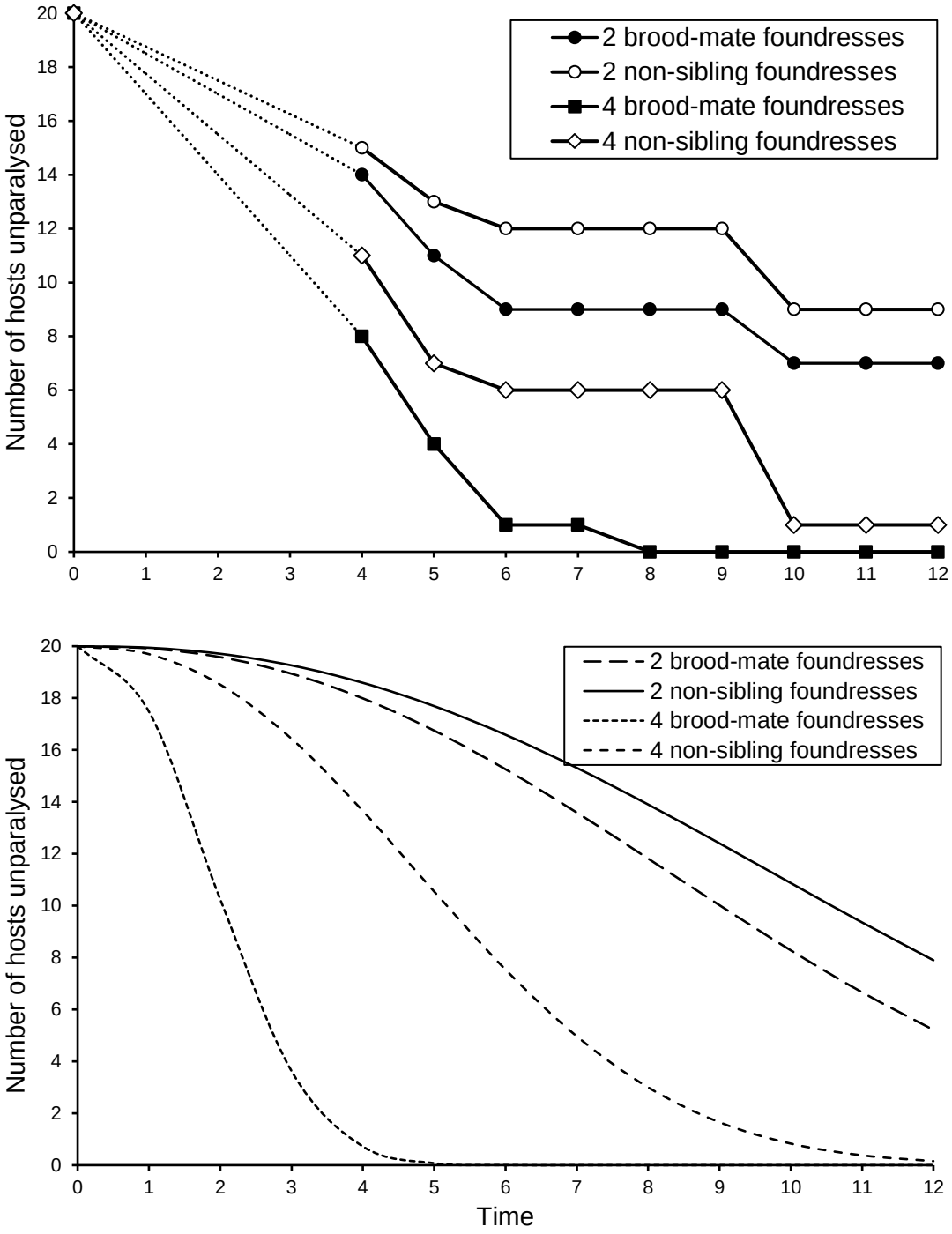


Figure 3.3 The effects of co-foundress relatedness and number on time to paralyse the host. Hosts were paralysed earlier when foundresses are related and when there were more foundresses. The upper panel shows the cohort data from the four treatments and the lower panel shows the fitted survival model, estimated from Weibull analysis with unparalysed hosts treated as censors. To avoid disruption of the females' behaviours no observations were taken on days 1, 2 and 3, and the statistical model was fitted assuming no hosts were paralysed during this period.

3.4.3 Oviposition

The percentage of paralysed hosts which were oviposited on was 92.06% (n=63). The probability of oviposition was not significantly affected by foundress relatedness (logistic ANOVA: G_1=0.15, P=0.701, %Dev=0.42), number (G_1=0.82, P=0.365, %Dev=2.35) or their interaction (G_1=1.12, P=0.29, %Dev=3.2). The time from host paralysis to the start of egg laying ranged up to 8 days and was not significantly affected by foundress relatedness (cohort survival analysis: G_1=3.2, P=0.0736, %Dev=3.8), number (G_1=0.1, P=0.7518, %Dev=0.07) or their interaction (G_1=0.2, P=0.655, %Dev=0.28).

3.4.4 Clutch size

Clutches laid by foundress groups ranged between 3 and 33 eggs (n=58, mean=16.24, S.E.=+0.83, -0.79) and did not vary according to foundresses relatedness (log-linear ANOVA: $F_{1,56}$=0.38, P=0.54, %Dev=0.70), initial foundress number ($F_{1,57}$=0.02, P=0.887, %Dev=0.04) or their interaction ($F_{1,55}$=0.14, P=0.707, %Dev=0.26). Although *C. cephalonica* hosts were selected to be of near uniform size (see above), we checked for an influence of host weight on clutch size: there was no significant relationship ($F_{1,56}$=0.98, P=0.327, %Dev=1.72).

The average number of eggs produced per initial foundress (clutch size divided by the initial number of foundresses, for the 58 replicates in which oviposition occurred) was not affected by foundresses relatedness (log-linear ANCOVA: $F_{1,56}$=0.56, P=0.457, %Dev=0.54) but was significantly smaller in 4-foundress replicates than in 2-foundress replicates ($F_{1,57}$=48.26, P<0.001, %Dev=46.84). Per-foundress egg production was not affected by an interaction between foundress relatedness and number ($F_{1,55}$=0.15, P=0.704, %Dev=0.14). Taking

into account that, due to foundress mortality, in some replicates foundress number at the time of oviposition was lower than the initial number, we similarly found that the number of eggs per foundress was unaffected by relatedness (log-linear ANCOVA: $F_{1,54}$=0.37, P=0.543, %Dev=0.28) and was significantly lower when foundress numbers were greater ($F_{3,57}$=27.81, P<0.001, %Dev=61.72, Figure 3.4), with no interaction between relatedness and the number alive at the time of oviposition ($F_{3,53}$=0.45, P=0.715, %Dev=1.01).

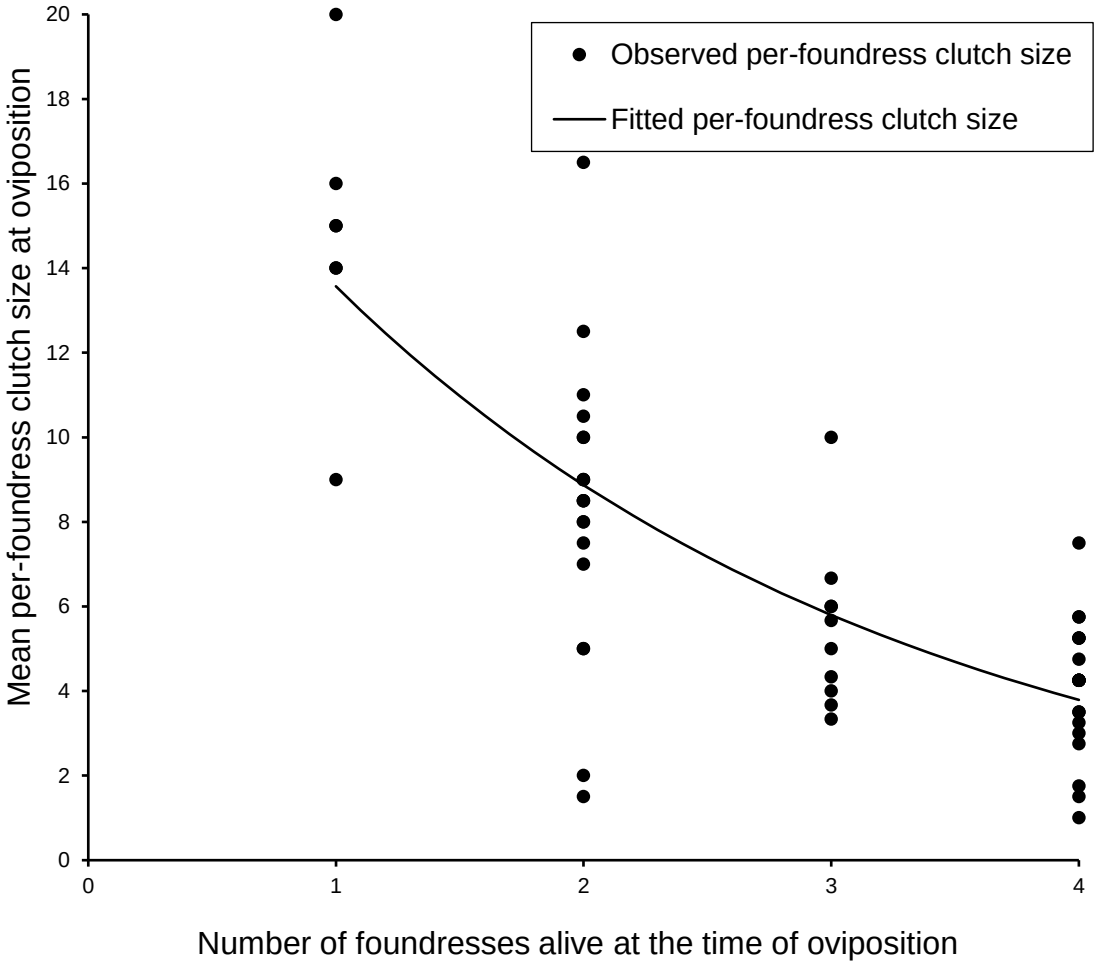


Figure 3.4 The effect of foundress number at the time of egg laying on per-foundress clutch size at oviposition. The mean number of eggs produced per foundress was lower when more foundresses were alive at the time of oviposition.

3.4.5 Offspring developmental mortality

The percentage of eggs that failed to develop to adulthood was 14.97% (n=58 clutches). Egg-to-adult mortality was not affected by foundress relatedness (logistic ANOVA: G_1=0.71, P=0.402, %Dev=1.24), foundress number (G_1=2.48, P=0.121, %Dev=4.3) or their interaction (G_1=0.47, P=0.496, %Dev=0.82).

3.4.6 Offspring production

The probability that at least some adult offspring were produced from a given host (i.e. reproductive success, defined as a binary response, for all provided hosts, n=80) was 0.66. This probablity was unaffected by foundresses relatedness (logistic ANCOVA: G_1=0.28, P=0.595, %Dev=0.30) but was significantly greater among 4-foundress replicates (mean=0.875) than among 2-foundress replicates (mean=0.575) (G_1=9.42, P=0.002, %Dev=10.01). There was no interaction between foundress relatedness and number (G_1=0.05, P=0.824, %Dev=0.05).

3.4.7 Brood size

The total number of adult offspring produced from a host which had been oviposited on (n=53) ranged between 3 and 32 (mean=15.11, S.E.=+0.71, -0.67). Brood size was not affected by foundresses relatedness (log-linear ANCOVA: $F_{1,51}$=2.56, P=0.116, %Dev=4.84), initial foundress number ($F_{1,52}$=1.26, P=0.266, %Dev=2.39) or by or their interaction ($F_{1,50}$=0.11, P=0.738, %Dev=0.21%).

In terms of the number offspring produced per foundress, production was significantly lower when there were initially four-foundresses than when there were two (log-linear ANOVA: $F_{1,52}$=82.37, P<0.001, %Dev=60.85). The effect of

foundress relatedness was non-significant ($F_{1,51}$=3.07, P=0.086, %Dev=2.27, n=53); but note the relatively low P-value and that with log-linear analyses estimated *P*-values are approximate rather than exact (Crawley, 1993). There was no significant interaction between foundress relatedness and initial number ($F_{1,50}$=0.12, P=0.730, %Dev=0.09%). Taking foundress mortality into account, we similarly found that the per-foundress production of offspring (brood size divided by the number of surviving foundresses at oviposition) was lower when there were more foundresses alive at the time of oviposition ($F_{1,52}$=119.74, P<0.001, %Dev=69.37) and that there was a marginally non-significant effect of relatedness, with brood-mate foundresses producing more offspring than non-sibling foundresses ($F_{1,51}$=3.74, P=0.059, %Dev=2.17, Figure 3.5). There was no significant interaction between foundress relatedness and number at oviposition ($F_{1,50}$=0.13, P=0.724, %Dev=0.07).

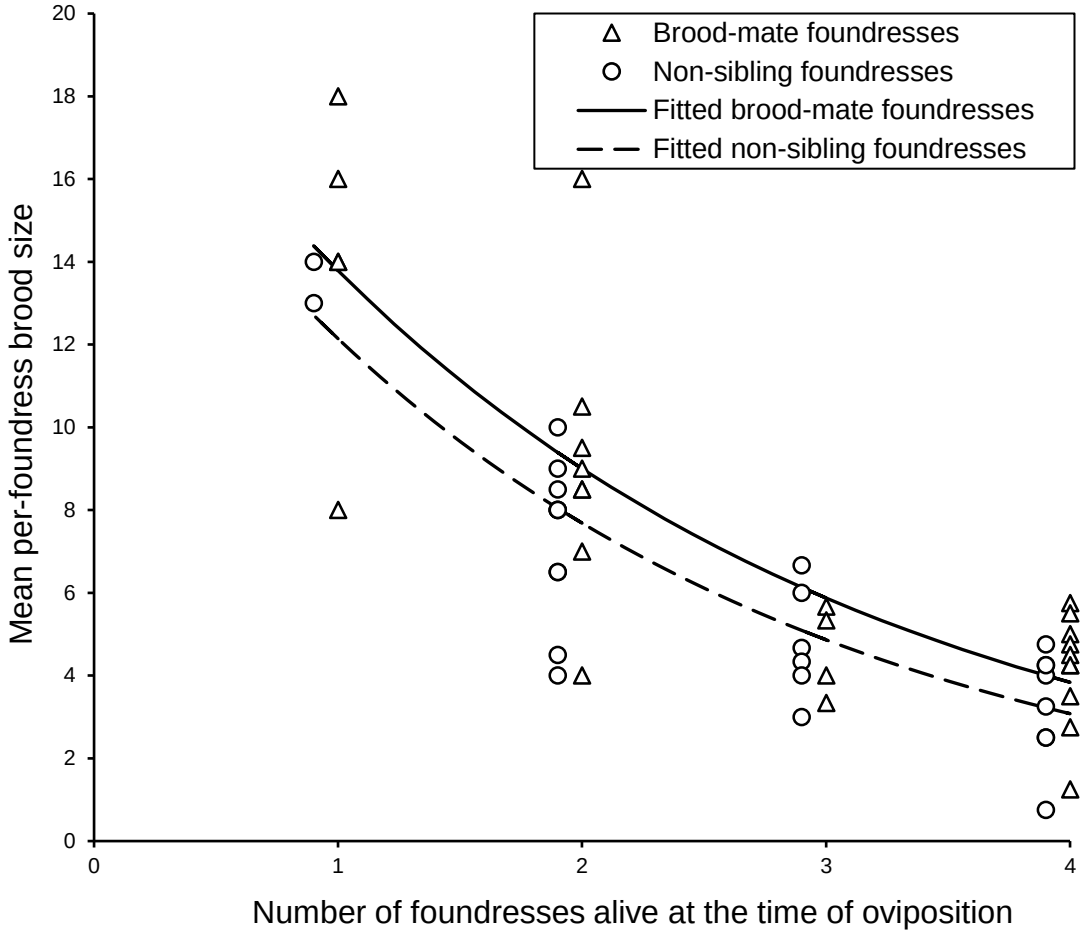


Figure 3.5 The effects of foundress number and relatedness on brood size produced per-foundress alive at the time of oviposition. For a given number of foundresses, broods were larger when foundresses were brood-mates. Data points are slightly laterally displaced to reduce overlap.

3.4.8 Brood developmental period

The time from oviposition to the emergence of adult offspring was affected by foundresses relatedness and initial foundress number via a significant interaction (cohort survival analysis of time to first emergence from each brood: Relatedness: G_1=2.0, P=0.157, %Dev=2.5; Number: G_1=0.5, P=0.479, %Dev=0.55; Interaction: G_1=9, P<0.01, %Dev=0.56): offspring developed most rapidly in 4-foundress replicates and the direction of influence of foundress relatedness was dependent on foundress number (Figure 3.6): for two-foundress broods development was most rapid when foundresses were non-

siblings while for four-foundress broods, non-sibling foundresses produced slower developing offspring. Taking in to account that in some replicates not all initial foundresses survived until eggs were laid, we similarly found that developmental time was affected by an interaction between relatedness and the number of foundresses alive at the time of oviposition (G_1=10.9, P<0.001, %Dev=17.11).

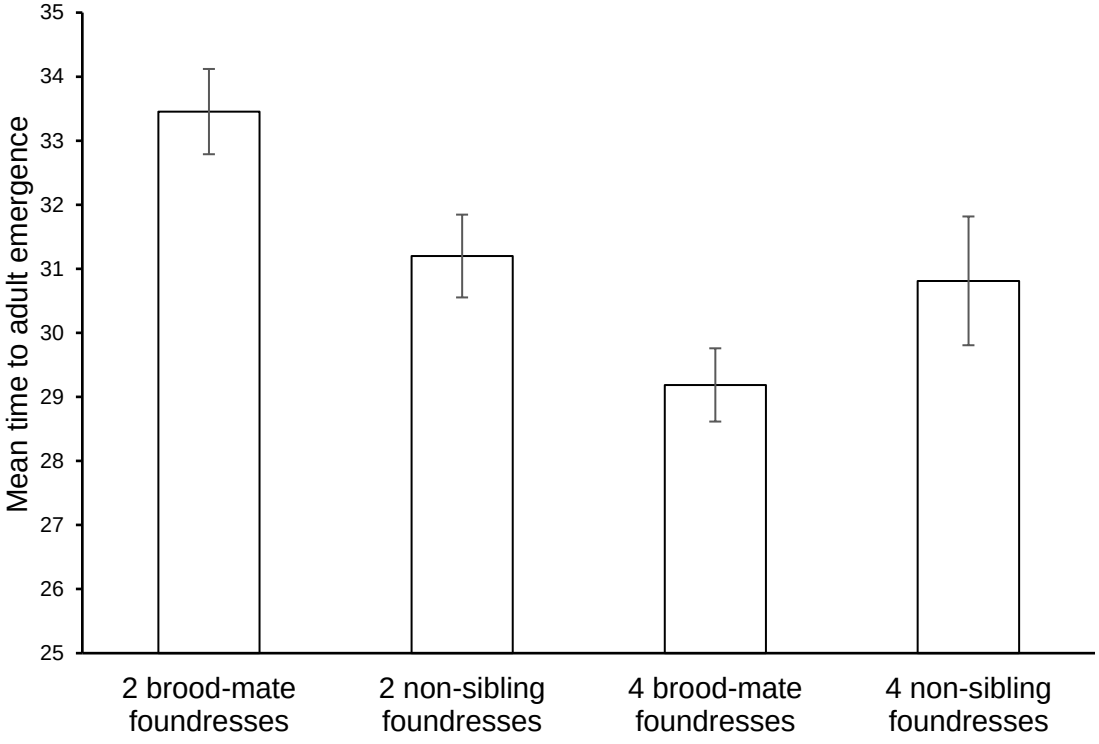


Figure 3.6 Developmental time of offspring. Among broods produced by two foundresses, offspring developed faster when the foundresses were not brood mates whereas among broods produced by four foundresses the opposite pattern was observed.

3.4.9 Adult offspring size

The average thoracic size of adult female offspring ranged between 0.58 and 0.91 mm (mean=0.764, S.E.± 0.011). The mean (per brood) size of female offspring was marginally non-significantly (positively) influenced by increasing per-offspring resource availability (host weight divided by the number of egg laid

onto the host) (standard regression: $F_{1,51}$=3.53, P=0.066, r^2=0.065, n=53 broods). Adults produced by non-sibling foundresses were significantly larger than those of brood-mate foundresses ($F_{1,50}$=9.12, P=0.004, r^2=0.14, Fig. 7) and body size was also negatively affected by the number of foundresses alive at the time of oviposition ($F_{1,50}$=4.27, P=0.045, r^2=0.066, Figure 3.7). There was no significant interaction between foundress number and relatedness ($F_{1,50}$=3.25, P=0.078, r^2=0.052).

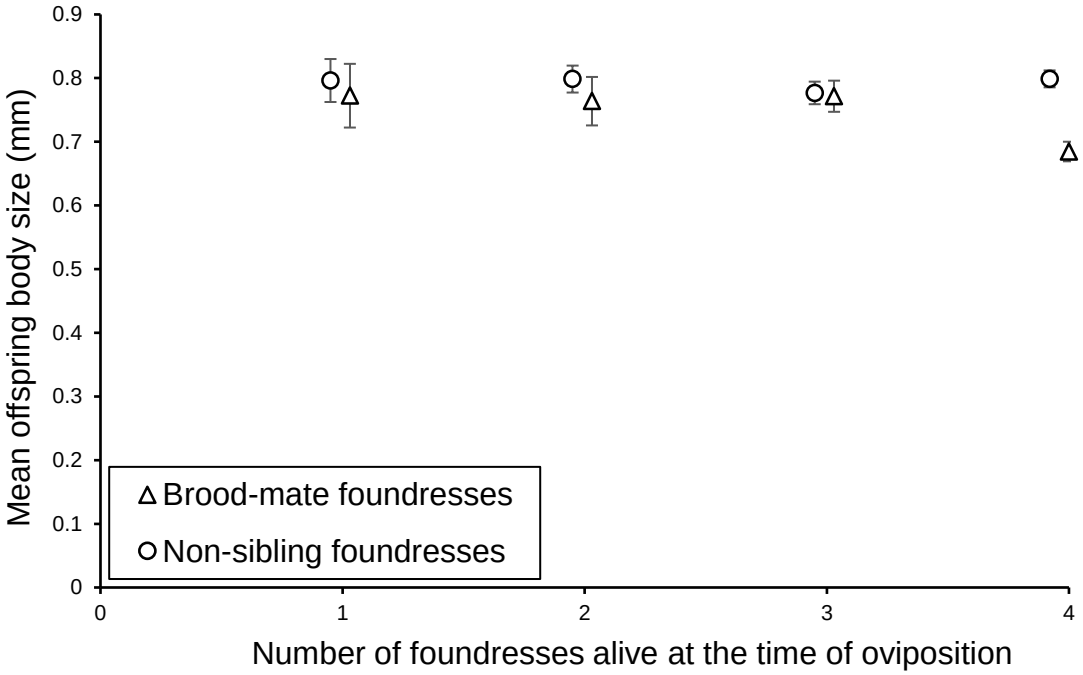


Figure 3.7 Influences on offspring body size. The size (thorax length) of adult female offspring was influenced by both relatedness and the number of foundresses alive at the time of oviposition. There was also a marginally non-significant influence of resource per developing offspring, such that offspring were larger when resources were less limited. Data for non-siblings are slightly laterally displaced to reduce overlap.

3.4.10 Sex ratio

Sex ratios of adult offspring groups were highly female-biased (mean proportion male=0.0787, SE=+0.0075, -0.0069, n=53) and were under dispersed (Heterogeneity Factor=0.571). Sex ratios were not significantly influenced by foundresses relatedness (logistic ANOVA: G_1=0.06, *P*=0.813, %Dev=0.11,

n=53), initial foundresses number ($G_{1=}$0.30, *P*=0.585, %Dev=0.60) or their interaction (G_1=1.08, *P*=0.305, %Dev=2.13). Taking foundress mortality into account, we similarly found that sex ratios were unaffected by the number of foundresses alive at the time of oviposition or by foundress relatedness (Number: G_1=1.34, *P*=0.253, %Dev=2.49; Relatedness: G_1=0.05, *P*=0.824, %Dev=0.09; Interaction: G_1=3.34, *P*=0.074, %Dev=6.22). Sex ratios were also not significantly affected by brood size ($F_{1,51}$=0.08, P=0.781, %Dev=0.0015) or by the percentage of developmental mortality experienced by the brood ($F_{1,51}$=1.10, P=0.299, %Dev=0.021).

Offspring groups that contained no adult males at emergence were not uncommon (mean proportion of all-female broods=0.1698, SE=+0.0557, -0.0442). The probability that an offspring group was all-female declined significantly with increase in brood size (G_1=53.60, P<0.001, %Dev=51.24) and increased significantly with the prevalence of developmental mortality within the brood (G_1=15.18, P<0.001, %Dev=22.93). Neither foundress number nor relatedness had any significant effects on these relationships.

Table 3.2 Summarized core experiment results

Reproductive trait	Relatedness	Foundress number	Interaction
Foundress mortality (proportion)	NS	NS	NS
Foundress mortality (timing) before host paralysis	NS	Marginal significance: Foundresses in small groups die earlier	NS
Foundress mortality (timing) after host paralysis	NS	Foundresses in large groups die earlier	NS
Paralysis of host (proportion)	NS	Large groups paralyse more hosts	NS
Time to paralysis of host	Brood-mates paralyse the host earlier	Large groups paralyse the host earlier	NS
Oviposition on host (probability)	NS	NS	NS
Time from paralysis to oviposition on host	Marginal significance: Non-siblings oviposit earlier	NS	NS
Clutch size [CS] (total)	NS	NS	NS
Per-foundress CS (using initial foundress number [FN])	NS	Smaller in large groups	NS
Per-foundress CS (using FN at oviposition)	NS	Smaller in large groups	NS
Offspring developmental mortality	NS	NS	NS
Reproductive success (probability)	NS	Greater in large groups	NS
Brood size [BS]	NS	NS	NS
Per-foundress BS (using initial FN)	Marginal significance: Brood-mates produce more offspring	Smaller in large groups	NS
Per-foundress BS (using FN at oviposition)	Marginal significance: Brood-mates produce more offspring	Smaller in large groups	NS
Brood developmental period (using initial FN)	Significance via interaction	Significance via interaction	Slower development when small groups are brood-mates, and when large groups are non-sibs
Adult offspring size (using FN at oviposition)	Non-siblings produce larger offspring	Small groups produce larger offspring	NS
Sex ratio of offspring	NS	NS	NS

NS = non-significant effect

3.5 Discussion

This study evaluated potential effects of co-foundress number and relatedness throughout the offspring-production cycle of the quasi-social parasitoid *Sclerodermus brevicornis*. Variation in *Sclerodermus* foundress number has been experimentally evaluated before (Tang *et al.*, 2014; Gao *et al.*, 2016b; Wang *et al.*, 2016; Wei *et al.*, 2017; Abdi *et al.*, Chapter 2), while variation in foundress relatedness has not. For foundress number, we found that groups of four foundresses were more successful, and also more rapid, in paralysing hosts than were pairs of foundresses. Hosts of *Sclerodermus* are typically large and dangerous to attack, with several prior studies reporting that hosts can kill the attacking female (Kühne & Becker, 1974; Liu *et al.*, 2011; Wei *et al.*, 2014), and our data additionally show that foundress mortality is typically higher pre-host paralysis than post-paralysis. It is unsurprising that larger groups of females perform better in host attack (as the probability of the host eventually becoming paralysed may equal the sum of independent probabilities of individual attack successes, or attacks may become successively more likely to succeed as the dose of venom injected into the host increases, or foundresses may coordinate their attacks as a team), and this result has been found in several prior studies on *Sclerodermus* species (Li & Sun, 2011; Tang *et al.*, 2014; Gao *et al.*, 2016b; Abdi *et al.*, Chapter 2). Although larger *S. brevicornis* foundress groups were more successful in terms of attacking hosts, similar numbers of offspring were produced from hosts paralysed by two or four females and thus the per-foundress reproductive output was lower. Tang *et al.* (2014) have previously shown that the costs and benefits to *Sclerodermus* females of sharing a host with other foundresses are host-size dependent,

essentially mediated by the lower probability of success for individual or small groups of foundresses when faced with larger, and likely more dangerous hosts (Liu *et al.*, 2011; Wei *et al.*, 2017).

For foundress relatedness, we found that many aspects of performance were unaffected by whether foundresses were brood-mates (which will often be siblings) or non-siblings (originating from different broods). Similarly, Wu *et al.* (2017) found that foundress-offspring relatedness had negligible effects on offspring development in *S. guani*. However, we found that groups of brood-mate foundresses paralysed hosts sooner than did non-sibling groups and also that, once hosts were suppressed, there was a (marginally significant) greater production of offspring per foundress when foundresses were prior brood-mates than when they were non-siblings. Greater numbers of offspring developing on a host of a given size led to adults produced by brood-mates having smaller body size, similar to results for S*. harmandi* (Liu *et al.*, 2011) and other bethylids (Mayhew, 1998) and small body size may constitute a fitness disadvantage (Gao *et al.*, 2016a).

Tang *et al.* (2014) concluded that *Sclerodermus* quasi-sociality (multi-foundress cooperation in brood production) could be explained in terms of direct fitness benefits accruing to females when hosts are too large to be likely to be suppressed by an individual foundress, but added that effects of kinship between foundresses may also be found to operate. The shorter latency to host paralysis observed among groups of prior brood-mates indicates that foundresses are more prone to take the risk of attacking and paralysing a host when the beneficiaries of a successful attack include their kin. We note that

evidence for earlier host attack by more closely related foundresses is also found in the accompanying study (Abdi *et al.*, Chapter 4).

The likelihood of *Sclerodermus* co-foundresses being kin in the field is unknown. Females may disperse from their natal patch individually and form new foundress groups effectively at random or they may tend to disperse as groups of brood mates, which will typically be kin: future work will be required to discover natural patterns of *Sclerodermus* dispersal. Because most *S. brevicornis* females are wingless but a minority have wings, we expected that wing-dimorphism might affect female's reproductive decisions. We consider alate females much more likely to disperse as individuals, and thus to typically reproduce with unrelated co-foundresses, and apterous females to be more likely to disperse and interact with relatives. We, however, found no convincing effects of this dimorphism on the behavioural reproductive characters assessed, although offspring developmental timing and offspring size were affected (Appendix 2). We also explored host-of-origin as a proxy for kinship given that the cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and that these profiles may in turn be used in kinship assessment. While we found effects of the developmental host on subsequent reproductive behaviours, we also found evidence for a wider set of developmental and physiological effects that confound confident interpretation in terms of kin selection (Appendix 1).

Our study contributes to the growing body of evidence for the operation kin effects in the reproductive ecology of parasitoid wasps. These have now been found at various life-history stages, including host attack and the defence of paralysed hosts by adults, between offspring developing on a shared host

resource (Giron *et al.*, 2004; Giron & Strand, 2004; Lizé *et al.*, 2012; Dunn *et al.*, 2014; Mathiron *et al.*, 2019) and some aspects of maternal care (Wu *et al.*, 2017). For *Sclerodermus*, the existence of kin-mediated host attack and effects on per-foundress offspring production suggest that co-foundresses may attune their behaviour according to inter-foundress relatedness at other life-history stages that have not yet been investigated in detail. For instance, once a dangerous host has been suppressed, the resources it represents may be open to selfish exploitation, such that some co-foundresses produce more offspring than others (reproductive skew). As foundresses may obtain indirect fitness benefits by facilitating the reproductive output of related foundresses, we might expect any such skew to be mediated by kinship.

Our experiment found that groups of brood-mate foundresses paralyse hosts more rapidly than non-sibling groups. The most likely explanation is that individual foundresses delay taking the risk of host attack according to their relatedness to other females present. Here we consider further a foundress's host attack decision by using the public goods framework of Hamilton's rule (Hamilton, 1964a; Cooper *et al.*, 2018) (Appendix 3).

Hamilton's rule asserts that a trait will spread if its fitness benefits to the recipients (b) multiplied by the relatedness (r) between social partners is greater than the fitness cost (c) to the actor ($rb - c > 0$). Public goods models explore the evolution of cooperation and consider situations in which a focal individual produces a costly public good, the benefits of which are then shared between the individual and its social partners. Inclusive fitness considerations (mediated by relatedness, r) can explain why individuals may be selected to carry out costly behaviours that benefit others, as opposed to not incurring costs and

possibly benefitting from public goods produced by others (Cooper *et al.*, 2018). Approaches to calculating *b* and *c* in Hamilton's rule are the subject of ongoing theoretical debate and development (Appendix 3) and it is, strictly, incorrect to estimate them directly from empirical data (Gardner *et al.*, 2011). We therefore proceed heuristically in making use of currently available evidence.

For *Sclerodermus*, we suggest that a paralysed host represents a public good with the fitness benefits (*b*), in terms of offspring production, shared by all foundresses present. We also suggest that the fitness cost (*c*) to a female in providing this public good can be represented by the risk of mortality involved in attacking a host. In the experiment on *S. brevicornis* reported here, host size was held essentially constant but both *b* and *c* are likely to vary with host size: in other *Sclerodermus* species larger hosts can support the development of more offspring (Liu *et al.*, 2011; Tang *et al.*, 2014) but are also more risky to attack (Liu *et al.*, 2011; Wei *et al.*, 2017). We use empirical results from these three prior studies to estimate the relationships between host size and costs and between host size and benefits (Appendix 3). Using these relationships and an open formulation of Hamilton's rule (in which the mechanism generating co-foundress relatedness is undefined (Cooper *et al.*, 2018)) suggests that selection for public goods host attack should depend interactively on both co-foundress relatedness and host size (

Figure **3.15**). The maximum size of a healthy and active host that individual foundresses should be prepared to attack alone and then share, if successful, is generally larger when co-foundresses are more closely related.

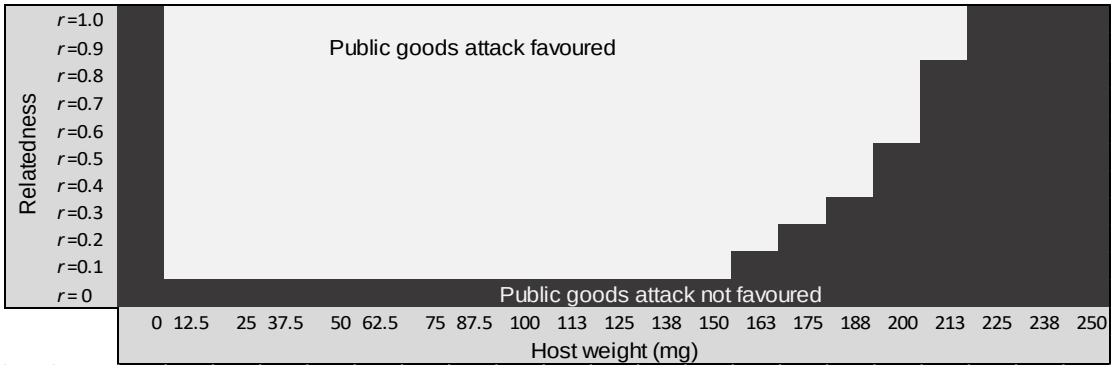
The simple model framework we employ heuristically is unlikely to capture exactly all of the selective forces that shape host-attack decisions in

Sclerodermus. Further, the model functions to indicate whether a female might be expected to attack and then share a suppressed host, not whether a female should attack a host to be exploited only by her own offspring. Females that do not obtain access to a paralysed host (whether shared or as a lone foundress) during their lifetime will have zero fitness and, as such, might be expected to attack even the most dangerous hosts when other options are unavailable (analogous to the Desperado Effect (Grafen, 1987)).

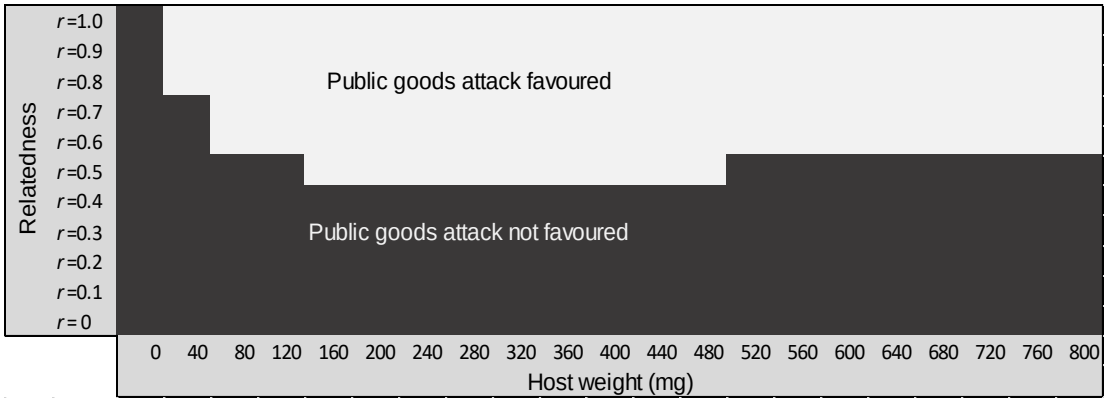
As the kinship effect that we have found empirically does not operate in terms of the eventual probability of host paralysis but in the timing of that suppression. We thus expect females encountering a dangerous host, and with limited alternative reproductive opportunities, to be selected to at first wait for other females to attack the host but eventually to attack it themselves, with reluctance to attack being reduced by inclusive fitness benefits when other females present are kin. We suggest that there is scope to usefully develop formal theory that considers how females' attack decisions may change over time as well as how attacks by individual females, whether separate or coordinated, may interact in terms of the probability of host suppression. Our expectation is that latency to attack a given host will be shorter when co-foundresses are more closely related and when the effects of individual attacks are non-independent.

Using Hamilton's rule to consider relatedness-based host attack in combination with cost and benefit estimates derived from prior studies has generated the testable prediction that the time-to-host attack will be affected interactively by host size and co-foundress relatedness.

(a) *Sclerodermus pupariae* attacking *Massicus raddi* (Wei *et al.*, 2014)



(b) *Sclerodermus harmandi* attacking *Monochamus alternatus* (Liu *et al.*, 2011)



(c) *Sclerodermus harmandi* attacking *Monochamus alternatus* (Tang *et al.*, 2014)

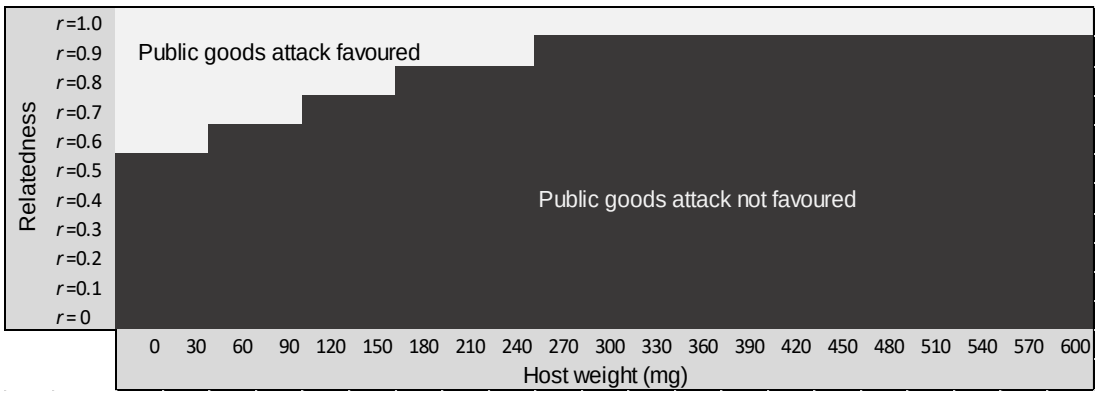


Figure 3.8 Public goods model of relatedness-mediated host attack. The panels illustrate how variation in relatedness (*r*) and in host weight combine to influence selection for public goods attack. Host weight affects the probability of a foundress dying prior to host suppression (cost, *c*) and also the fitness benefits obtained from paralysed hosts (benefit, *b*). Light areas indicate combinations that satisfy Hamilton's rule ($rb - c > 0$). These illustrations derive from three separate empirical studies, of two different *Sclerodermus*-host species associations that assessed different ranges of host sizes: details on data sources and the relationships between host size and the cost and benefit parameters are provided in (Appendix 3).

3.6 Conclusion

This study was designed to identify potential roles of foundress kinship in a quasi-social reproductive system that has hitherto been explained in terms of direct fitness advantages to co-founding broods on large hosts and without recourse to inclusive fitness considerations. We find that inter-foundress relatedness influences the timing of host attack by *S. brevicornis* females: groups of brood-mate females paralyse hosts earlier than similar-sized groups of non-sibling foundresses. A simplistic use of a public goods model framework appears to capture the essence of a female's decision to attack (and, if successful, subsequently share) a host according to her relatedness to co-foundresses but further information on the natural life-histories of *Sclerodermus* species will be required to assess and develop its key assumptions. In our experiment, host size was held constant but the public goods model framework suggests that both host size and inter-foundress kinship will interactively influence host attack behaviour. We test this assertion in the accompanying empirical study (Abdi *et al.*, Chapter 4).

3.7 Appendix 1: Effects of foundress number and host species-of-origin

The mechanistic basis of parasitoid kin recognition may operate via the chemical composition of the adult exoskeleton, which may be influenced by an individual's genetics and/or by its developmental environment (Ode *et al.*, 1995; Lizé *et al.*, 2012; Khidr *et al.*, 2013). In *Sclerodermus* (Stockermans & Hardy, unpublished data), and in other bethylids (Khidr *et al.*, 2013), the profile of cuticular hydrocarbons in the adult integument is affected by the species of host

on which the individual developed. For an assessor, a conspecific perceived to have developed on a different host, especially on a different species of host, is unlikely to be as closely related as a conspecific perceived to have developed on the same individual host. Hence, we expected that *Sclerodermus* foundresses may assess propinquity of relatedness to co-foundresses according to their host-of-origin and modify their cooperative reproductive behaviour accordingly.

3.7.1 Intrinsic effects of developing on a beetle larva or moth larva

Using only data from replicates containing two non-sibling foundresses (Table 3.1, rows B and F, n=40), we first checked for effects of foundress development on different host species in the absence of variation in foundress number or relatedness; such differences might indicate that host-of-origin is not a simple proxy for the assessment of relatedness. We found no significant effects on any of the life-history stages examined (paralysis, clutch size, foundress mortality, reproductive success, developmental mortality, brood size, time to adult emergence, adult offspring size, and sex ratio) except for time to oviposition. The time taken for foundresses to oviposit on the hosts was significantly shorter when foundresses had been reared on *P. h. hilaris* than when they had been reared on *C. cephalonica* (cohort survival analysis: G_1= 19.14, P<0.001, %Dev=51.04, Figure 3.9). We interpret this to indicate that *S. brevicornis* females that have developed on a beetle host may have an intrinsically enhanced metabolic profile (e.g. allowing them to mature eggs more rapidly), compared to those reared on a factitious lepidopteran host. More broadly, this result indicates that further effects of host-of-origin (in the analyses using a wider set of data below) may not be the result of kinship assessment alone.

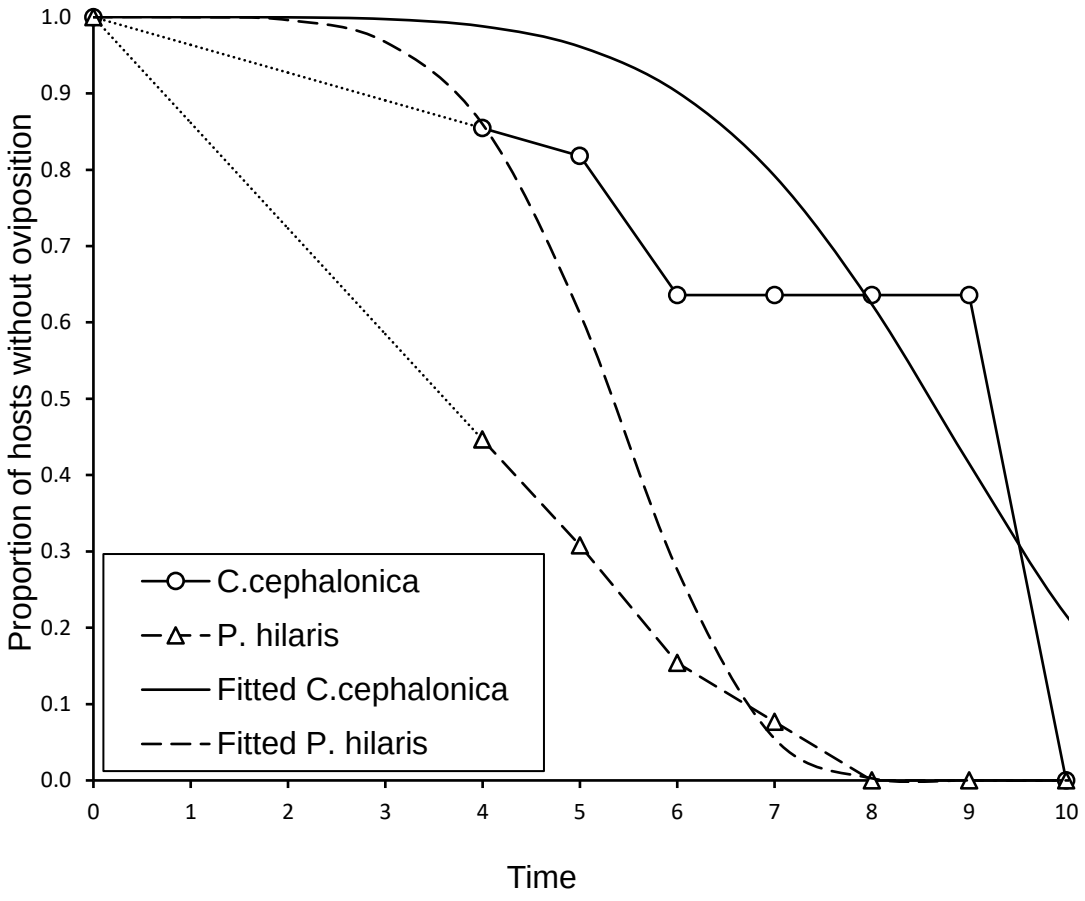


Figure 3.9 The effect of host species-of-origin on time taken to oviposition by two non-sibling foundresses. *Corcyra cephalonica* hosts were oviposited on more rapidly when foundresses were reared on beetle larvae.

Further analyses of host-of-origin effects on the behaviour and performance of unrelated co-foundresses used data from rows B, E and F (Table 3.1) (with rearing host species fitted as a factor with 3 levels) for the 2-foundress case only, and from rows B, D, E and H (with rearing host species fitted as a factor with 2-levels) for both 2 and 4 foundress cases (Table 3.3).

3.7.2 Foundress mortality

Foundress mortality (proportion) was not affected by host species-of-origin (logistic ANOVA: 3-level factor: G_2=1.38, P=0.261, %Dev=4.61, n=60; 2-level factor: G_1=1.66, P=0.202, %Dev=2.08, n=80) or by an interaction with foundress number (G_1=0.00, P=1.00, %Dev=0.00). Time to foundress mortality

was not significantly affected by rearing host species-of-origin (cohort survival analysis: 3-level factor: G_2=5, P=0.082, %Dev=3.64; 2-level factor: G_1=2.9, P= 0.089, %Dev=1.2) or by an interaction with foundress number (G_1=0.1, P=0.752, %Dev=0.04).

3.7.3 Paralysis

The proportion of hosts paralysed was not affected by the host species on which foundresses developed (logistic ANOVA: 3-level factor: G_2=0.65, P=0.522, %Dev=1.67; 2-level factor: G_1=1.56, P=0.212, %Dev=1.95) or by an interaction with foundress number (G_1=0.82, P=0.366, %Dev=1.02). Time to paralysis was significantly affected by rearing host species (cohort survival analysis: 2-level factor: G_1=12.9, P<0.001, %Dev=16.42) and by foundress number (G_1=22.5, P<0.001, %Dev=21.75), with hosts being most rapidly paralysed when there were four co-foundresses that had developed on a mix of host species (Figure 3.10). There was, however, no significant interaction with foundress number (G_1=0.7, P=0.403, %Dev=1.95). There was no significant effect when host-of-origin was treated as a factor with three levels (G_2=3.3, P=0.192, %Dev=3.49, n=60).

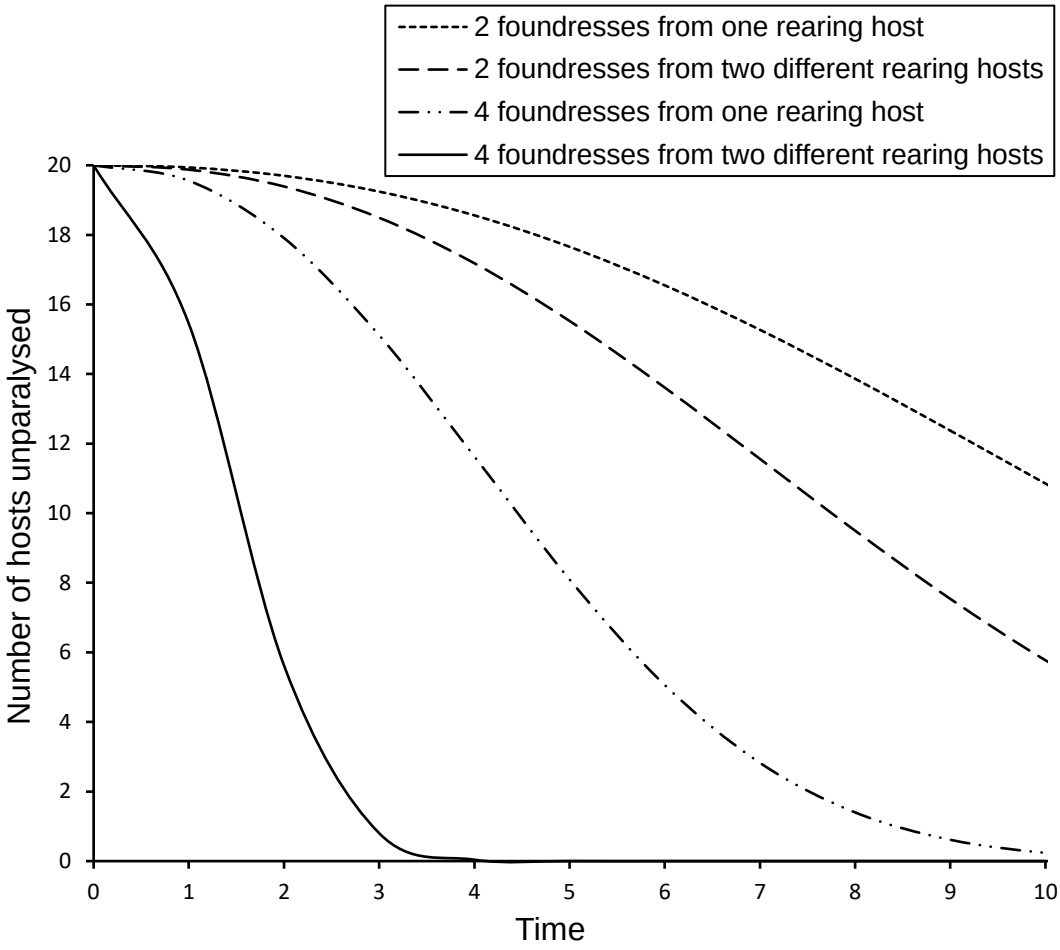


Figure 3.10 The effect of host species-of-origin on time taken to paralyse hosts. *Corcyra cephalonica* hosts were paralysed more rapidly when foundresses had been reared on a mixture of host species, and when there were more foundresses. The figure shows the fitted cohort survival curves from Weibull analysis, with hosts unparalysed on the 10th day treated as censors.

3.7.4 Oviposition

The probability of oviposition was significantly affected by host species-of-origin, such that co-foundresses that had been reared from two different host species were more likely to lay eggs than those had been reared on *C. cephalonica* (logistic ANCOVA: 2-level factor: G_1=4.85, P=0.028, %Dev=16.20, n=64). There was a significant interaction effect between foundress number and host species-of-origin, with oviposition probability being higher when foundress numbers were greater and co-foundresses had been reared on different host

species, but lower when co-foundresses were reared in *C. cephalonica* (G_1=5.42, P=0.020, %Dev=18.12). The probability of oviposition was not affected by host species-of-origin treated as a factor with three levels (G_2=1.23, P=0.292, %Dev=11.65, n=39).

In terms of time to oviposition, when host species-of-origin was treated as a 2-level factor, there was a significant interaction between foundress number and host species (cohort survival analysis: Number: G_1= 1.00, P=0.3173, %Dev=1.18; Host-of-origin: G_1= 7.00, P<0.01, %Dev=9.21; Interaction: G_1= 4.2, P<0.05, %Dev=5.97), with eggs being laid earliest when there were four co-foundresses that had been reared on different host species (Figure 3.11). Time to egg laying was, however, unrelated to the foundresses host species-of-origin, when treated as a 3-level factor (G_2=4.1, P=0.129, %Dev=8.09).

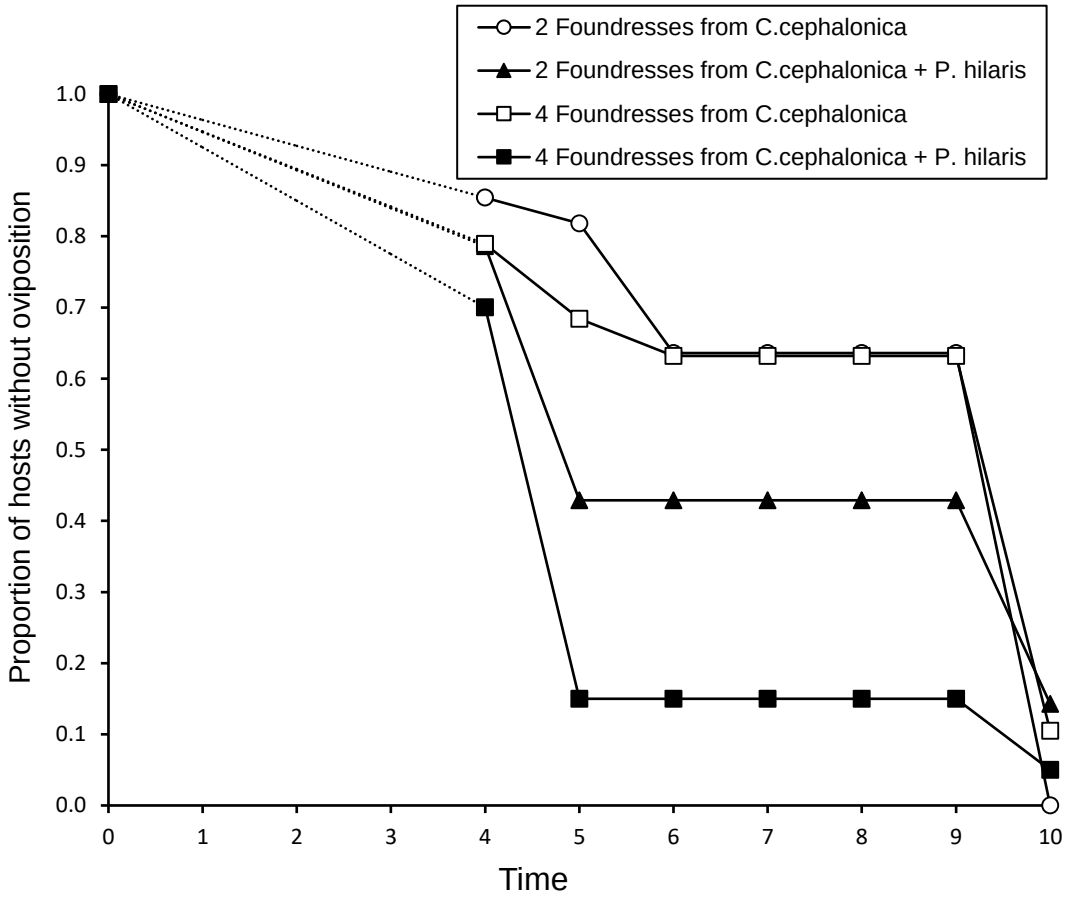


Figure 3.11 The effects of host species-of-origin and foundress number on time taken to oviposition. *Corcyra cephalonica* hosts were oviposited on more rapidly when foundress groups of four comprised individuals reared from different host species.

3.7.5 Clutch size

There were no effects of host species-of-origin on clutch size (log-linear analysis: 3-level factor: $F_{2,34}$=1.05, P=0.362, %Dev=5.81, n=37; 2-level factor: $F_{1,58}$=1.26, P=0.266, %Dev=2.11, n=61), nor was there an interaction with foundress number ($F_{1,57}$=0.98, P=0.325, %Dev=1.64). Per-foundress egg production was unaffected by host-of-origin (3-level factor: $F_{2,34}$=1.05, P=0.362, %Dev=5.81; 2-level factor: $F_{1,57}$=2.15, P=0.148, %Dev=1.57) and there was no interaction with foundress number ($F_{1,58}$=0.99, P=0.325, %Dev=0.72). Per-foundress clutch sizes were significantly smaller wen foundress numbers were larger ($F_{1,58}$=77.27, P<0.001, %Dev=7.18).

3.7.6 Offspring developmental mortality

Offspring mortality was greatest when co-foundresses had been reared on a mix of host species (logistic ANOVA: 3-level factor: G_2=5.96, P=0.006, %Dev=25.96, n=37; 2-level factor: G_1=7.17, P=0.010, %Dev=8.53, n=61). There was also a significant interaction between host species-of-origin and foundress number (G_1=8.63, P=0.005, %Dev=10.26), such that the number of offspring produced by co-foundresses reared from different host species was lower when foundress number was higher, whereas the numbers of offspring produced by co-fondresses reared from the same host species were relatively little affected by foundress number (Figure 3.12).

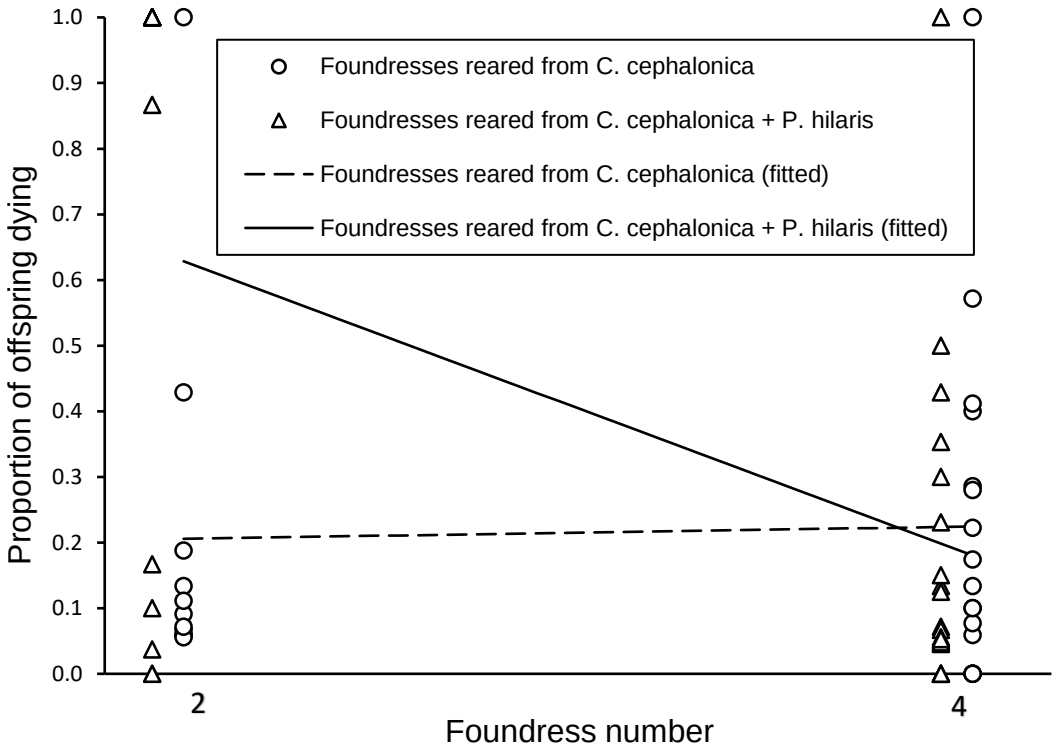


Figure 3.12 The effects of host species-of-origin and foundress number on offspring developmental mortality. Mortality decreased with an increase in foundress number when offspring were produced by co-foundresses reared from different host species, whereas when co-foundresses had been reared from the same host species, mortality was little affected by foundress number.

3.7.7 Reproductive success

The probability of reproductive success (production of ≥1 offspring) was unrelated to foundresses' host-of-origin (3-level factor: G_2=1.11, P=0.329, %Dev=2.71, n=60; 2-level factor: G_1=0.07, P=0.789, %Dev=0.07, n=80), but it was significantly affected by foundress number: the probablity of success was greater for 4-foundress groups (mean=0.875) than 2-foundress groups (mean=0.375) (G_1=20.79, P<0.001, %Dev=19.84). Host-of-origin had a marginally non-significant interaction with foundress number (G_1=3.79, P=0.051, %Dev=3.62) suggesting that reproductive success for co-foundresses reared from the same host species is less sensitive to foundress number variation.

3.7.8 Brood size

Foundresses' host-of-origin had no significant effects on brood size (log-linear analysis: 3-level factor: $F_{2,25}$=1.64, P=0.217, %Dev=12.45, n=26; 2-level factor: $F_{1,50}$=0.01, P=0.908, %Dev=0.024, n=51) and there was no interaction with foundress number ($F_{1,48}$=0.60, P=0.442, %Dev=1.26). Per-foundress offspring production was not affected by host-of-origin (3-level factor: $F_{2,25}$=1.64, P=0.217, %Dev=12.45, n=26; 2-level factor: $F_{1,50}$=0.14, P=0.707, %Dev=0.17, n=51) or by an interaction with foundress number ($F_{1,48}$=0.68, P=0.414, %Dev=0.78).

3.7.9 Brood developmental period

Adult offspring emerged within a significantly shorter time when broods were produced by a larger number of foundresses (cohort survival analysis of time to first emergence per brood: G_1=12.7, P<0.001, %Dev=0.11, n=65). Brood

developmental period was influenced by host species-of-origin (as a 2-level factor) via a significant interaction with foundress number (host of origin: G_1=0.1, P=0.752, %Dev=0.18; Interaction: G_1=18.1, P<0.001, %Dev=17.96, Figure 3.13): offspring produced by 4-foundress groups that has been reared from a mix of host species emerged earliest. There was no significant effect of host-of-origin fitted as a 3-level factor (G_2=0.85, P=0.654, %Dev=2.7).

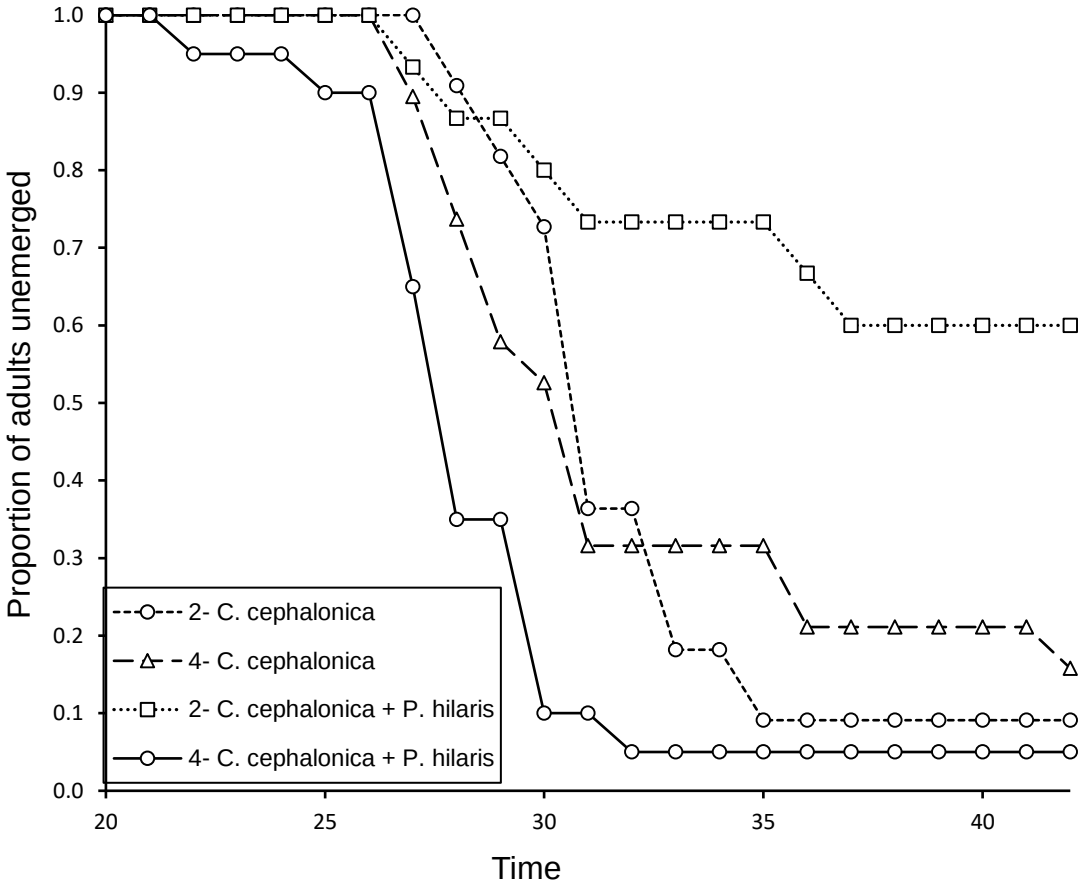


Figure 3.13 The effect of foundress number and host species of origin on post-oviposition brood developmental time. Groups containing four foundresses and reared from two different host species-of-origin produced their offspring within a significantly shorter period compared to those containing pairs of foundresses.

3.7.10 Adult offspring size

Adult female offspring were smaller when produced by co-foundresses reared from a mix of host species when host-of-origin was treated as a 3-level factor

($F_{2,23}$=4.43, P=0.025, r^2=0.28, n=26). However, in the case of the 2-level factor, there was no effect ($F_{1,47}$=1.67, P=0.203, r^2=0.03, n=51) nor was there an interaction with foundress number ($F_{1,49}$=1.22, P=0.276, r^2=0.02).

3.7.11 Sex ratio

Sex ratios were not affected by the host species that foundresses developed on (3-level factor: G_2=0.06, *P*=0.946, %Dev=0.48, n=26; 2-level factor: G_1=2.23, *P*=0.142, %Dev=4.38, n=51), or by the interaction between host-of-origin and foundress number (G_1=1.01, *P*=0.320, %Dev=1.01).

Table 3.3 Summarized host species-of-origin results.

Reproductive trait	Host-of-origin (2-levels)	Host-of-origin (3-levels)	Foundress number (FN)	Interaction with Foundress number (2-levels)
Mortality (proportion)	NS	NS	NS	NS
Mortality (timing)	NS	NS	NS	NS
Paralysis (proportion)	NS	NS	NS	NS
Paralysis (timing)	Foundresses from different hosts attacked faster	NS	Larger groups attacked earlier	NS
Oviposition (probability)	Foundresses from different hosts laid more eggs	NS	Significance via interaction	With an increase of foundress number, co-foundresses from different hosts oviposited more, whereas those from one host oviposited less.
Oviposition (timing)	Foundresses from different hosts oviposited earlier	NS	Larger groups laid eggs earlier	Eggs were laid earlier when there were four co-foundresses that had been reared on different host species
Clutch size (CS)	NS	NS	NS	NS
Per-foundress CS	NS	NS	Smaller with larger groups	NS
Offspring developmental mortality	Greater when foundresses were from different hosts	NS	Smaller groups died more	Mortality was similar among all 4 foundress broods and 2 foundress broods produced by foundresses reared from the same host species, but mortality was higher when offspring were produced by 2 foundresses reared from different host species
Reproductive success (probability)	NS	NS	Higher with larger groups	Marginally NS: Reproductive success for co-foundresses reared from the same host species is less sensitive to foundress number variation
Brood size (BS)	NS	NS	NS	NS
Per-foundress BS	NS	NS	NS	NS
Brood developmental period	Significance via interaction	NS	Adults produced by larger groups emerged earlier	Offspring produced by 4-foundress groups that has been reared from a mix of host species emerged earlier
Adult offspring size (FN at oviposition)	NS	Foundresses from different hosts produced smaller adults	NS	NS
Sex ratio	NS	NS	NS	NS

NS = Non-signficiant effect

3.7.12 Discussion

Host-of-origin was explored as a proxy for kinship given that the cuticular hydrocarbon profiles of foundresses are likely to be affected by their developmental host and that these profiles may in turn be used in kinship assessment. While we found effects of developmental host on the subsequent reproductive behaviour of foundresses, we also found evidence for intrinsic effects of developing on a beetle larva or moth larva (summarized in Table 3.3). Prior studies of parasitoid reproduction have also concluded that the species a female is reared on has consequences in terms of host-feeding and parasitism capacity (Dai *et al.*, 2014), host acceptance (Henter *et al.*, 1996), longevity and offspring developmental mortality (Corrigan & Laing, 1994), and sex ratios (Othim *et al.*, 2017). Such intrinsic effects generate confounding explanations for apparent effects of kinship. Interpretation of any effects of host-of-origin should therefore be made with this caveat taken into account.

Overall, our results suggest that *S. brevicornis* co-foundresses performed better when reared on different host species; paralysing hosts earlier, ovipositing earlier, laying more eggs and producing adult offspring earlier. These results are the opposite to what would be expected if females use hydrocarbon cues to assess their relatedness to co-foundresses and attune their reproductive behaviours accordingly but would align to expectation if females that develop on beetle larvae perform better than females that have developed on a factitious lepidopteran host. Offspring developmental mortality, however, was higher when co-foundresses had been reared on different host species, possibly due to infanticide. Future studies will be required to investigate this possibility further.

3.8 Appendix 2: Effects of *Sclerodermus* females wing dimorphism

Most *Sclerodermus* females are wingless (apterous) and most males are winged (alate). However, wing dimorphism is common throughout the genus, thus some females are alate and some males are apterous (Kühne & Becker, 1974; Evans, 1978; Papini, 2014; Tang *et al.*, 2014; Wang *et al.*, 2016; Lupi *et al.*, 2017; Wei *et al.*, 2017). As the possession of wings likely enhances capacity for post-eclosion dispersal (Wang *et al.*, 2015) but see (Kühne & Becker, 1974), we expect that alate females more commonly co-found broods with non-siblings than do apterous females, and thus winged and wingless foundresses may differ in their of cooperative behaviour and sex allocation strategies.

Analyses in this Appendix use replicates from Table 3.1 in the core part of this chapter, rows F and G, to test effects of foundress wing morphology for the 2-foundress non-sibling case (Table 3.4).

3.8.1 Foundress mortality

Foundress mortality (proportion) was not affected by foundresses wing dimorphism (logistic ANOVA: G_1=1.24, P=0.272, %Dev=3.17, n=40 broods), nor was the longevity of foundresses affected by their wing morphology (cohort survival analysis: G_1=2.6, P=0.107, %Dev=2.64, n=80 foundresses).

3.8.2 Paralysis

The proportion of hosts paralysed was not affected by wing morphology (G_1=1.31, P=0.252, %Dev=3.07, n=40). Nor did wing morphology affect the time taken to paralyse the host (cohort survival analysis: G_1=0.9, P=0.343, %Dev=1.43).

3.8.3 Oviposition

Wing morphology did not affect the probability of oviposition (G_1=0.02, P=0.887, %Dev=0.14, n=31) or the time to oviposition (cohort survival analysis: G_1=3.00, P=0.084, %Dev=6.6) but note the marginal non-significance suggesting that wingless foundresses may oviposit earlier than winged foundresses.

3.8.4 Clutch size

Clutch sizes were unaffected by foundress wing morphology (log-linear ANOVA: $F_{1,27}$=0.25, P=0.622, %Dev=0.92, n=29) nor did wing morphology affect the mean number of eggs laid per-foundress ($F_{1,27}$=0.25, P=0.622, %Dev=0.92, n=29).

3.8.5 Offspring developmental mortality

Offspring developmental mortality was unaffected by foundress wing morphology (logistic ANOVA: G_1=0.13, P=0.717, %Dev=0.49, n=29) or by clutch size (G_1=3.62, P=0.062, %Dev=5.79).

3.8.6 Reproductive success

Foundresses wing morphology did not affect the probability of reproductive success (logistic ANOVA: G_1=2.71, P=0.100, %Dev=5.11, n=40).

3.8.7 Brood size

Brood sizes were not affected by foundresses wing morphology (log-linear ANOVA: $F_{1,23}$=1.26, P=0.111, %Dev=10.68, n=25) nor did foundresses wing morphology affect mean per-foundress offspring production ($F_{1,23}$=0.00, P=0.948, %Dev=0.02).

3.8.8 Brood developmental period

Offspring matured earlier when produced by winged foundresses than when produced by wingless foundresses (cohort survival analysis of time to first emergence per brood: G_1= 6.5, P<0.05, %Dev=15.07, Figure 3.14).

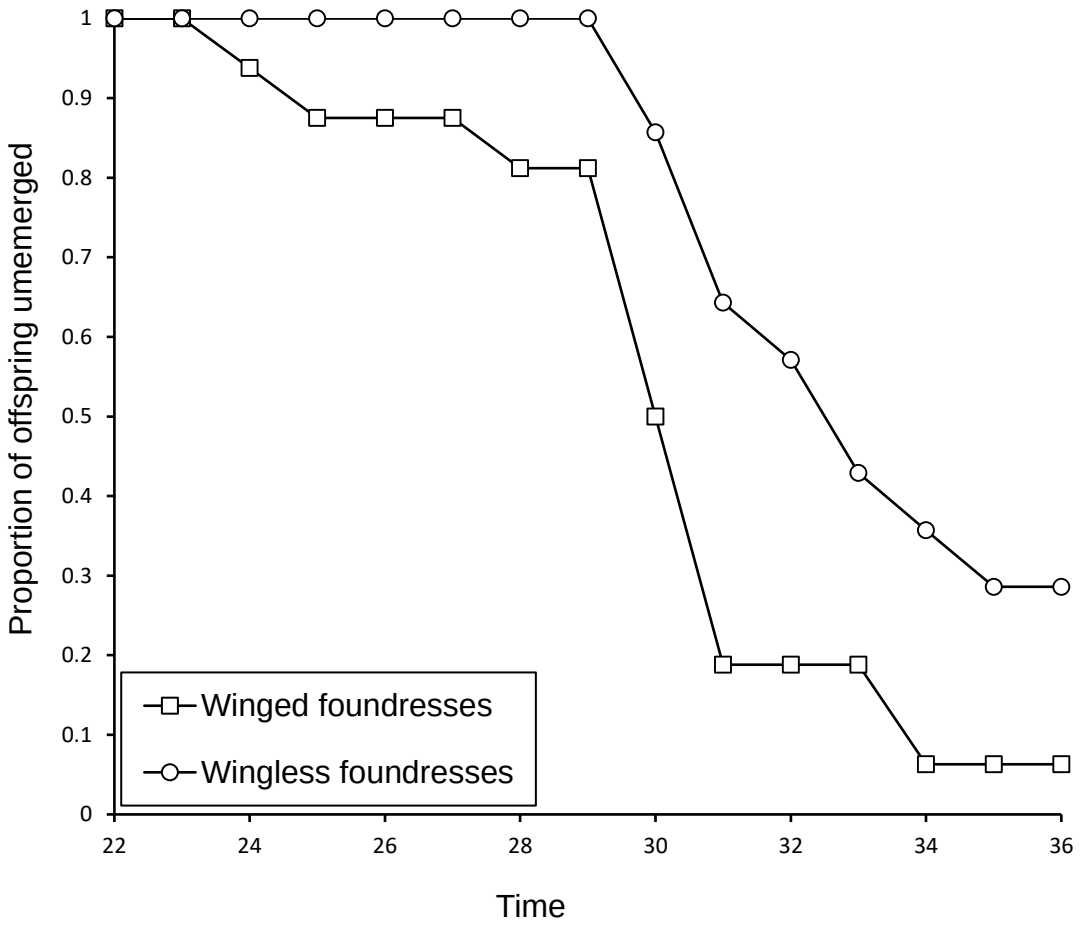


Figure 3.14 The time taken from oviposition to offspring emergence according to foundress wing dimorphism. Offspring produced by winged foundresses taken significantly less time to emerge.

3.8.9 Adult offspring size

Adult female offspring were significantly larger when foundresses were wingless than when they were winged (standard ANOVA: $F_{1,23}$=6.50, P=0.018, r^2=0.22, n=25).

3.8.10 Sex ratio

Sex ratios were not affected by foundresses wing morphology (logistic ANOVA: G_1=0.16, *P*=0.690, %Dev=0.71, n=25).

Table 3.4 Summarized wing dimorphism results.

Reproductive trait	**Wing dimorphism**
Mortality proportion	NS
Mortality timing	NS
Paralysis proportion	NS
Time to paralysis	NS
Oviposition probability	NS
Time to oviposition	Marginal significance: Wingless foundresses oviposit earlier than winged foundresses
Clutch size (CS)	NS
Per-foundress CS	NS
Offspring developmental mortality	NS
Reproductive success probability	NS
Brood size (BS)	NS
Per-foundress BS	NS
Brood developmental period	Offspring produced by winged foundresses matured earlier
Adult offspring size (FN at oviposition)	Adults produced by wingless foundresses were larger in size
Sex ratio	NS

NS = non-signfiicant effect

3.8.11 Discussion

As alate foundresses are capable of post-eclosion dispersal, they were expected to co-found broods with non-siblings more often than do apterous females, and thus differ in their cooperative behaviour and sex allocation strategies. We found no convincing effects of this dimorphism on the reproductive behaviours assessed. In terms of development, however, the offspring of winged foundresses matured earlier and were smaller than those of wingless foundresses. The scarcity of winged females is typical across *Sclerodermus* species (Tang *et al.*, 2014; Lupi *et al.*, 2017) and precluded us from including all possible combinations of wing-dimorphism and other factors into the experimental design. Current evidence does not suggest that the wing morphology of *S. brevicornis* is strongly associated with host attack behaviour or the degree of cooperation between foundress females.

3.9 Appendix 3: Public goods models of host attack

Hamilton's rule states that a trait will spread if its fitness benefits to the recipients (*b*) multiplied by the relatedness (*r*) between social partners is greater than the fitness cost (*c*) to the actor ($rb - c > 0$). There are several approaches to calculating *b* and *c* in Hamilton's rule and these are at the core of recent controversy over the general importance of kin selection in evolutionary ecology (Rousset, 2015; Nowak *et al.*, 2017; Van Veelen *et al.*, 2017). While some methods involve regression, the regressions are applied to theoretical models rather than empirical data sets and the slope of the regression represents the benefit, *b*, and its intercept the cost, *c* (Gardner *et al.*, 2011; Rousset, 2015; Nowak *et al.*, 2017; Van Veelen *et al.*, 2017). Here we estimate proxies of *b* and *c* from separate empirical (statistical) regressions, one for the effect of host size on offspring production (*b*) and the other for the effect of host size on the mortality risk to attacking foundresses (*c*). As benefits and costs are affected by host size, our proxies of *b* and *c* are in the form of regression equations rather than being fixed values applied across all host sizes (fitness costs and benefits are thus identified in terms of different currencies (Woodford, 2019)). Further, the theoretical regression method (Gardner *et al.*, 2011) assumes linearity while some of the empirical regression results to do not (e.g. because some relationships are polynomial). We refer the reader to the following papers for details on the conceptual issues at stake: (Gardner *et al.*, 2011; Nowak & Allen, 2015; Rousset, 2015; Marshall, 2016; Birch, 2017; Nowak *et al.*, 2017; Van Veelen *et al.*, 2017; Van Veelen, 2018; Woodford, 2019). We emphasise that our purpose is not to critique or to test Hamilton's rule (Bourke, 2014; Van

Veelen *et al.*, 2017; Van Veelen, 2018) but to provisionally embrace its qualitative utility.

We proceed heuristically in making use of currently available empirical evidence from three prior studies in combination with the public goods framework provided by Hamilton's rule to explore possible influences on *Sclerodermus* host attack decisions. We note that these studies assessed different ranges of host sizes and used different host and wasp species and that they were not specifically designed to provide data for our current purpose. Nonetheless, all three provide estimates of (or provide information that allows to estimate) the host size dependent probabilities of a foundress dying prior to host suppression (≈*c*) and the fitness benefits obtained from paralysed hosts (≈*b*) across a range of host sizes. Were possible we use relationships (regression equations) reported in the original papers; in the remaining cases we estimate them from raw or reconstructed data using generalized linear modelling. We use these relationships to explore, qualitatively, how variation in co-foundress relatedness (*r*) might affect selection for host attack as a public good (Figure 3.15 and see Figure 3.8 in the main part of the chapter). For this, we use an open formulation of Hamilton's rule in which the mechanism generating co-foundress relatedness is undefined (Cooper *et al.*, 2018).

For *Sclerodermus pupariae* attacking *Massicus raddei*, mortality of single foundresses was estimated by logistic regression of data reconstructed from Table 1 in Wei *et al.* (2014) using the mean host weight for each host size class. Foundress mortality increased significantly as host size increased (G_1=28.6, P<0.001) and was described by the following equation: probability of dying =1/(1+(1/(EXP((0.03201×host weight)-7.2)))) (Figure 3.15, panel A). The

benefit provided by hosts of a given size is described by the polynomial regression of offspring produced by a single foundress on host size in Wei et al.'s Figure 1c: $y= -0.02x^2 + 0.052x -3.012$. This relationship peaks at 28.488 and is shown in panel D of Figure 3.15 divided by this maximum to scale benefits as a proportion. Panel G in Figure 3.15 suggests that selection favours public goods attack when $r > 0$, except when hosts are large and that, as r increases, the maximum size of a host that a female should attack as a public good also increases.

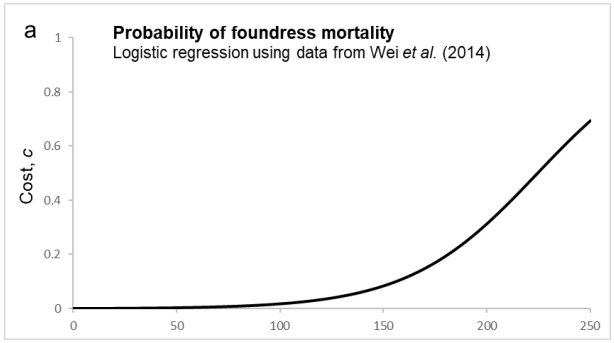
For *Sclerodermus harmandi* attacking *Monochamus alternatus*, mortality of single foundresses was estimated by logistic regression of data reconstructed from Table 1 in Liu *et al.* (2011) using the mean host weight for each host size class. Foundress mortality increased significantly as host size increased (G_1=5.96, P=0.015) and was described by the equation: probability of dying =1/(1+(1/(EXP((0.00373×host weight)-2.618)))) (Figure 3.15, panel B). The benefit provided by hosts of a given size is described by the standard regression equation given Lui et al.'s Figure 1c: y= 0.1203x +5.6948. This relationship reaches 101.93 offspring for hosts at the maximum of the range Liu et al. considered and benefits, shown in panel E, are divided by this number to scale as a proportion. Panel H of Figure 3.15 suggests that selection typically favours public goods attack when r>0.5 and that when r=0.5 a female should not attack hosts in the upper half of the weight range studies as a public good. These values also suggest that very small hosts should not be attacked by any female as a public good.

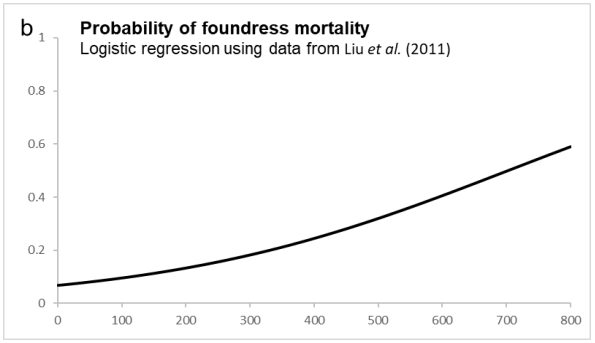
A second set of estimates for *S. harmandi* attacking *M. alternatus* were obtained using Tang *et al.* (2014) data. Mortality of single foundresses was estimated

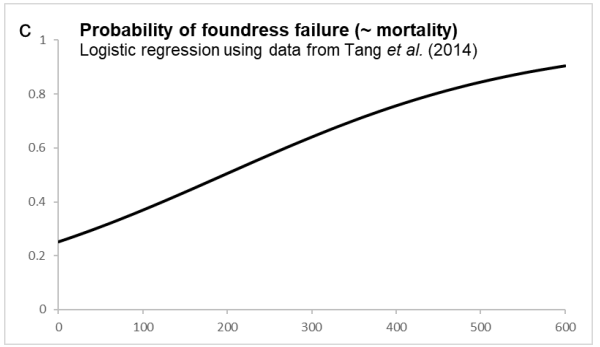
using host attack failure as a close proxy (given that failure will likely be due to parasitoid death). Foundress mortality increased significantly as host size increased (G_1=4.23, P=0.04) and was described by: probability =1/(1+(1/(EXP((0.00557×host weight)-1.095)))) (Figure 3.15, panel C). The benefit provided by hosts of a given size was obtained using data on adult offspring production across all numbers of foundresses studied. Offspring production increased marginally non-significantly with host weight (log-linear regression using quasi-Poisson errors to correct for over-dispersion: $F_{1,110}$=3.12, P=0.080) and was not polynomial (quadratic term: $F_{1,109}$=0.92, P=0.34). The regression equation was =EXP((0.001205×host weight)+3.547): this is shown in panel G of Figure 3.15 divided by the maximum predicted brood size for the host size range considered (71.52 offspring) to scale benefits as a proportion. Panel J of Figure 3.15 suggests that, unless foundresses are genetically identical, selection favours public goods host attack only when relatedness is high (0.75) and hosts <160mg. Under other conditions, females are not expected to pay the private cost of a public good.

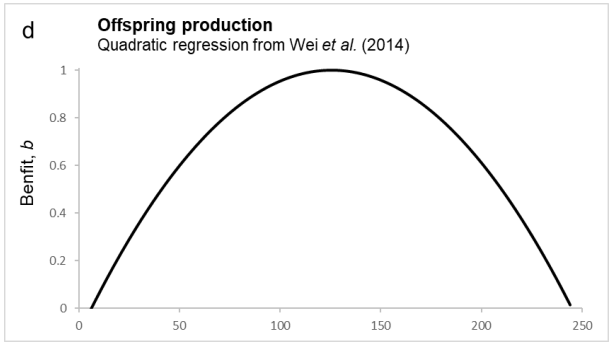
Figure 3.8 in the core of this chapter further illustrates combinations of parameters for which Hamilton's rule is satisfied for each other the parasitoid-host associations considered.

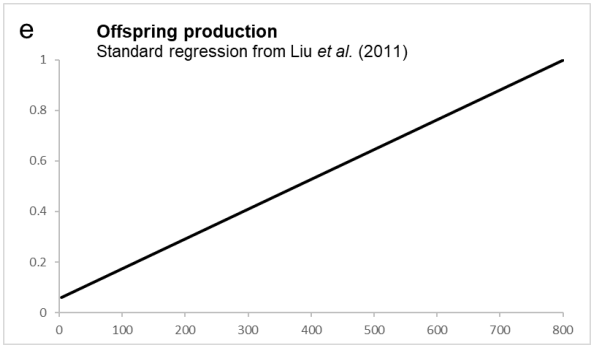
Sclerodermus pupariae attacking *Massicus raddei*

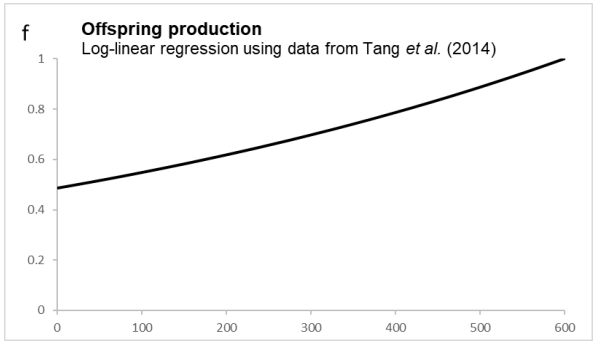


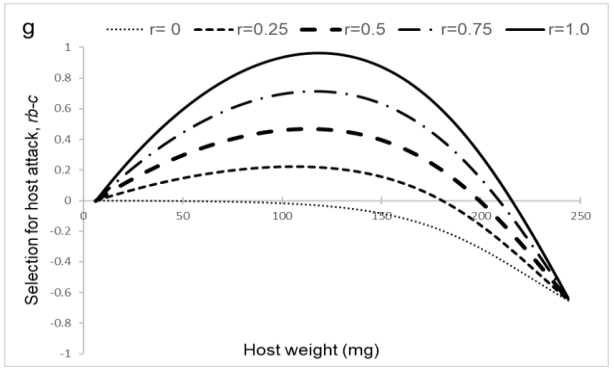


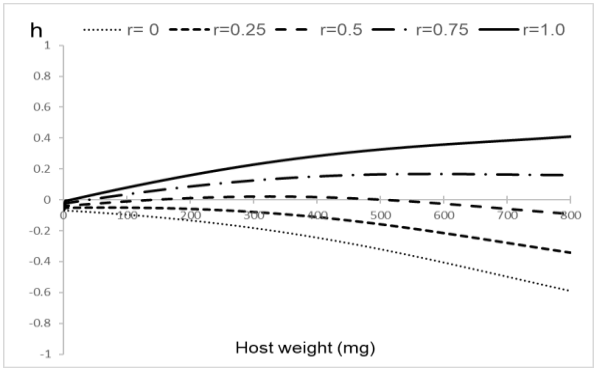


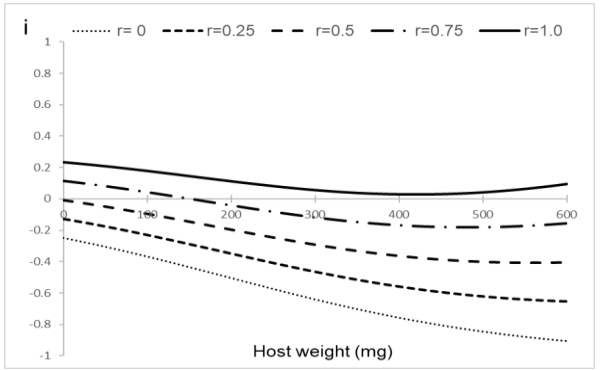












Sclerodermus harmandi attacking *Monochamus alternatus*

Figure 3.15 Public goods model of relatedness-mediated host attack. We obtained estimates of host size dependent probabilities of a foundress dying prior to host suppression (cost, *c*) and the fitness benefits obtained from paralysed hosts (benefit, *b*) across a range of host sizes and then used these in Hamilton's rule ($rb - c > 0$) to explore how variation in co-foundress relatedness (*r*) might affect selection for host attack as a public good. These estimates derive from three separate studies, of two different *Sclerodermus*-host species associations, that assessed different ranges of host sizes. In all three cases, foundress mortality increased as host size increased (panels A, B, C). The benefits (scaled to a maximum of 1) provided by suppression of a host were also host-size dependent; either increasing with host size (panels E & F) or in a curvilinear relationship (panel D). The lower panels combine costs and benefits to indicate whether public goods attack is selectively favoured. Panel G suggests public goods attack when $r>0$, except when hosts are large and that, as *r* increases, the maximum size of a host that a female should attack as a public good also increases. Panel H suggests that selection typically favours public goods attack when $r>0.5$ and that when $r=0.5$ a female should not attack hosts in the upper half of the weight range studied as a public good. These values also predict that very small hosts should not be attacked by any female as a public good. Panel I suggests that, unless foundresses are genetically identical, selection favours public goods host attack only when relatedness is high (0.75) and hosts are small. Under other conditions, females are not expected to pay the private cost of a public good. Overall, public goods attack is expected to depend on a combination of host size and relatedness in all three examples considered.

Chapter 4: Co-foundress relatedness and host dangerousness interactively affect host exploitation in a quasi-social parasitoid

4.1 Abstract

Sclerodermus brevicornis is a parasitoid of beetle larvae that exhibits cooperative multi-foundress brood production. Prior work showed that the time lag to paralysis of small sized hosts is shorter when co-foundress relatedness is higher. A public goods model, using data from congeners attacking a range of host sizes, has predicted that the greater risks and greater benefits of attacking larger hosts would combine with co-foundress relatedness to determine the limits to the size of a host that a female is selected to attack to provide essential reproductive resources to co-foundresses as well as to herself. It was also predicted that the time to host attack would, in consequence, be affected by an interaction between host size and relatedness. Here we show empirically that both host size and kinship affect *S. brevicornis* reproduction and that they interact to influence the timing of host attack. We also find effects of co-foundress relatedness after hosts have been successfully suppressed. A public goods model using parameters estimated for *S. brevicornis* again suggests that selection for individual foundresses to attack and then, if successful, share hosts will be dependent on both size of the host and the foundresses' relatedness to any co-foundresses present. Females will not be selected to bear the individual cost of a public good when hosts are large and dangerous nor when their relatedness to co-foundress relatedness is low. We conclude that while reproductive behaviours exhibited by *Sclerodermus* females can be cooperative, they are unlikely to be exhibited without reference to kinship or to the risks involved in attempting to suppress and share large and dangerous hosts.

4.2 Introduction

Sclerodermus (Hymenoptera: Bethylidae) are among the most socially complex parasitoids known (Bridwell, 1920; Wheeler, 1928; Mamaev, 1979). Females do not only exhibit maternal care to their own offspring, but a group of 'foundress' females may cooperate to tend, apparently indiscriminately, a group of offspring produced collectively on a single host (Bridwell, 1920; Kühne & Becker, 1974; Casale, 1991; Hu *et al.*, 2012; Wu *et al.*, 2014). *Sclerodermus* are considered quasi-social as they exhibit cooperative brood care without generational overlap or apparent division of labour (Tang *et al.*, 2014; Costa, 2018). Tang *et al.* (2014) showed that co-exploitation of large hosts provides direct fitness benefits (in terms of average reproductive success) to individual females within *Sclerodermus harmandi* foundress groups and thus that quasi-sociality could be explained without reference to inclusive fitness benefits, an often invoked factor in social evolution studies (Hamilton, 1964a; Costa, 2006; Davies *et al.*, 2012; Costa, 2018).

While Tang *et al.* (2014) showed that kin selection need not be required to explain *Sclerodermus* sociality, a role for inclusive fitness effects in the reproductive cycle was not precluded. Abdi *et al.* (Chapter 3) subsequently showed that, in *Sclerodermus brevicornis,* kinship among foundress groups affects the timing of host attack: paralysis of standard sized hosts was more rapid when co-foundress relatedness is higher. Attacking hosts carries considerable mortality risks for *Sclerodermus* females, especially when hosts are large (Kühne & Becker, 1974; Liu *et al.*, 2011; Tang *et al.*, 2014; Wei *et al.*, 2014) and, while females must ultimately access a host in order to reproduce, they may be reluctant to take risks when reproductive benefits may be shared

with non-kin. Using data from several *Sclerodermus* species, Abdi *et al.* (Chapter 3) employed a simple public goods model based on Hamilton's rule (Hamilton, 1964a; Cooper *et al.*, 2018) to explore how three parameters, the greater risks and greater benefits of attacking larger hosts and co-foundress relatedness, could combine to determine the size ranges of hosts that a female is selected to attack to provide reproductive resources to co-foundresses as well as to herself. This suggested that when foundresses are more closely related, larger hosts will be attacked by individuals as a public good. It also suggested that, in general, effects of kinship would be least marked when hosts are small and relatively safe to attack and may be greater when hosts are large and dangerous to attack, and thus that co-foundress relatedness and host dangerousness should interact to affect the timing of host attack.

Here we test empirically the combined effects of host size and *S. brevicornis* co-foundress relatedness. We hold foundress number constant, at two females, and vary relatedness such that co-foundresses were either brood-mates or non-siblings. We provide foundress pairs with a single host, drawn from a wide range of host sizes, larger hosts being more dangerous to attack. We evaluate the timing and success of host attack behaviour and other aspects of reproductive success, and find effects of kinship including an interaction between co-foundress relatedness and host dangerousness. We also assay the success of single foundresses attacking hosts of different sizes, the timing of attack and the probability of dying during attack, confirming that larger hosts are more dangerous for individual females to attack. We use our empirical estimates of the host-size dependent risks of host attack and on offspring production on successfully supressed hosts in a new model of host attack by *S. brevicornis*.

As with models for other *Sclerodermus* species, this suggests host size and foundress kinship will interact to influence host attack behaviour and, specifically, that individual *S. brevicornis* foundresses will be selected to attack and then share hosts as a public good only when their relatedness to co-foundresses is high and the host is neither large nor very small.

4.3 Materials and Methods

4.3.1 Insect culturing

Sclerodermus brevicornis (Kieffer) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid wasp which is a parasitoid of European long-horned beetle larvae (Coleoptera: Cerambycidae) (Kieffer, 1914; Lupi *et al.*, 2017) that can be reared on several non-native, invasive cerambycids, including the larvae of yellow long-horned beetle, *Psacothea hilaris hilaris* (Pascoe) (Lupi *et al.*, 2017) which was used as a host in this study. Stocks of *P. h. hilaris* were maintained according to Lupi *et al.* (2017) on the standard diet described by Limonta *et al.* (2009). Stocks of *S. brevicornis* were reared on late instar larvae of *P. h. hilaris*. All cultures and experiments were maintained in a climate chamber at 25±1°C, and 16H: 8H L:D photoperiod and 60±5% relative humidity. To synchronize parasitoid and host availability, newly emerged *S. brevicornis* adults were stored in a refrigerator at 5±0.5°C for a maximum of 7-10 days prior to use in experiments: we have found that such storage does not affect their subsequent performance (D.L. & C.J. pers. obs.).

4.3.2 Two-foundress experiment

The experiment followed a 2×3 factorial design, varying foundress relatedness and host size and holding foundress number constant. In each replicate, a larva

host was placed individually into a plastic container (5 cm diameter and 4 cm depth) in which there was a thin layer (*ca.* 1.5 mm) of cork oak granules to maintain the relative humidity and to allow the parasitoids to access the part of the host which otherwise would be in contact with the container. Hosts were classified as small (mean weight = 0.0476 g, range = 0.0213 to 0.0825 g, SD = 0.0197), medium (mean = 0.2851 g, range = 0.2142 to 0.3671 g, SD = 0.0415) or large (mean = 0.7020 g, range = 0.4921 to 1.0522 g, SD = 0.1367); the weight of the host in each replicate, not just its size class, was also recorded. Note that our 'small' size class corresponds closely to the standardized sizes of the factitious host used by Abdi *et al.* (Chapter 3) and also to the 'small' host size class of *P. hilaris* used by Lupi *et al.* (2017). Then two adult female *S. brevicornis* were added into each vial. The relatedness between co-foundresses was varied such that either foundress were brood-mates (often sisters) that had developed in the same brood or were non-siblings that had developed in different broods. To give adequate overall statistical power (Smith *et al.*, 2011), we set up 20 replicates of each foundress relatedness and host size combination (20 replicates × 2 foundress relatedness levels × 3 host size levels = 120 replicates overall).

Replicates were left undisturbed for two days as observing the wasps within this period has previously been found to disrupt their reproductive behaviours (D.L. & C.J. pers. obs.). We then observed each replicate once per day until the 23rd day. At each observation, we recorded whether the host had been paralysed, whether any oviposition occurred, whether any foundresses had died and the number and sex of adult offspring produced.

4.3.3 Single-foundress assay

The assay consisted of presenting single female *S. brevicornis* with a host of known weight and varying host weight considerably across replicates (range = 0.0227 to 0.9408 g, mean = 0.3536, SD = ±0.2723, n=37). In each replicate, a larva host was placed individually into a plastic container with cork granules, as described above. Then a single adult female *S. brevicornis* was added into each container. The container was covered with a white plastic cover and left undisturbed for 7 days. We then observed each replicate once per day for a further 14 days. At each observation, we recorded whether the host had been paralysed, whether any oviposition occurred, whether the foundresses had died and whether dead foundresses had been bitten into two parts by the host.

4.3.4 Statistical analyses

4.3.4.1 Single and two-foundress experiments

In the two-foundress experiment, the explanatory variable foundress relatedness was always fitted as a factor with two levels. In analyses of single-foundress and two-foundress data, host size was fitted as a factor with three levels in some analyses and as a continuous variable in others. The response variables were the parameters recorded during the daily observations and at the end of the experiment.

Binary data (on host paralysis, acceptance, foundress mortality and bisection) were explored using logistic analyses assuming binomially distributed errors and a logit link function; here the test statistic is change in deviance, *G*, which is approximately χ^2 distributed (Crawley, 1993). Other logistic analyses of proportional data with denominators larger than unity (e.g. sex ratio) assumed

quasi-binomial error distributions and adopted a logit link function; the test statistic was the *F*-ratio (Crawley, 1993; Wilson & Hardy, 2002). Logistic analyses comparing foundress mortality during the successive stages of host handling (2-foudress experiment) employed mixed models (GLMM and Wald χ^2 statistics), with replicate fitted as a random factor. Integer data (e.g. number of offspring produced) were explored using log-linear analyses assuming quasi-Poisson error distributions, adopting a log-link function and generating *F*-ratio test statistics (Crawley, 1993). Data derived from integers (e.g. offspring production per foundress) that were likely to be approximately Poisson-distributed were also analysed using log-linear models (Faraway, 2006; Tang *et al.*, 2014). Following logistic and log-linear analyses, we give the percentage deviance explained (% Dev) as a descriptor analogous to r^2.

Time-to-event data on host paralysis, oviposition and foundress mortality were analysed using parametric cohort survival analyses with censoring (Aitkin *et al.*, 1989; Crawley, 1993). We first compared exponential models (constant hazard function) against Weibull models (time dependent hazard function): in all cases, the Weibull models provided a significantly better description of the data. The influences of the candidate explanatory factors (see above) were then explored by their inclusion into the Weibull models (Aitkin *et al.*, 1989; Crawley, 1993). In one case, we used a non-parametric log-rank test due to poor fit to parametric assumptions.

All statistical tests were two sided. All analyses were carried out using the statistical software package *GenStat* (version 17, VSN International, Hemel Hempstead, UK).

4.3.4.2 Public goods modelling

We consider the selective forces that might combine to influence an individual female *S. brevicornis* host attack decision by using a public goods model. Public goods models consider situations in which a focal individual produces, at personal cost, a benefit which is then shared between the individual and its social partners. Hamilton's rule asserts that a trait will spread if its fitness benefits to the recipients (*b*) multiplied by the relatedness (*r*) between social partners is greater than the fitness cost (*c*) to the actor ($rb - c > 0$) (Hamilton, 1964a; Cooper *et al.*, 2018). As approaches to calculating *b* and *c* are under ongoing debate (Gardner *et al.*, 2011; Abdi *et al.*, Chapter 3), we proceed heuristically in making use of our empirical estimates of the host size dependent probability of a foundress dying during host attack (cost, *c*) and of the fitness obtained from a successfully paralysed host of a given size (benefit, *b*) to explore how co-foundress relatedness (*r*) might affect selection for host attack as a public good.

4.4 Results

4.4.1 Two-foundress experiment

4.4.1.1 Foundress mortality

Approximately a quarter (23.75%, 57/240) of foundresses died by the twenty-third day after presentation with a host. Foundresses that were brood-mates died earlier than non-sibling foundresses (cohort survival analysis with surviving females treated as censors: $G_1 = 5.7$, $P = 0.020$, %Dev = 1.98, Figure 4.1). In this analysis, the longevity of each of the pair of foundress was treated as if it were independent of the other; such pseudo-replication could have generated

the significance. We therefore ran an additional analysis non-parametric log-rank test on the mean longevity of females in each replicate: this also indicated that brood-mates died earlier than did non-siblings (z = 4.919, d.f. = 1, P = 0.027). Time to death was not significantly affected by host size (G_2 = 2.4, P = 0.301, %Dev = 0.88) or by its interaction with foundress relatedness (G_2 = 2.9, P = 0.235, %Dev = 1.10).

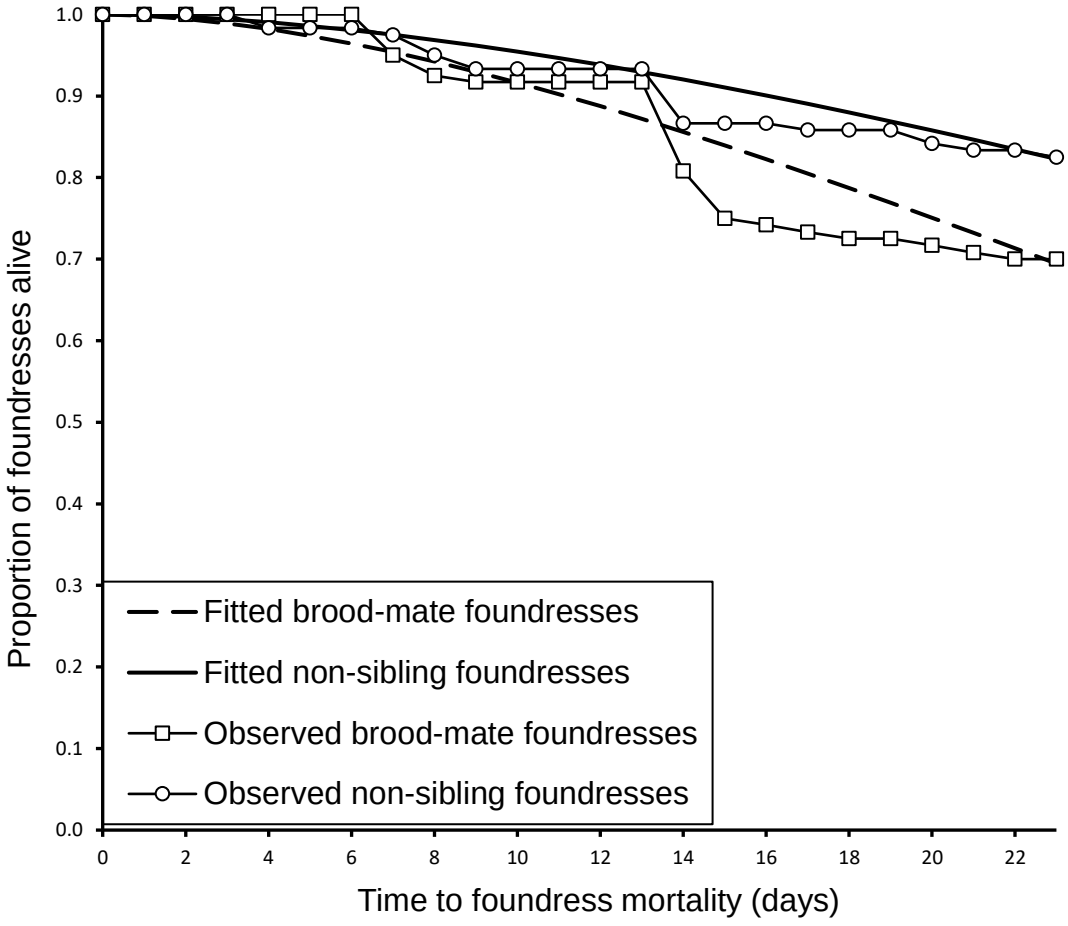


Figure 4.1 The effect of relatedness on foundress survival over time.

Similarly, the proportion of foundresses in a replicate dying (number dying/2) was significantly higher when foundresses were brood-mates (logistic ANOVA: G_1 = 5.23, P = 0.022, %Dev = 3.15, Figure 4.2) but was not affected by host

size (G_2 = 1.14, P = 0.321, %Dev = 1.37) or its interaction with foundress relatedness (G_2 = 1.52, P = 0.218, %Dev = 1.84). However, separate analyses within each host size class indicated that mortality of brood-mate foundresses was significantly higher when presented with medium sized hosts (logistic ANOVA: G_1 = 7.62, P = 0.006, %Dev = 12.71, Figure 4.2) but did not differ when hoster were large (G_1 = 0.62, P = 0.430, %Dev = 1.09) or small (G_1 = 0.08, P = 0.774, %Dev = 0.18).

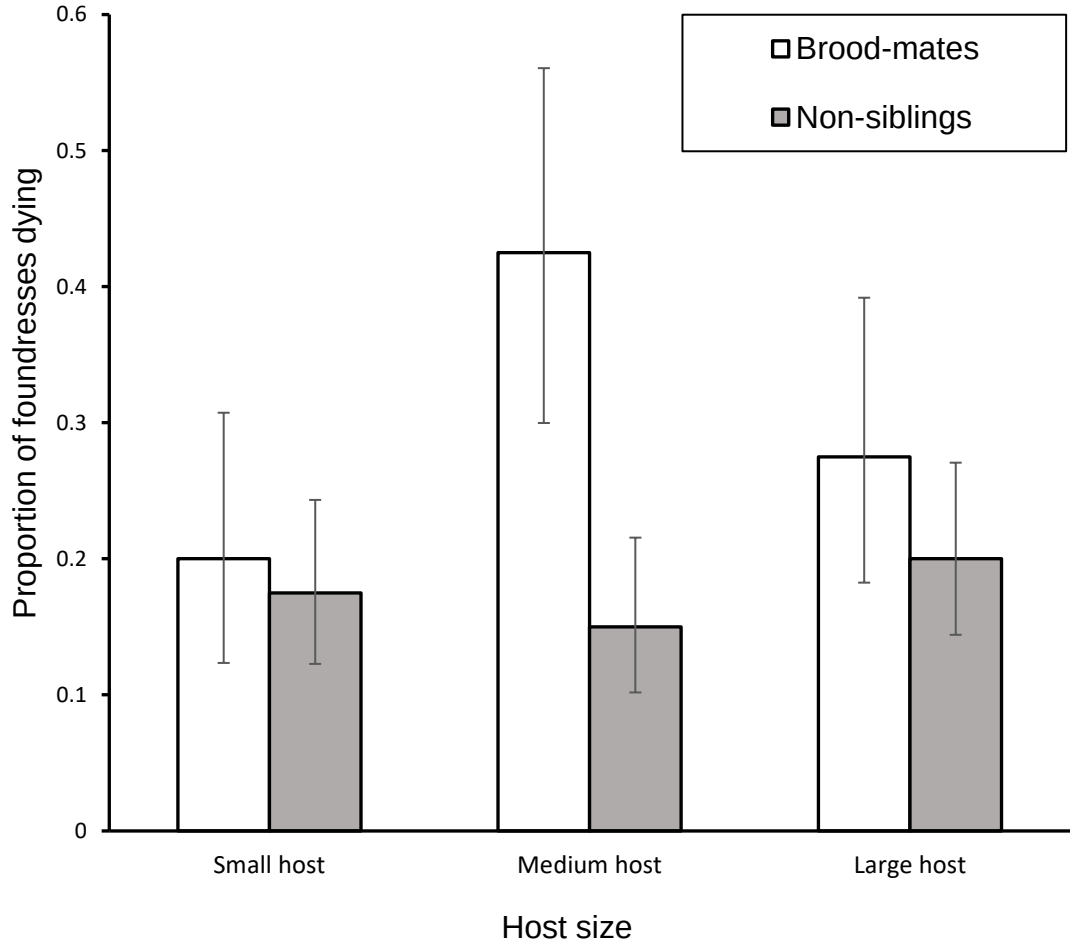


Figure 4.2 Foundress mortality according to host size and relatedness. Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale. Differences between brood-mates and non-siblings on small or large hosts were not significant.

We further explored foundresses mortality in relation to the successive host-handling periods: pre-paralysis (host presentation to host paralysis), paralysis-to-oviposition and post-oviposition (Figure 4.3). The proportion of foundress that died differed significantly according to relatedness (mortality was highest amongst brood mates, GLMM: Wald $\chi^2 = 4.06$, d.f. = 1, $P = 0.046$) and between periods (post-oviposition mortality was lowest, $\chi^2 = 17.29$, d.f. = 2, $P < 0.001$) but without significant interaction between relatedness and period ($\chi^2 = 2.12$, d.f. = 2, $P = 0.348$). Host size affected mortality through an interaction with period (size: $\chi^2 = 2.59$, d.f. = 2, $P = 0.279$; size × period: $\chi^2 = 15.48$, d.f. = 4, $P = 0.005$), with pre-paralysis mortality being lowest when foundresses were presented with small hosts. There was no significant interaction between relatedness and size ($\chi^2 = 2.81$, d.f. = 2, $P = 0.250$) or between all three main effects ($\chi^2 = 0.04$, d.f. = 3, $P = 0.998$).

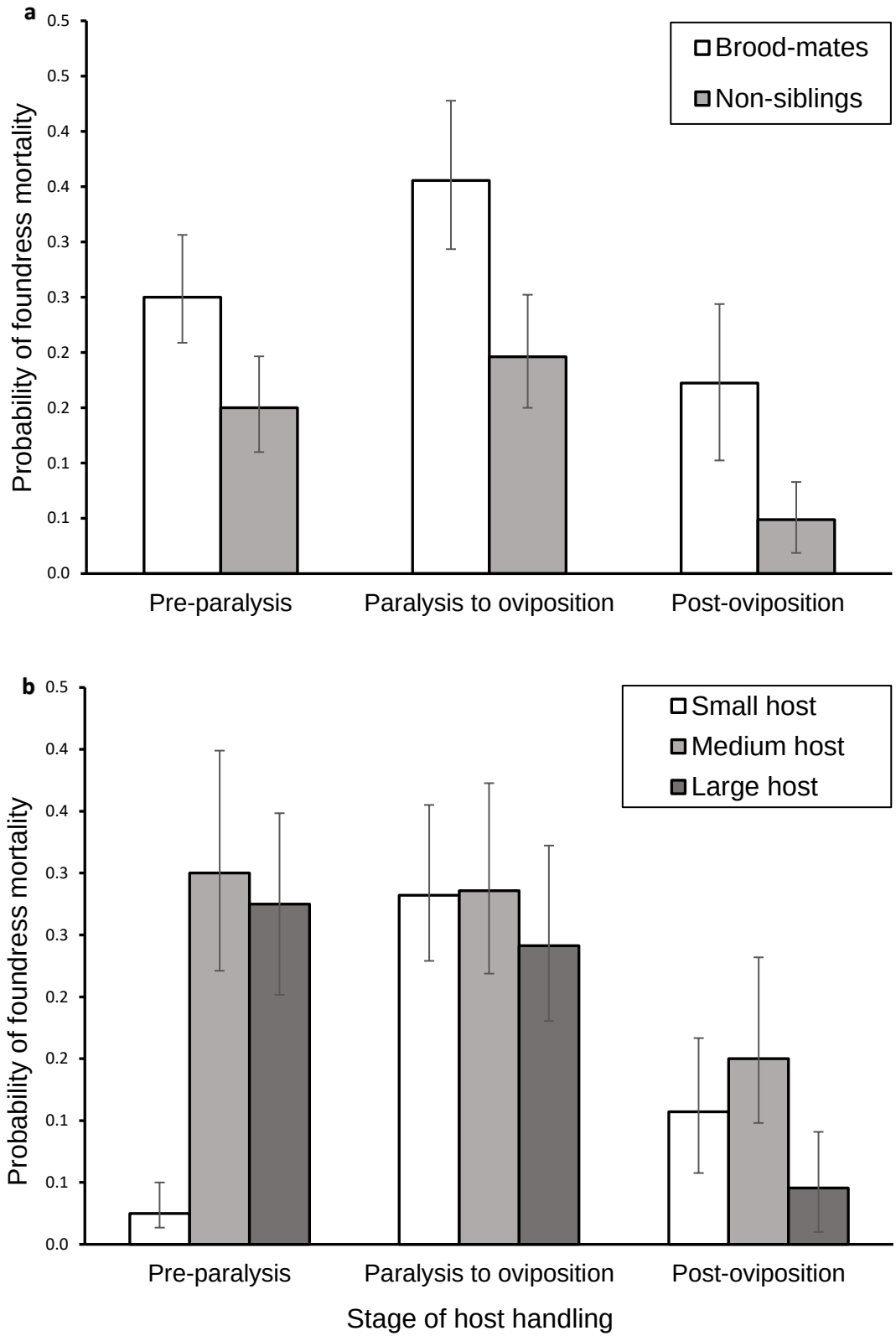


Figure 4.3 Foundress mortality according to relatedness (a) and host size (b) during the successive stages of host handling. Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale.

4.4.1.2 Paralysis

The percentage of hosts that were paralysed by *S. brevicornis* foundresses was 83.33% (n = 120). This proportion decreased significantly with an increase in host size (logistic ANCOVA: G_1 = 15.90, P<0.001, %Dev = 14.70, Figure 4.4) but was unaffected by relatedness (G_1 = 0.20, P = 0.653, %Dev = 0.19) or its interaction with host size (G_1 = 1.55, P = 0.213, %Dev = 1.43).

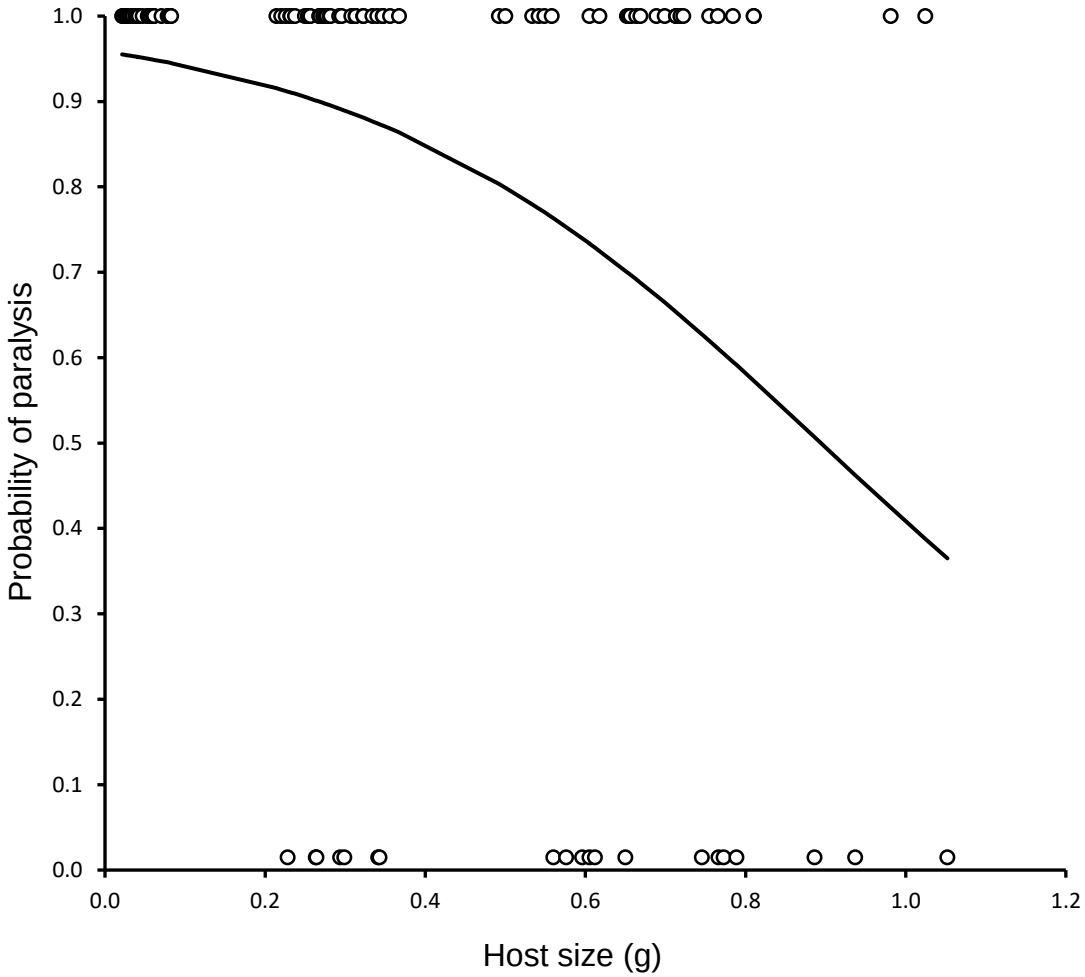
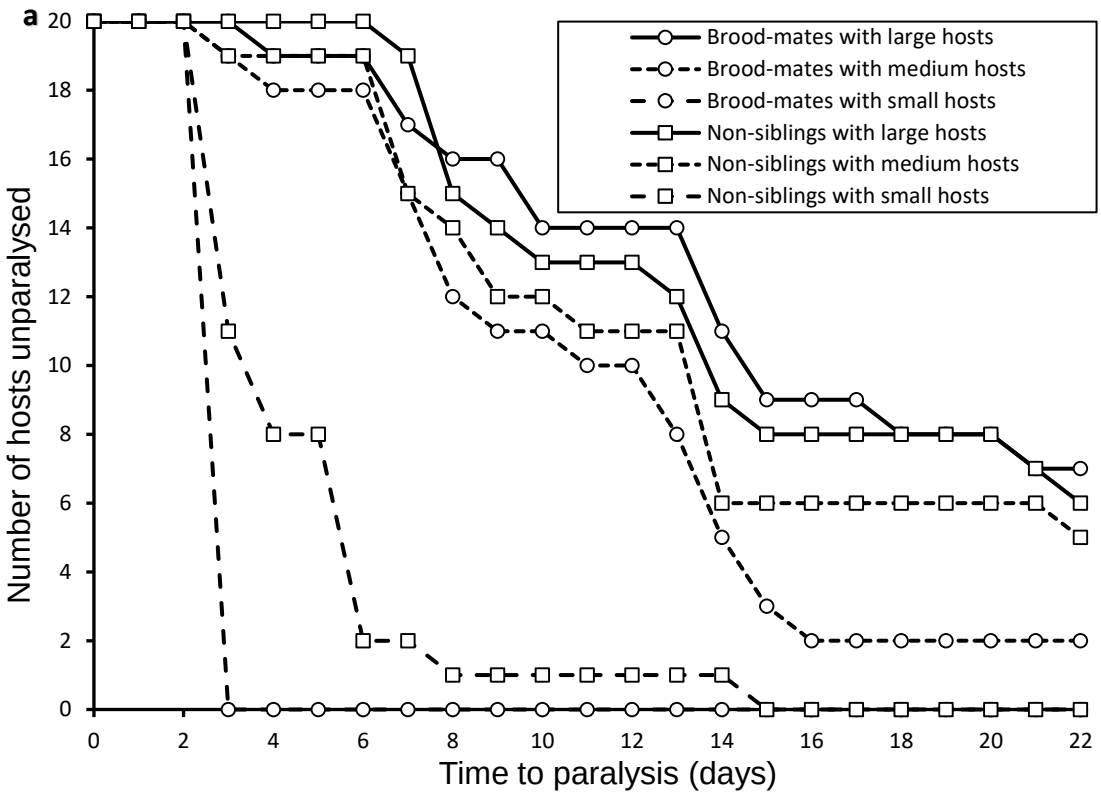


Figure 4.4 The effect of host size on probability of host paralysis.

Among the 100 replicates in which host paralysis occurred, the time taken to paralyse the host ranged up to 22 days. The 20 replicates in which there was no paralysis observed were treated as censors in the subsequent cohort survival analysis. Time-to-paralysis was significantly affected by host size (G_2 = 96.5, P<0.001, %Dev = 37.36), with smaller hosts paralysed faster than larger hosts. Foundress relatedness also affected time-to-paralysis (G_1 = 9.5, P = 0.002, %Dev = 5.97) and there was a significant interaction between relatedness and host size (G_2 = 9.5, P = 0.009, %Dev = 7.80): for small hosts, paralysis was significantly more rapid when foundresses were brood-mates (G_1 = 20.4, P < 0.001, %Dev = 46.99), for medium sized hosts, paralysis by brood-mates was non-significantly earlier (G_1 = 2.10, P = 0.147, %Dev = 3.61) and for large sized hosts paralysis by brood-mates was non-significantly later (G_1 = 0.1, P = 0.752, %Dev = 0.16) (Figure 4.5). We checked these results by repeating the analysis with host size fitted as a variate rather than as a categorical factor: the interpretation remained the same (Host size: G_1 = 60.1, P<0.001, %Dev = 29.09; Relatedness: G_1 = 4.3, P = 0.038, %Dev = 2.64; Interaction: G_1 = 4.1, P = 0.043, %Dev = 2.47).


a
Brood-mates with large hosts
Brood-mates with medium hosts
Brood-mates with small hosts
Non-siblings with large hosts
Non-siblings with medium hosts
Non-siblings with small hosts
Number of hosts unparalysed
Time to paralysis (days)

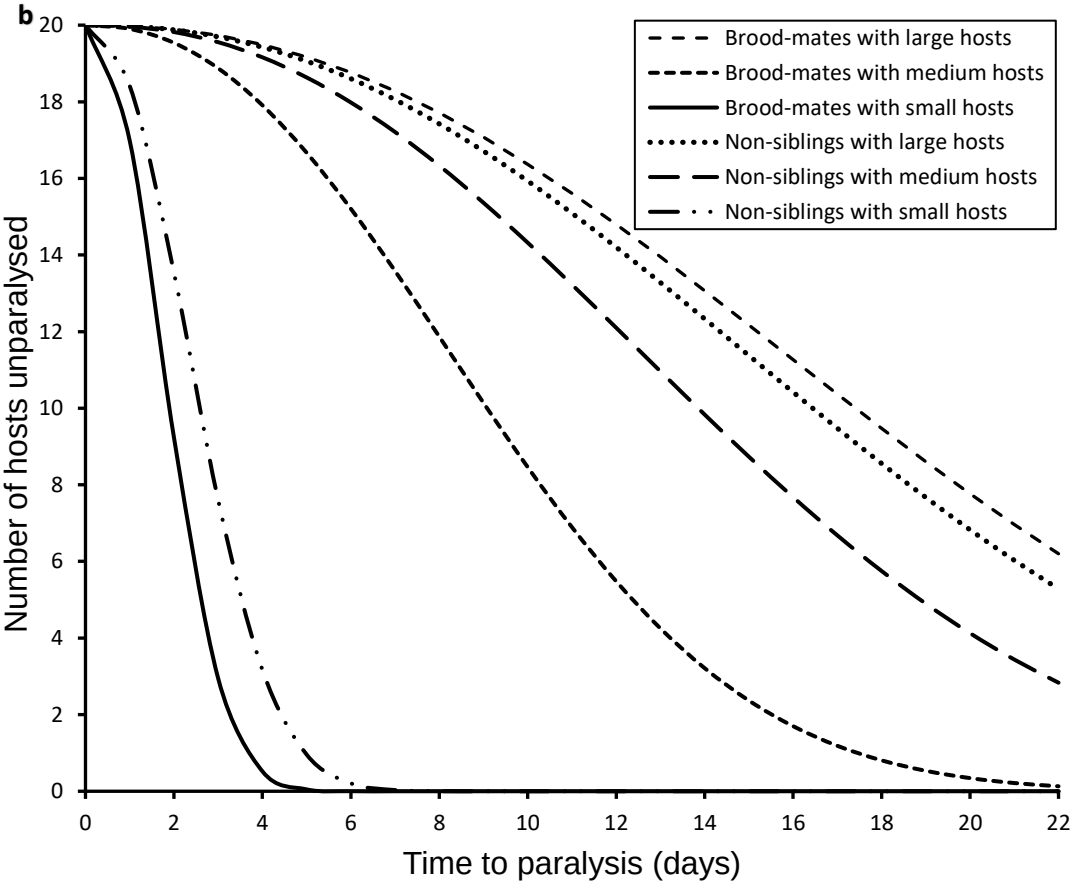

b
Brood-mates with large hosts
Brood-mates with medium hosts
Brood-mates with small hosts
Non-siblings with large hosts
Non-siblings with medium hosts
Non-siblings with small hosts
Number of hosts unparalysed
Time to paralysis (days)

Figure 4.5 The effects of co-foundress relatedness and host size on time to paralyse the host. The upper panel shows the cohort data from the six treatment combinations and the lower panel shows the fitted survival model, estimated from Weibull analysis with unparalysed hosts treated as censors. To avoid disruption of the females' behaviours no observations were taken during the first two days and the statistical model was fitted assuming no hosts were paralysed during this period. Foundress relatedness and host size interacted to influence time-to-paralysis: effects of kinship were significant only when hosts were small.

4.4.1.3 Oviposition

The percentage of presented hosts which were oviposited on was 73.33% (n = 120), excluding the 20 hosts that were not paralysed, the percentage was 88%. The probability of oviposition on paralysed hosts was not significantly affected by host size (logistic ANCOVA: $G_1 = 0.07$, P = 0.791, %Dev = 0.10, n = 100), relatedness ($G_1 = 1.36$, P = 0.243, %Dev = 1.86) or by an interaction between host size and relatedness ($G_1 = 2.41$, P = 0.121, %Dev = 3.29). Once a host was paralysed, time to oviposition was not affected by host size (cohort survival analysis with foundresses that did oviposit by day 20 treated as censors, $G_2 = 5.2$, P=0.074, %Dev = 3.58) or by an interaction between host size and relatedness ($G_2 = 2.4$, P = 0.301, %Dev = 1.93) but non-siblings oviposited significantly earlier ($G_1 = 7.70$, P = 0.006, %Dev = 5.50). There was, however, no difference between non-siblings and brood mates in the time taken to oviposition when data were analysed from the time of host presentation $G_1 = 0.2$, P = 0.655, %Dev = 0.10).

4.4.1.4 Brood size

The total number of adult offspring produced from a host which had been oviposited on (n = 59) ranged between 1 and 163. Total brood size had a significantly curvilinear response to increasing host size (log-linear ANCOVA including a quadratic term: host size: $F_{1,55} = 14.50$, $P < 0.001$, %Dev = 13.95, host size^2: $F_{1,55} = 32.41$, $P < 0.001$, %Dev = 31.18, Figure 4.6 (a)) and was also affected by foundresses relatedness via a significant interaction with host size (relatedness: $F_{1,55} = 0.356$, $P = 0.064$, %Dev = 3.43; interaction: $F_{1,55} = 6.42$, $P = 0.014$, %Dev = 6.18, Figure 4.6 (a)); non-siblings typically produced larger broods. Examining the same data in terms of mean per-foundress offspring production (brood size/number of foundresses surviving at the time of oviposition) also found a curvilinear response to increasing host size (host size: $F_{1,55} = 10.08$, $P = 0.002$, %Dev = 10.41; host size^2: $F_{1,55} = 27.22$, $P < 0.001$, %Dev = 28.10) and an interaction between foundress relatedness and host size (relatedness: $F_{1,55} = 9.88$, $P = 0.003$, %Dev = 10.20; interaction: $F_{1,55} = 11.40$, $P = 0.001$, %Dev = 11.77, Figure 4.6 (b)); non-siblings typically smaller broods than brood-mate foundresses when hosts were small but relatively larger broods when hosts were large.

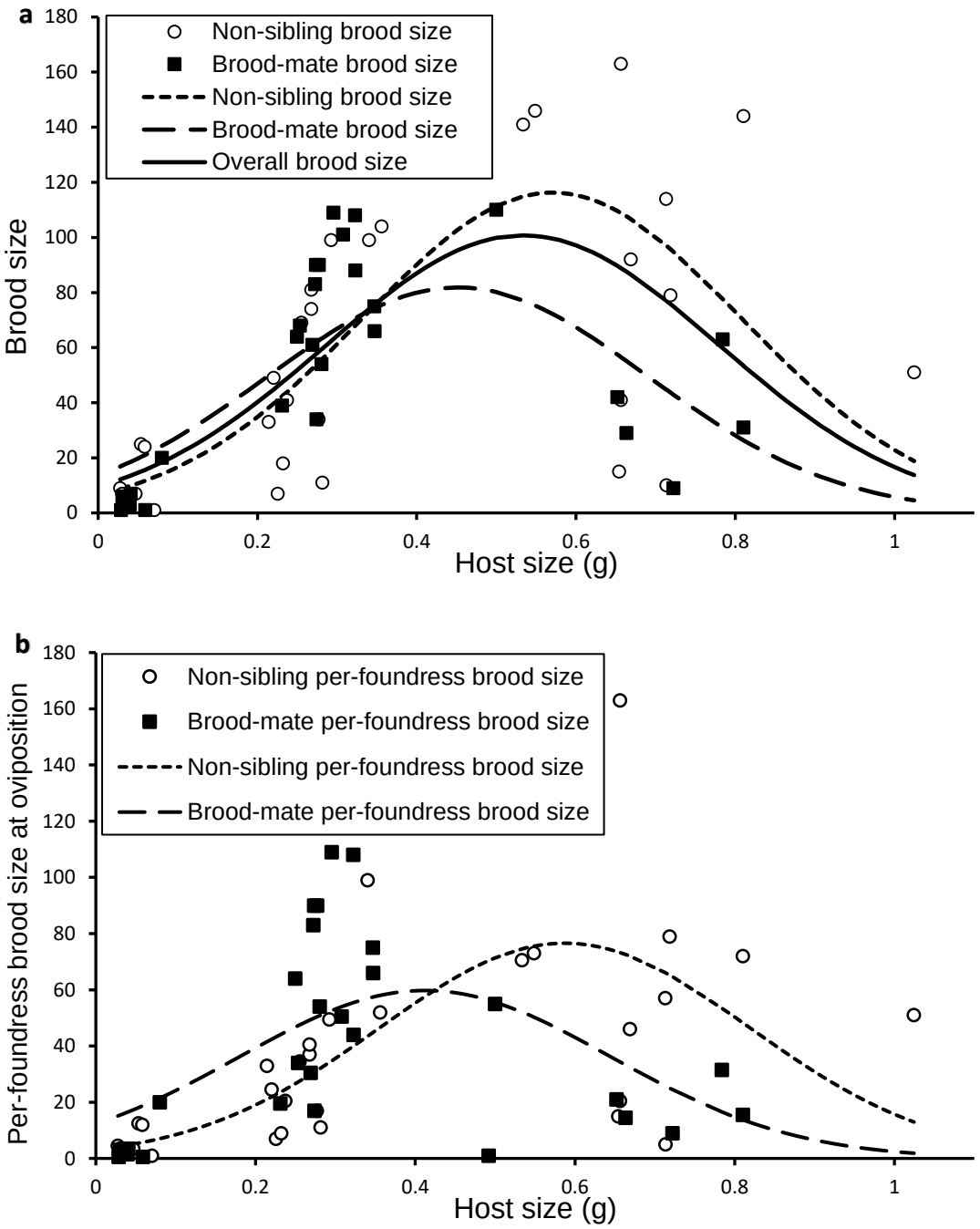


Figure 4.6 Adult offspring produced according to relatedness and host size. (a) Total brood size. (b) Per-foundress offspring production. Regression lines were obtained by log-linear ANCOVAs including a quadratic term. Offspring production differed according to relatedness so separate lines are shown for brood-mate and non-sibling foundresses. The regression line for overall brood size on panel (a) is used as the host-size dependent benefit, b, in the public goods model: fitness benefits obtained from paralysed host = EXP (8.82 × host size – 8.27 × host size^2 + 2.261).

4.4.1.5 Sex ratio

Offspring broods sex ratios were strongly female biased: the mean proportion of offspring that were male was 0.0339 (+S.E. = 0.0039, -S.E. = 0.0035, n = 59). Due to the mortality of some foundresses before oviposition, broods were either produced by a single surviving foundress from a sibling group; a single surviving foundress from a non-sibling group; two surviving sibling foundresses or two surviving non-sibling foundresses. The effect of varying foundress number at oviposition was explored by fitting it as a 2-level factor along with relatedness and host size in a logistic 3-way ANOVA. Sex ratios were less female biased when pairs of foundresses oviposited together ($F_{1,58}$ = 4.08, P = 0.049, %Dev = 7.19, Figure 4.7) but were not significantly affected by relatedness ($F_{1,55}$ = 0.00, P = 0.948, %Dev = 0.01), host size ($F_{2,57}$ = 0.40, P = 0.670, %Dev = 1.42) or by any interactions between these main effects.

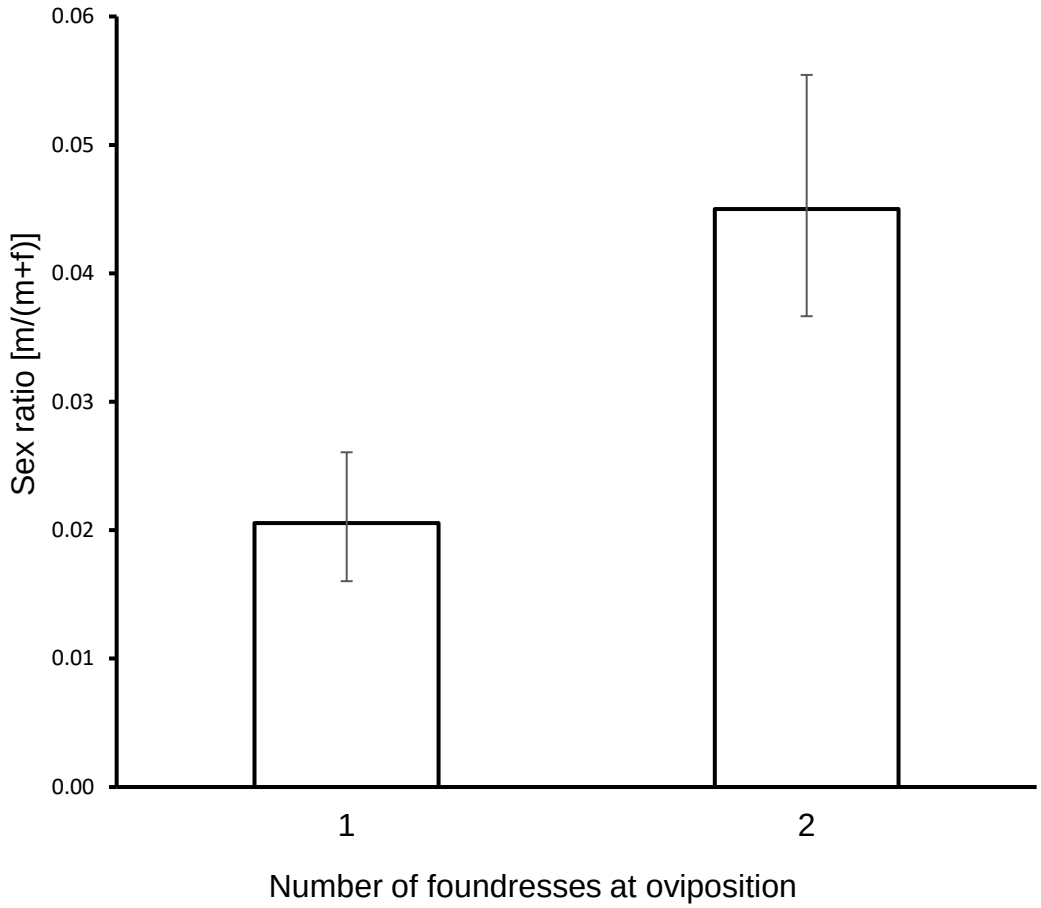


Figure 4.7 Sex ratios of broods according to the number of foundresses surviving to the time of oviposition. Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale.

Sex ratios were not significantly affected by brood size (logistic regression: $F_{1,57}$ = 3.08, P = 0.085, %Dev = 5.12). The number of male offspring produced increased significantly with an increase of brood size (log-linear regression: $F_{1,58}$ = 25.39, P < 0.001, %Dev = 30.82, Figure 4.8). No other measured variables or their interactions affected the number of males per brood significantly.

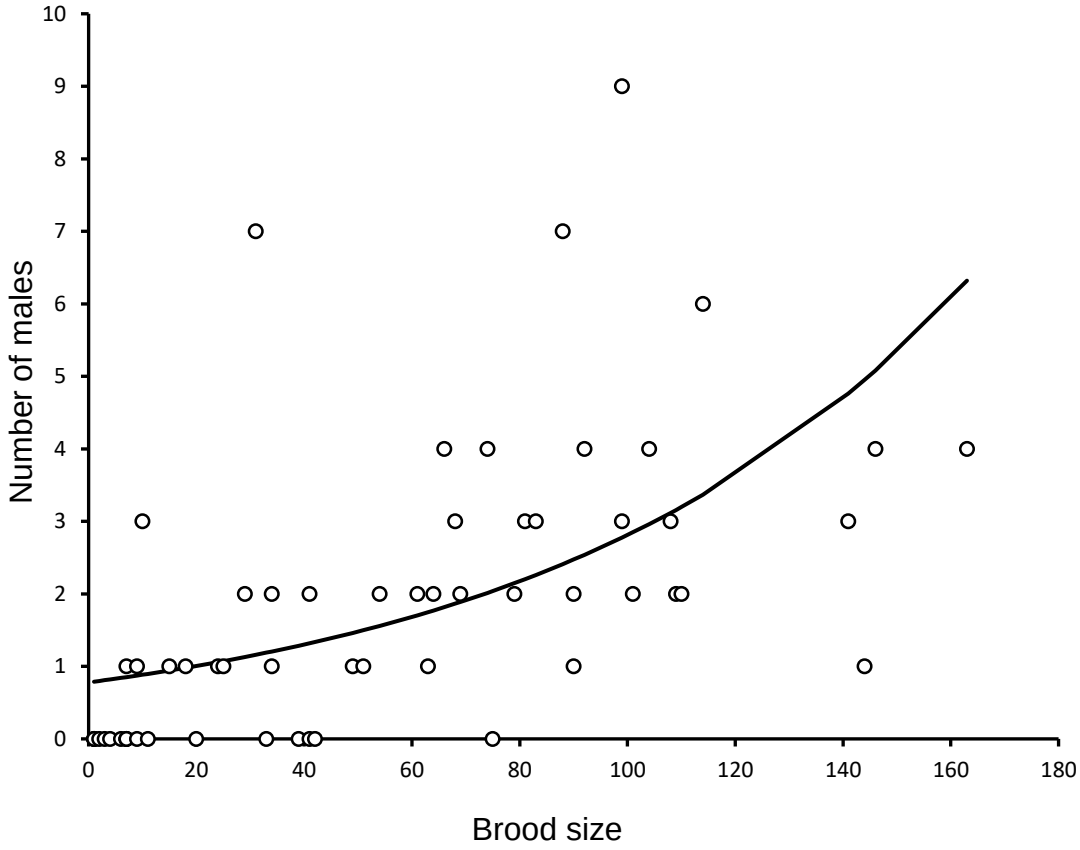


Figure 4.8 The relationship between the number of males in a brood and brood size.

4.4.2 Single-foundress assay

4.4.2.1 Probabaility of paralysis and oviposition

Amost 60% (22/37) of hosts prestented to a single *S. brevicornis* foundress became paralysed. The probabaility of paralysis decreased significantly with an increase of host size (logistic regression: G_1 = 11.68, P<0.001, %Dev = 23.39, Figure 4.9 (a)). Once a host was paralysed, the probablity that the foundress oviposited on it was also almost 60% (13/22) and was not affected by the size of the host (logistic regression: G_1 = 0.00, P=0.957, %Dev = 0.01).

4.4.2.2 Foundress mortality

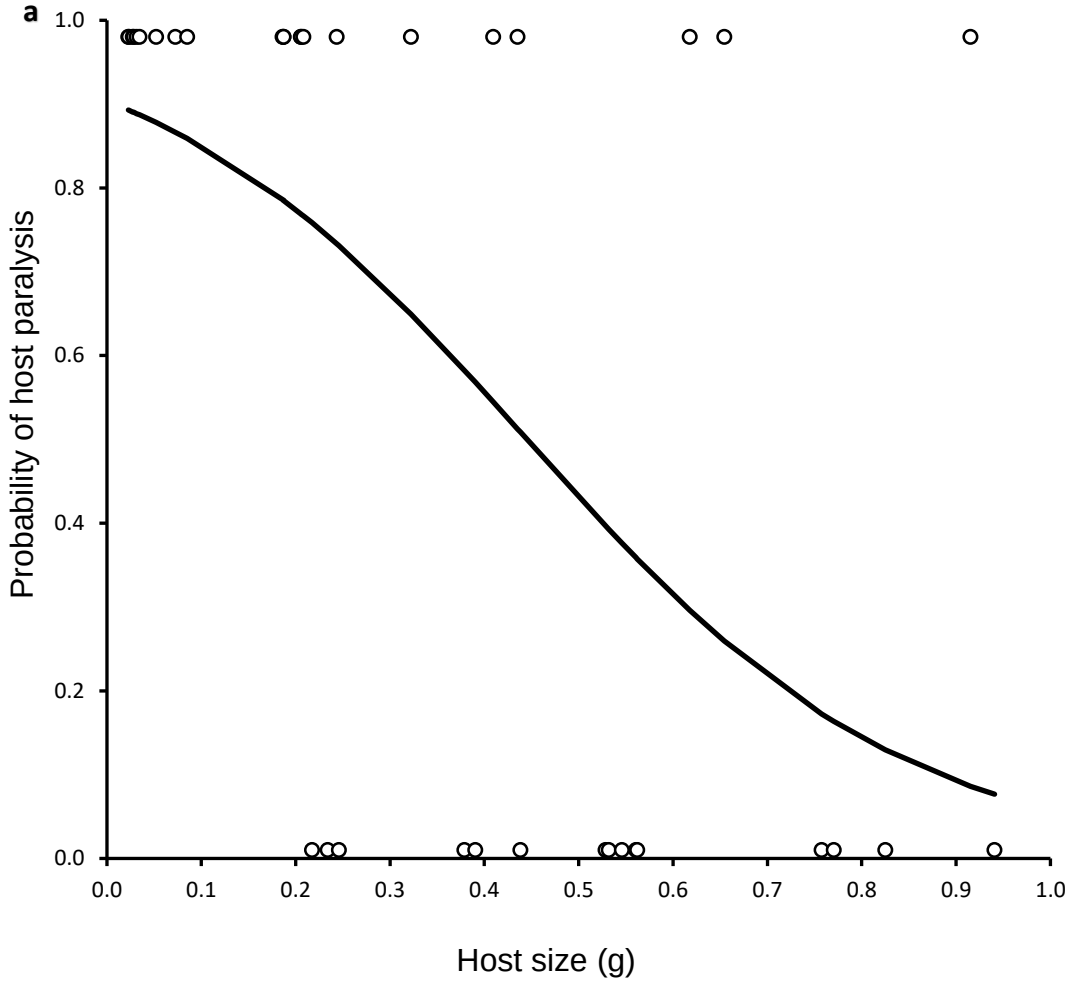
Typically, when a female *S. brevicornis* attacks a host, either the host is paralysed or the wasp is killed (Table 4.1). In three of the 37 replicates the

female apparently did not attack the host within the 21 day observation period as neither the host was paralysed nor foundress died (Table 4.1); these were excluded from estimation of the probability of mortality as a consequence of attack (cost parameter, c, in the public goods model, see below). Among the remaining replicates, around 40% (14/34) of the foundresses died. In two cases, the foundress died and the host was paralysed (Table 4.1): in one of these the foundress was cut in half, suggesting the host killed it during wasp attack and in the other case the foundress died intact suggesting that the cause of death may have been unconnected to host attack. Whether or not these replicates were excluded from analysis, the probability of mortality increased significantly with an increase in host size (all data: G_1 = 5.18, P=0.023, %Dev = 11.25; excluding only the foundress that died intact: G_1 = 7.03, P = 0.008, %Dev = 15.54; excluding both females that died along with host paralysis: G_1 = 8.22, P=0.004, %Dev = 18.62; Figure 4.9 (b) shows the regression for the second case). We observed that some dead females had been severed into two parts, such as the head being separated from the rest of the body or the head and thorax separated from the abdomen. The probability that a dead female was bisected increased significantly with an increase of host size (logistic regression: G_1 = 7.97, P=0.005, %Dev = 43.67, n=14, Figure 4.9 (c)).

Table 4.1 Outcomes of single-foundress interactions with hosts.

Foundress status	Host not paralysed	Host paralysed but not oviposited on	Host paralysed and oviposited on
Foundress died (bisected)	8	1	0
Foundress died (intact)	4	1	0
Foundress lived	3	7*	13

*Six of these hosts may have been too small (0.0005–0.0592mg, mean=0.0287, SD=0.0215) to support offspring development.



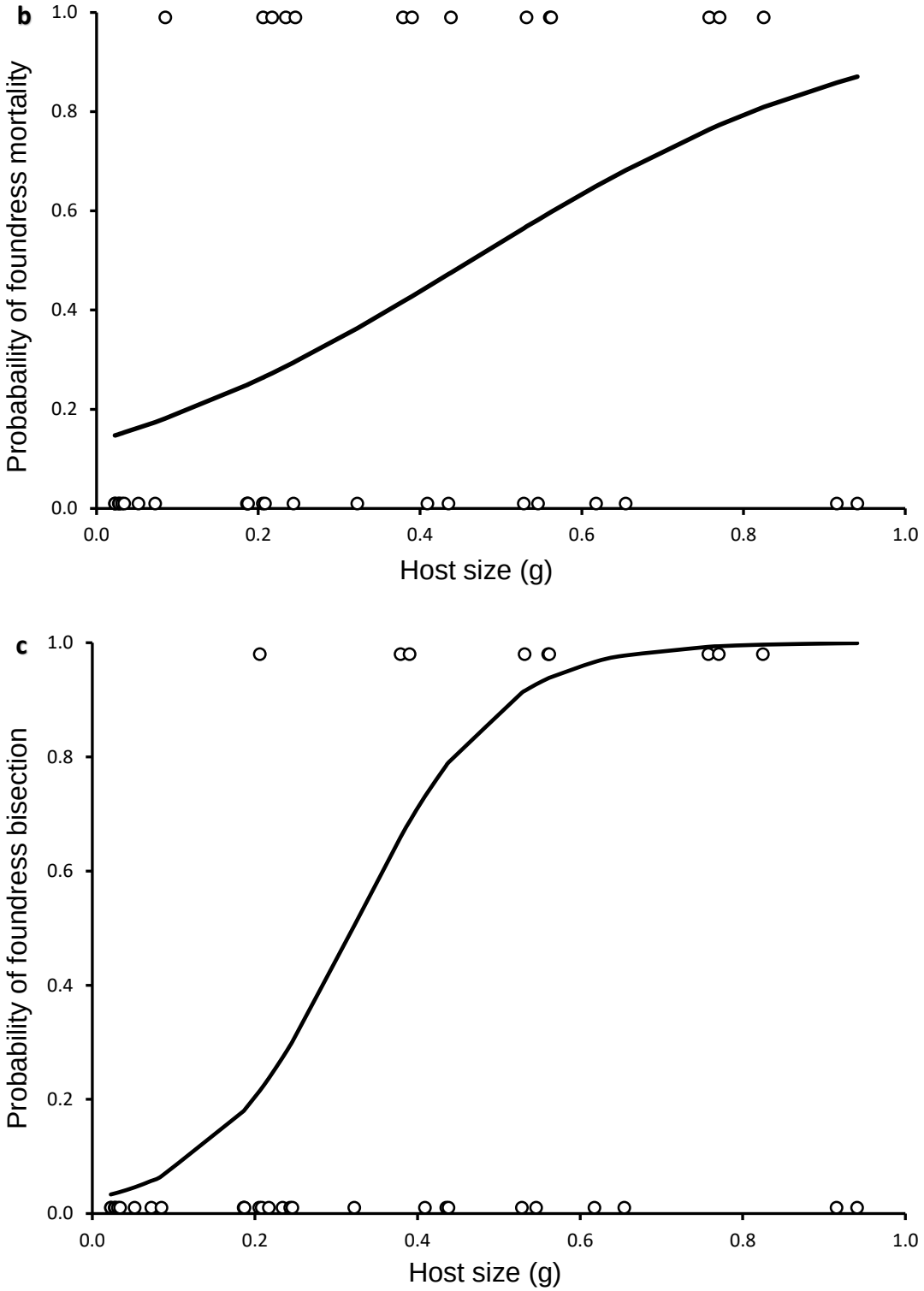


Figure 4.9 Probabilities of host paralysis [n=37] (a), foundress mortality [n=34] (b) and the foundress being bitten in half by the host [n=14] (c). The logistic regression line in panel b is used as the host-size dependent cost, c, in the public goods model: probability of death when attacking host =1/(1+(1/(EXP((3.99×host weight)-1.847)))), excluding the replicate where the female may have died after successful host attack.

4.4.2.3 Timing of paralysis and mortality

Among the 22 replicates in which host paralysis occurred, the time taken to paralyse the host ranged up to 19 days. Time-to-paralysis was significantly affected by host size, with smaller hosts being paralysed earlier than larger hosts (G_1 = 18.4, P<0.001, %Dev = 37.15); in this analysis replicates in which the foundress died before host paralysis, or both the foundress and the host were alive at the end of the experiment were treated as censors). Time to foundress mortality also ranged up to 19 days but was unaffected by host size (G_1 = 2.8, P=0.095, %Dev = 5.34, females that did not die were treated as censors). There was no relationship between time to foundress mortality and whether a female was bisected by the host (logistic analysis: G_1 = 0.00, P = 0.947).

4.4.3 Public goods model of host attack

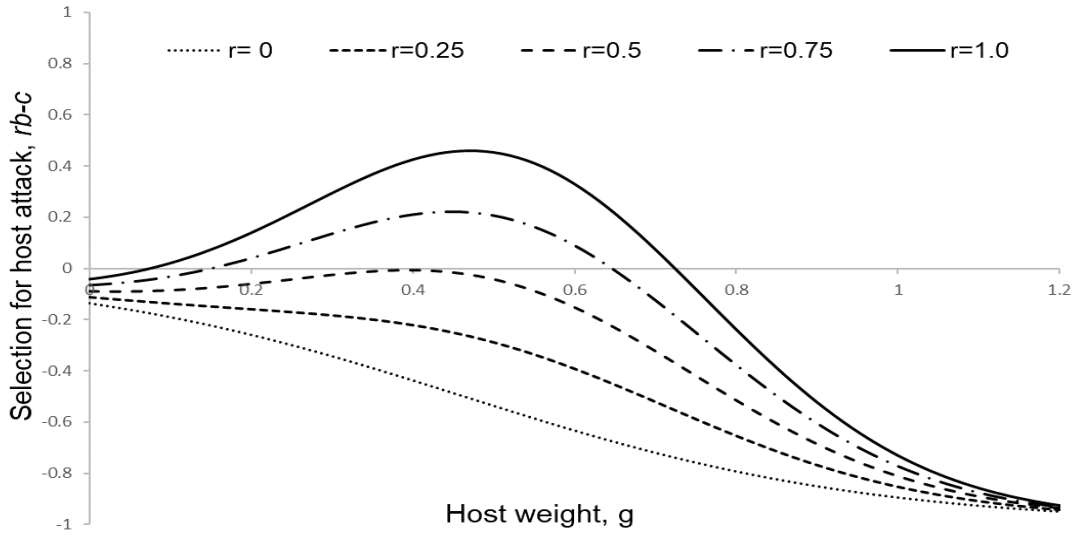
An estimate of the host size dependent probability of a foundress dying during host attack (cost, *c*) was obtained from the single-foundress assay (Figure 4.9) and an estimate of how fitness benefits obtained from a successfully paralysed host (benefit, *b*) are influenced by host size was obtained from the two-foundress experiment (Figure 4.6a). Following Abdi *et al.* (Chapter 3), these relationships were then used in Hamilton's rule ($rb - c > 0$) (Hamilton, 1964a) to explore how variation in *S. brevicornis* co-foundress relatedness (*r*) might affect selection for host attack as a public good (Cooper *et al.*, 2018).

The result (Figure 4.10) suggests that selection for public goods attack should depend on a combination of host size and relatedness. Specifically, the model suggests that individual foundresses will be selected to attack and then share hosts as a public good only when the host is neither large nor very small (i.e.

within the range *ca*. 0.1 to 0.7g) and further, only when their relatedness to co-foundresses is high (r>0.5); with a wider range of host sizes attacked when inter-foundress relatedness is higher.

As the relationships between host size and benefits differ according to co-foundresses relatedness, (Figure 4.6), we constructed a version of model using the fitted quadratic log-linear regression for broods mate foundresses when relatedness, r, is equal to 0.75 or 1, and the regression for non-sibling foundresses when r = 0.0 or 0.25. The form of the prediction was very similar to results shown in Figure 4.10, but brood-mate females would be expected to carry out public good host attack on very small hosts and to have a slightly lower upper-host size that they should attack as a public good.

a



b

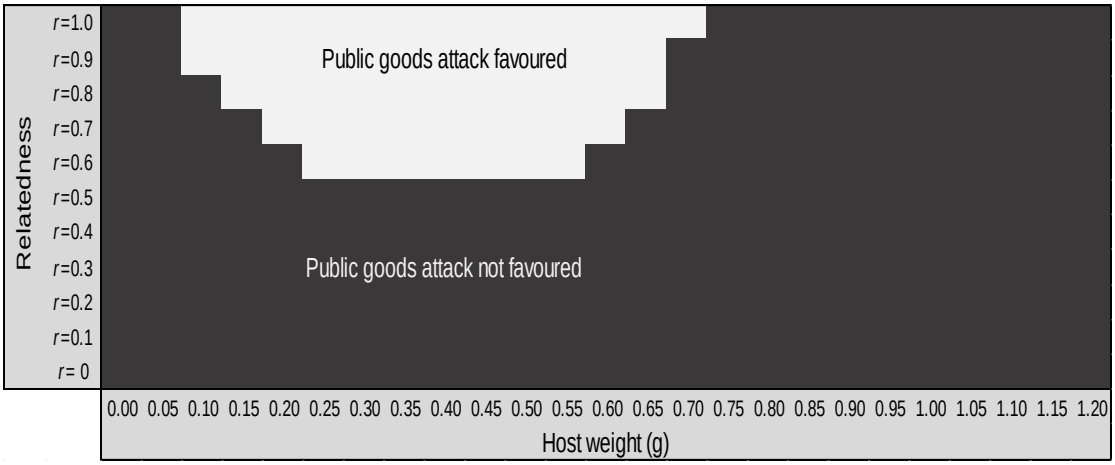


Figure 4.10 Public goods model of relatedness mediated host attack for *Sclerodermus brevicornis*. The model combines the estimate of the host size dependent probability of a foundress dying during host attack (cost, c), presented in Figure 4.9 (b), with the estimate of fitness benefits of successful attack (*b*), shown in Figure 4.6, into Hamilton's rule ($rb - c > 0$) to illustrate how co-foundress relatedness (*r*) might affect selection for host attack as a public good. The model suggests that selection for public goods attack depends on a combination of host size and co-foundress relatedness, and will be strongest when hosts are mid-sized and relatedness is >0.5. In **panel (a)** values from Hamilton's rule are plotted against host weight and the benefit curve was scaled to a maximum of 1, following Abdi *et al.* (Chapter 3). **Panel (b)** highlights the combinations of relatedness and host weight when public goods attack is favoured.

4.5 Discussion

For *Sclerodermus* females, attacking a host is a challenging step in the reproductive cycle and yet, to be able to reproduce, a female parasitoid must gain access to a suppressed host. Our estimates indicate that, when presented with a healthy host, 25-40% of *S. brevicornis* foundresses die prior to host suppression. Further, foundress mortality is related to host size, with larger hosts being considerably more dangerous to attack, as is also the case for other *Sclerodermus* species (Liu *et al.*, 2011; Wei *et al.*, 2014). The observation that many of the dead wasps in the single-foundresses assay were severed into two pieces indicates that hosts engage in active, and often successful, behavioural defence against parasitism, as also observed by (Li & Sun, 2011; Liu *et al.*, 2011).

A female may avoid the risks of host attack by utilizing a host that has been successfully attacked by another female ('free-rider' behaviour, Rankin *et al.* (2007)). *Sclerodermus* females have not been observed to defend paralysed hosts against usage by conspecifics (in contrast to some sub-social bethylids, Hardy *et al.* (2013)) and post-ovipositional brood care is cooperative, without apparent inter-foundress conflict (Hu *et al.*, 2012; Wu *et al.*, 2014): as such the reproductive resource represented by a paralysed host is shared between co-foundress females. In *S. brevicornis* (this study) and *S. pupariae* (Wei *et al.*, 2014) there is a domed relationship between offspring production and host size while for *S. harmandi* larger hosts, once suppressed, provide a larger collective reproductive benefit for co-foundresses (Liu *et al.*, 2011; Tang *et al.*, 2014). While there are several estimated forms of the relationship between host size

and *Sclerodermus* brood size, in all cases the total reproductive benefits of host exploitation are host-size dependent (Abdi *et al.* (Chapter 3); and this study). While a female may avoid host attack risks by joining one or more conspecifics in the exploitation of a previously suppressed host, the converse question concerns whether a female should be prepared to attack a host to obtain a resource that will then be shared with others. A guide to whether individuals should attack and then share a host of a given size (with the associated risk of death and benefits of successful suppression), is provided by the public goods modelling approach using Hamilton's rule. A discussion of the caveats associated with using empirically obtained parameter estimates in Hamilton's rule is provided by Abdi *et al.* (Chapter 3) and we further note that defining fitness benefits, *b*, simply in terms of numerical offspring production does not take into account that host size may affect further components of fitness. One example is the size of female offspring (Wei *et al.*, 2014) which, in turn, may influence performance, including the ability to attack hosts of a given size (affecting *r*). Using Hamilton's rule nonetheless suggests that both host size and co-foundress relatedness will be important influences on a *S. brevicornis* female's attack decision. When hosts are very small, the risks of host attack are low but the benefits of host suppression are also small and, even when the relatedness of a female to any co-foundresses present is high, a female is unlikely to attack a host as a public good, because the personal fitness costs to the female outweigh the fitness benefits. When hosts are medium sized, the risks during host attack are intermediate and the benefits of success are large. Under these circumstances, a female is expected to pay the private cost of providing a public good but only if her relatedness to co-foundresses is high.

Using empirically estimated cost and benefit parameters suggests that inter-foundress relatedness of 0.75 (the relatedness of full sisters under haplo-diploid genetics, Hamilton (1964a)) is sufficient for selection to favour public goods attack on medium sized hosts. When hosts are large and very dangerous, such that most attacking females are killed, and the benefits of successful host suppression are small, females are not expected to engage in public goods attack. We note that we have implicitly considered host attack an individual activity, carried out by one foundress while others present stand to benefit from her actions, but it remains possible that multi-female *Sclerodermus* attack, whether simultaneous or sequential, has a synergistic effect on the probability of success.

The public goods approach functions to indicate whether a *S. brevicornis* female should attack and then share a host, rather than whether a female should attack a host to support only her own offspring. Females that do not obtain access to a suppressed host (whether shared or not) cannot reproduce and thus might be expected to attack all sizes of hosts when there are no other options. Following Abdi *et al.* (Chapter 3) we interpret the public goods model to suggest patterns in the latency of host attack, i.e. how long a female encountering a dangerous host should wait for other females to attack the host before eventually attacking it themselves. We might expect a female's reluctance to attack to be reduced by potential inclusive fitness benefits when other females present are kin and, indeed, we found from the two-foundress experiment that females tended to die both earlier and with a higher probability when they were with brood-mates rather than non-siblings (in contrast to the lack of effect found by Abdi *et al.* (Chapter 3), assessing attack on small hosts only).

The models constructed by Abdi *et al.* (Chapter 3) for other *Sclerodermus* species and the model constructed here for *S. brevicornis* all suggest that co-foundress relatedness and host size will interact to influence the time that females wait before host attack. We have found empirically that time-to-paralysis is affected by host size (in accord with Lui et al. studying *S. harmandi* (Liu *et al.*, 2011) by relatedness (in accord with Abdi *et al.* (Chapter 3)) and also by their interaction, as predicted. We found the greatest effect when hosts were small, with hosts being paralysed more rapidly when co-foundresses relatedness was higher, whereas the model for *S. brevicornis* suggests greater effects for medium-sized hosts. When hosts are large, both the model and the time-to-paralysis data suggest that relatedness has little effect on host attack rates. We conclude that using empirically estimated benefit and cost parameter's in Hamilton's rule provides a broadly useful heuristic to understanding host attack in *Sclerodermus*.

Following the successful suppression of a host, mortality among pairs of foundresses was common, especially during the period between paralysis and oviposition. We suggest that this mortality may result from inter-foundress conflict over resource exploitation, even though such conflict is not visually obvious. Further work is required to probe the apparently cooperative nature of post-paralysis behaviour and brood production in *Sclerodermus* (e.g. to test for disproportionate reproduction by some foundresses [reproductive skew]) and any mediating effects of co-foundress kinship. While we detected no effect of relatedness on foundress mortality after host suppression, we did find that brood-mate females produced slightly greater numbers of offspring on small hosts (as also found by Abdi *et al.* (Chapter 3)) and on larger hosts brood sizes

were considerably larger when co-foundresses were not siblings. We suggest that *Sclerodermus* oviposition decisions may comprise a dynamic game between co-foundresses, involving a trade-off between brood-size and offspring fitness and a tragedy of the commons (TOC) scenario, whereby individual foundresses tend to selfishly add their own offspring leading to broods being larger than the size that would optimise group fitness; we also expect that such behaviour would be mediated by kinship (Rankin *et al.*, 2007; Van Dijk *et al.*, 2014; Ferrari *et al.*, 2015; Smith & Schuster, 2019). Further, as communal brood production in *Sclerodermus* usually leads to some reproductive output, we suggest that a TOC would be a 'component tragedy' in which selfishness reduces lower average fitness partially but not completely (Rankin *et al.*, 2007). Maturing offspring had extremely female-biased sex ratios, as previously observed in *S. brevicornis* (Lupi *et al.*, 2017; Abdi *et al.*, Chapter 3) and congeners (Kühne & Becker, 1974; Li & Sun, 2011; Liu *et al.*, 2011; Hu *et al.*, 2012; Tang *et al.*, 2014; Wei *et al.*, 2014; Gao *et al.*, 2016b; Kapranas *et al.*, 2016). We detected no effects of co-foundress relatedness on sex ratios but, in common with (Tang *et al.*, 2014), we found a slight decrease in bias as foundress number increased. Sex ratio bias in *Sclerodermus* is likely to be selected for by the advantages accrued by co-foundresses from communal exploitation of hosts that are too large for single females to reproduce on alone (i.e. mutually beneficial female–female interactions increase the reproductive value of daughters, Tang *et al.* (2014); Kapranas *et al.* (2016)); however, there are currently no models of optimal sex allocation in *Sclerodermus* and therefore no formal predictions concerning the likely influences of co-foundress relatedness.

4.6 Conclusion

Tang *et al.* (2014) showed that it is to the direct fitness advantage of individual female *Sclerodermus* to exploit hosts as part of a multi-foundress group, provided that the host is not small. This is because individual females have very low probabilities of successfully suppressing and reproducing on medium and large sized hosts, while larger groups of foundresses have greater collective success and the mean per foundress production of offspring is higher than when females attempt to reproduce alone. However, Tang *et al.* (2014) did not assess variation in behaviour or success within foundress groups, nor did they control inter-foundress relatedness, and it remains possible that individuals within apparently cooperative groups may attune their contributions to their own direct and inclusive fitness interests.

This study set out to evaluate the importance of host size on the host attack and further reproductive behaviour of *Sclerodermas brevicornis*. This followed a prior study (Abdi *et al.*, Chapter 3) which identified that co-foundress relatedness affects the timing of host attack and suggested (using data on reproductive patterns in congeners and a public goods approach), that host size should interact with relatedness and thus mediate the observed effect of kinship. Here we have shown that effects of both host size and kinship operate during several of the stages of *S. brevicornis* host attack and reproduction and that they interact to influence the timing of host attack, as predicted. A public goods model of host attack using parameters estimated for *S. brevicornis* gives broadly similar results to the previous models, again suggesting that selection for an individual foundresses to attack and then, if successful, share their hosts will depend on both size of the host and the foundresses' relatedness to any co-

foundresses present. We also found that co-foundress relatedness influence some reproductive behaviours following host suppression, and we suggest that future studies are likely to reveal further effects.

Our overall conclusion is that females attune their behaviour to the risks involved in attempting to suppress large and dangerous hosts and that while reproductive behaviours exhibited by *Sclerodermus* females can be cooperative, they will not always be exhibited without reference to kinship

4.7 Coda: Cooperative reproduction in the genus *Sclerodermus*

Tang *et al.* (2014) showed that it is to the direct fitness advantage of individual female *Sclerodermus* to exploit hosts as part of a cooperative multi-foundress group, provided that the host is not small. This is because individual females have very low probabilities of successfully suppressing, and reproducing on, medium and large sized hosts, while larger groups of foundresses have greater collective success, such that the mean per foundress production of offspring is higher than when females attempt to reproduce alone. However, Tang *et al.* (2014) did not assess variation in behaviour or success within foundress groups, nor did they control inter-foundress relatedness. Our new evidence suggests that individuals within apparently cooperative foundress groups may attune their contributions to their own direct and inclusive fitness interests and thus that there is conflict within cooperation, as observed in numerous other biological societies (West & Ghoul, 2019).

Table 4.2 *Sclerodermus* reproductive strategy: a synthesis

	Small host	Large host
High relatedness	**Attack and share host** Conflict free cooperation	**Attack and share host** Conflict free cooperation
Low relatedness	**Attack host but do not share host** No cooperation	**Avoid host attack but share host attacked by others** Conflict within cooperation

We suggest that the current understanding of *Sclerodermus* cooperative reproduction can be broadly summarized by considering inter-foundress relatedness simply as ‘high’ or ‘low’ and hosts as ‘small’ or ‘large’, and by classifying cooperation very simply in terms of inner conflict (Table 4.2); we note that when hosts are either very small or very large, any selection for cooperation may break down (due to very low benefits or very high costs of attack, respectively). Depending on the combination of inter-foundress relatedness and host size, a female may be selected to attack a host or to leave host attack to others, and also to share or not share a successfully attacked host. When hosts are small, *Sclerodermus* may share them with related females due to inclusive fitness but attempt to exclude non-relative females to maximise direct fitness gains from host exploitation (as in sub-social bethylids, (Hardy *et al.*, 2013), although aggressive inter-female behaviour has not currently been reported in S*clerodermus*). When hosts are large, females may gain both direct and inclusive fitness from sharing with relatives but will only gain direct fitness when co-foundresses are not kin. In the latter case, cooperation may not be conflict free and is likely to be characterised by forms of exploitation and enforcement.

Part three: Kinship among subsocial parasitoids

Chapter 5: Co-foundress confinement elicits kinship effects in a naturally sub-social parasitoid

5.1 Abstract

Parasitoids in the bethylid genus *Goniozus* are sub-social, with single foundress females exhibiting post-ovipositional maternal care via short-term aggressive host and brood defence against conspecific females. Contests are not normally fatal and aggression is reduced when competing females are kin. Here we test for further effects of kinship, along with varying host size; both can be associated with the evolution of parasitoid sociality. We confined groups of sibling or non-sibling *Goniozus nephantidis* females together with a host for 5+ days. Confinement led to the death of some females (11% overall) but only when host resources were most limiting. Mortality of foundresses was less common when foundresses were siblings. Developmental mortality among offspring was considerably higher in multi-foundress clutches but was unaffected by foundress relatedness. Groups of sibling foundresses produced larger clutches than non-sibling groups. There was little advantage for individual females to reproduce in multi-foundress groups: single foundresses suppressed even the largest hosts presented and had the highest *per capita* production of adult offspring. Despite single-foundress reproduction being the norm, *G. nephantidis* females in multi-foundress groups appear to attune sex allocation according to both foundress number and foundress relatedness: broods produced by sibling foundresses had similar sex ratios to broods produced by single foundresses (*ca*. 11% males) whereas the sex ratios of broods produced by non-sibling females were approximately 20% higher and broadly increased with foundress number. We conclude that relatedness and host size may combine to reduce selection against communal reproduction on hosts and that, unlike other studied parasitoids, *G. nephantidis* sex ratios conform to predictions of both classical and extended Local Mate Competition theory.

5.2 Introduction

Hymenopteran parasitoids are typically socially solitary but a few taxa exhibit traits associated with advanced sociality. For example, *Copidosoma* wasps in the family Encyrtidae have polyembryonic clonal development and non-reproductive larval castes which defend their reproductive caste kin (Cruz, 1981; Grbic *et al.*, 1992; Giron *et al.*, 2004; Giron & Strand, 2004), representing reproductive division of labour. In contrast, species in the bethylid genus *Sclerodermus* have evolved quasi-sociality, in which multiple foundresses parasitize the same host and exhibit cooperative brood care (Tang *et al.*, 2014), without apparent division of labour. Species in the bethylid genus *Goniozus* exhibit a lower level of sociality termed sub-social: individual mothers care for their own offspring, for a period of time immediately after oviposition and until they develop to the pupal stage, by aggressively excluding conspecific females from the vicinity of their hosts (Hardy & Blackburn, 1991; Mayhew & Hardy, 1998; Goubault *et al.*, 2007; Bentley *et al.*, 2009; Venkatesan *et al.*, 2009b; Hardy *et al.*, 2013).

A series of experiments (reviewed in Hardy *et al.* (2013)) has explored factors affecting the outcomes of competitive interactions between pairs of *Goniozus* females, e.g. a brood guarding host 'owner' and an 'intruder', in the context of testing game-theoretic models of contest behaviour. This body of work examined relatively short-term interactions (<3h) and considered initial contest outcomes to be decisive, such that losers would be permanently excluded. While the results are largely compatible with model predications, the possibility of longer-term, or repeated, interactions between competing females remains. Subsequent observations made during microcosm experiments, involving

multiple females and multiple hosts confined together during the entire period of offspring development (Sreenivas & Hardy, 2016; Velasco-Hernandez *et al.*, unpublished), indicate that it is within the behavioural repertoire of females to tolerate each other's presence on a host, even though overall offspring production can be reduced (Legner & Warkentin, 1988; Venkatesan *et al.*, 2009b; Sreenivas & Hardy, 2016). Sharing a breeding site is a characteristic of communality, a more advanced level of sociality than sub-social, but which does not involve cooperative brood care (Wilson, 1971; Choe & Crespi, 1997; Costa, 2018).

Here, we utilize naturally sub-social *Goniozus* wasps to explore the life-history effects of three factors that may have influenced the evolution of sociality in the Bethylidae: host size, foundress number and foundress relatedness. First, host size appears to be correlated with sociality, in that the socially solitary bethylid *Laelius pedatus* attacks small hosts (˜1.5 mg) and lays clutches of 1-6 eggs (Klein *et al.*, 1991; Mayhew & Godfray, 1997; Mayhew, 1998; Mayhew & Hardy, 1998; Mayhew & Heitmans, 2000), sub-social species of *Goniozus* oviposit on medium-sized hosts (~35 mg) with clutches ranging up to approximately 18 eggs (Hardy *et al.*, 1992; Sreenivas & Hardy, 2016), and quasi-social species of *Sclerodermus* can attack large hosts (up to ~250mg) and lay clutches of more than 100 eggs (Wei *et al.*, 2014). Evolutionary transitions between solitary and sub-social behaviour may be due to only medium or large sized hosts being worth the opportunity cost of brood deference (Hardy & Blackburn, 1991) while transitions to quasi-sociality are likely favoured by the inability of individual females to suppress and exploit very large hosts (Liu *et al.*, 2011; Tang *et al.*, 2014; Wei *et al.*, 2014; Abdi *et al.*, Chapter 3; Abdi *et al.*, Chapter 4).

Second, across all parasitoid wasps, larger numbers of females attempting to exploit a host are usually associated with greater competition which, in gregarious species, typically reduces clutch sizes produced by each female (Godfray, 1994) or, as in *Goniozus*, may lead to the aggressive exclusion of some females from the host resource and subsequent brood defence (parental care) (Enquist & Leimar, 1987; Petersen & Hardy, 1996; Humphries *et al.*, 2006; Hardy *et al.*, 2013). However, multiple foundresses can also exhibit cooperation in host exploitation: cooperation between foundresses of the quasi-social *Sclerodermus* results in direct (*per capita*) fitness benefits when hosts are large (Tang *et al.*, 2014).

Third, there is a substantial literature on the role of kinship (genetic relatedness) in the evolution of social behaviour and sociality (Hamilton, 1964a; Hamilton, 1964b; Queller, 2000; Abbot *et al.*, 2011; Bourke, 2014; Birch, 2017). Kin selection theory predicts that individuals will tend to show less aggression and more altruism towards relatives (Hamilton, 1963; Hamilton, 1964a). Kin recognition abilities have been found in many animal species; among parasitoid hymenopterans, kinship has been found to mediate intra-clonal attack by the soldier caste in polyembryonic encrytids (Giron *et al.*, 2004; Giron & Strand, 2004; Dunn *et al.*, 2014), the aggresiveness of female-female contests in the socially solitary Eupelmid *Eupelmus vuilletti* (Mathiron et al., 2019) and the sub-social *Goniozus legneri* (Lizé *et al.*, 2012) and, most recently, the time to host attack by groups of quasi-social *Sclerodermus* foundresses (Abdi *et al.*, Chapter 3; Abdi *et al.*, Chapter 4).

All of the above social taxa experience group structured mating among their offspring with consequent selection for female bias, whether due to Local Mate

Competition (LMC) or Local Resource Enhancement (LRE) (Hamilton, 1967; Taylor, 1981; Grbic *et al.*, 1992; Hardy & Cook, 1995; West, 2009; Tang *et al.*, 2014). Extentions to classical LMC theory have predicted that selection will favour greater female bias when closer kin interact (Frank, 1985; Taylor & Crespi, 1994; Burton-Chellew *et al.*, 2008; West, 2009). We therefore also evaluate the effects of host size, foundress number and relatedness on *Goniozus* sex ratios.

In the present study, we confine *Goniozus nephantidis* (Muesebeck) females (experimentally varying number and relatedness) together with one host (experimentally varying size) to discover whether prolonged female-female contact elicits behavioural and reproductive responses (such as foundress mortality, host attack, offspring production and sex ratio) that are commensurate with those observed in species with naturally higher-levels of sociality. We thus force sub-social parasitoids into a range of conditions that probe their incipient propensity to transition to communal, or even quasi-social, reproduction and to respond to kinship in the direction predicted by sex ratio theory.

5.3 Materials and Methods

5.3.1 Insects

The natural hosts of *Goniozus nephantidis* are the mid-to-late larval stages of *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), a defoliator of coconut in the Indian sub-continent (Cock & Perera, 1987). Although it appears to be monophagous in the field (Shameer *et al.*, 2017), *G. nephantidis* can be laboratory-reared on the rice moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Galleridae): developmental parameters, such as clutch size, adult offspring

production and sex ratio, are similar whether the natural host or either of these factitious hosts is utilized (Mohan & Shameer, 2003). Our *G. nephantidis* stock had been maintained in laboratory culture reared for over 10 years on *C. cephalonica* but in our experiments we used a culture of *G. mellonella*, obtained from a local pet-food retailer, as the host because we wished to assess the influence of a wide range of host sizes on parasitoid behaviour: *G. mellonella* larvae commonly reach >500mg before pupation while *C. cephalonica* larvae >90mg are unusual (Hardy *et al.*, 1992).

Both *C. cephalonica* and *G. mellonella* were reared on a diet of wheat bran, fine corn meal, brewer's yeast granules, honey and glycerol (Sreenivas & Hardy, 2016). All cultures and experimental replicates were kept in a climate room at the University of Nottingham, maintained at 26 °C, 18L: 6D and 60% - 80% RH.

5.3.2 Experiment

We evaluated whether foundress, mortality, behaviour and offspring production were affected by the number of foundresses, the relatedness between foundress females, and the size of the host. Hosts from a wide range of weights (measured using a digital microbalance to an accuracy of 0.0001 g) were selected and placed individually into glass vials (1.0 cm diameter × 5.0 cm long) stoppered with nylon gauze and cotton wool. Adult *G. nephantidis* females (foundresses) were then added into the vials: there were replicates with 1, 2, 3, 4, 6 and 8 foundresses. The relatedness between foundresses in multi-foundress replicates was also varied, such that either all foundress were brood-mate sisters that had developed on the same host (siblings) or all had developed in different broods within the culture (non-siblings). To give adequate overall statistical power (Smith *et al.*, 2011), we set up 20 replicates of each

foundress number and relatedness combination ([20 replicates × 6 foundress number treatments × 2 relatedness treatments] – 20, due to foundress relatedness being invariant in single-foundress replicates, = 220 replicates overall). We ensured that a wide range of host sizes was represented within the 20 replicates for each foundress number and relatedness combination. This experimental design thus followed that of (Tang *et al.*, 2014), studying the quasi-social bethylid *Sclerodermus harmandi*, but with more foundress number treatments and the addition of variation in foundress relatedness.

The parasitoids and hosts within each replicate were examined once daily. We recorded the number of foundresses alive, the paralysis of the host, whether foundresses were on or next to the host or where positioned away from the host, the occurrence of oviposition, the number of eggs laid, the duration of offspring development (from the first day an egg was observed on a host to the first day an adult offspring emerged) and the number of adult male and female offspring produced. We also recorded the average size (thorax length) of females within each offspring group.

5.3.3 Statistical analysis

The influences of foundress number, foundress relatedness and host size on foundress mortality, behaviour, offspring production and sex allocation were explored using generalized linear modeling (Crawley, 1988; Faraway, 2006) in the *GenStat* statistical package (v17.1, VSN International Ltd., Hemel Hempsted, UK). Foundress relatedness in multi-foundress replicates was treated as a factor with two levels (siblings or non-siblings). Foundress number was fitted as a factor in most statistical analyses but as a continuous variable in sex ratio analyses. Host weight was fitted as a continuous explanatory variable

except in time-to-event data analysis, such as time to paralysis and time to oviposition, in which host size was categorized as small, medium or large. First order interactions between main effects were included in the initial statistical models. Hypothesis testing was carried out using backwards elimination of explanatory variables from initial models and by aggregation of factor levels to find minimum adequate statistical models (Crawley, 1988; Wilson & Hardy, 2002). In analyses of effects of inter-foundress relatedness, we excluded single foundress replicates. In log-linear and logistic analysis, of small integer and proportional response variables respectively, over-dispersion was taken into account, where appropriate, via empirical estimation of scaling parameters and thus the assumption of quasi-Poisson and quasi-binomial error distributions (Crawley, 1988; Wilson & Hardy, 2002). Following logistic and log-linear analyses, we give the percentage deviance explained (%Dev) as a descriptor analogous to r^2.

Time-to-event data on host paralysis and oviposition were analysed using parametric cohort survival analyses with censoring (Crawley, 1988; Aitkin *et al.*, 1989). We first compared exponential models (constant hazard function) against Weibull models (time dependent hazard function): in all cases, the Weibull models provided a significantly better description of the data. The influences of the candidate explanatory factors (see above) were then explored by their inclusion into the Weibull models (Crawley, 1988; Aitkin *et al.*, 1989).

5.4 Results

5.4.1 Foundress mortality

Two of the 20 single foundresses died by the end of the fifth day after host presentation. Similarly, 11.30% (104/920) of foundresses in multi-foundress

groups died during this period. Mortality was more common among foundresses in non-sibling groups than among siblings and this difference was significant from the first day though to the fifth day (cumulative mortality: First: G_1=15.32, P<0.001, %Dev=7.32; Second: G_1=12.21, P<0.001, %Dev=5.99; Third: G_1=17.11, P<0.001, %Dev=8.11; Fourth: G_1=2817, P<0.001, %Dev=12.68; Fifth: G_1=34.16, P<0.001, %Dev=14.97, Figure 5.1).

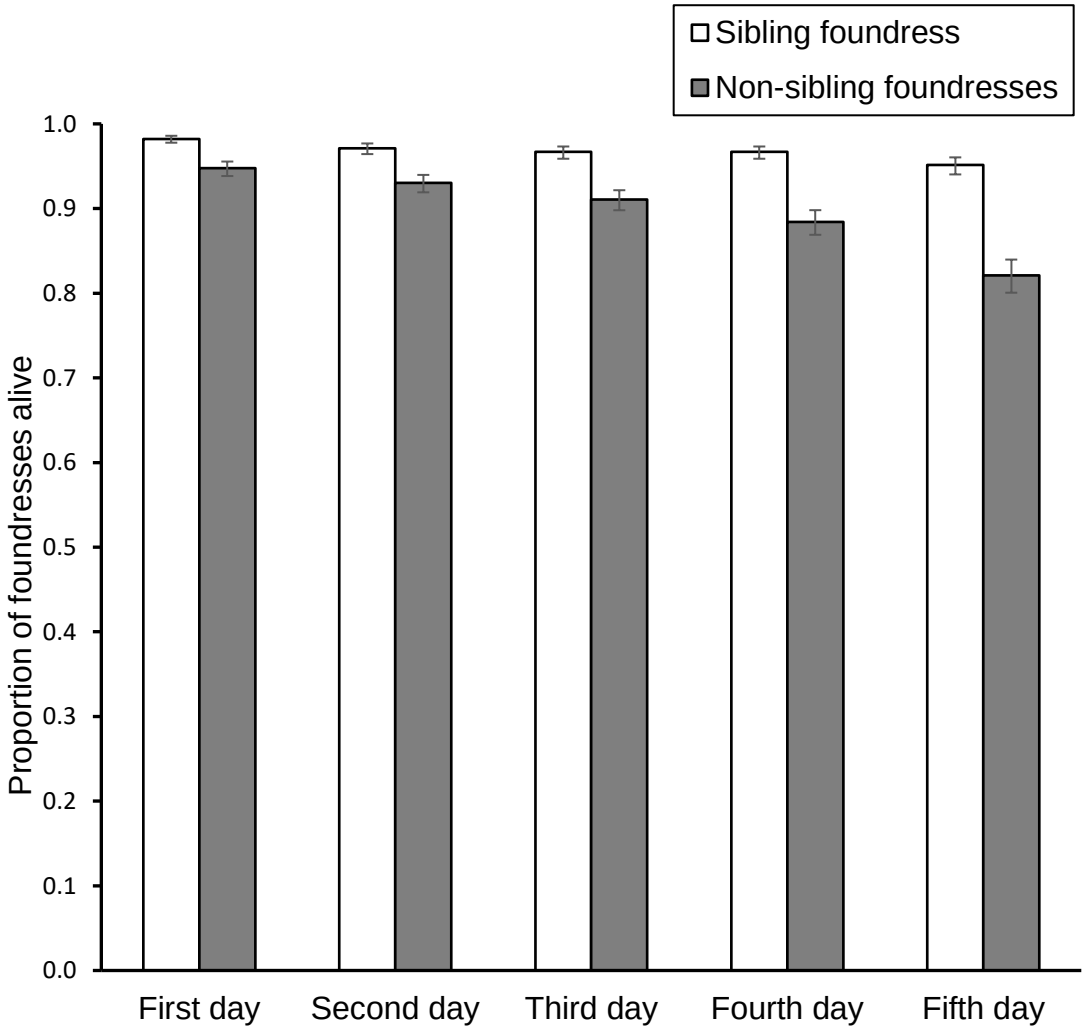


Figure 5.1 Increasingly apparent effects of co-foundress relatedness on cumulative foundress mortality over the first five days of confinement. Bars represent standard errors of means and are asymmetrical due to back transformation from the logit scale.

The probability of mortality (by the end of day 5) was also progressively higher as foundress number increased (G_5=4.35, P<0.001, %Dev=8.87) and was lower when hosts were larger (G_1=15.78, P<0.001, %Dev=6.44) but there was no interaction between these main effects (Interaction: G_5=0.71, P=0.615, %Dev=1.45). Considering the amount of resource available per foundress (host weight/initial number of foundresses) showed that foundress mortality was entirely absent when resources were relatively abundant (>0.1g per foundress) and was more common among non-siblings than among siblings when resources were more limiting (Resource availability: G_1=35.43, P<0.001, %Dev=13.23; Relatedness: G_1=35.43, P<0.001, %Dev=13.23; Figure 5.2). The causes of foundress mortality could not be identified but in some replicates small hosts died and foundress mortality followed. We also observed female-female contests in at least one replicates of each multiple-foundress treatment combination.

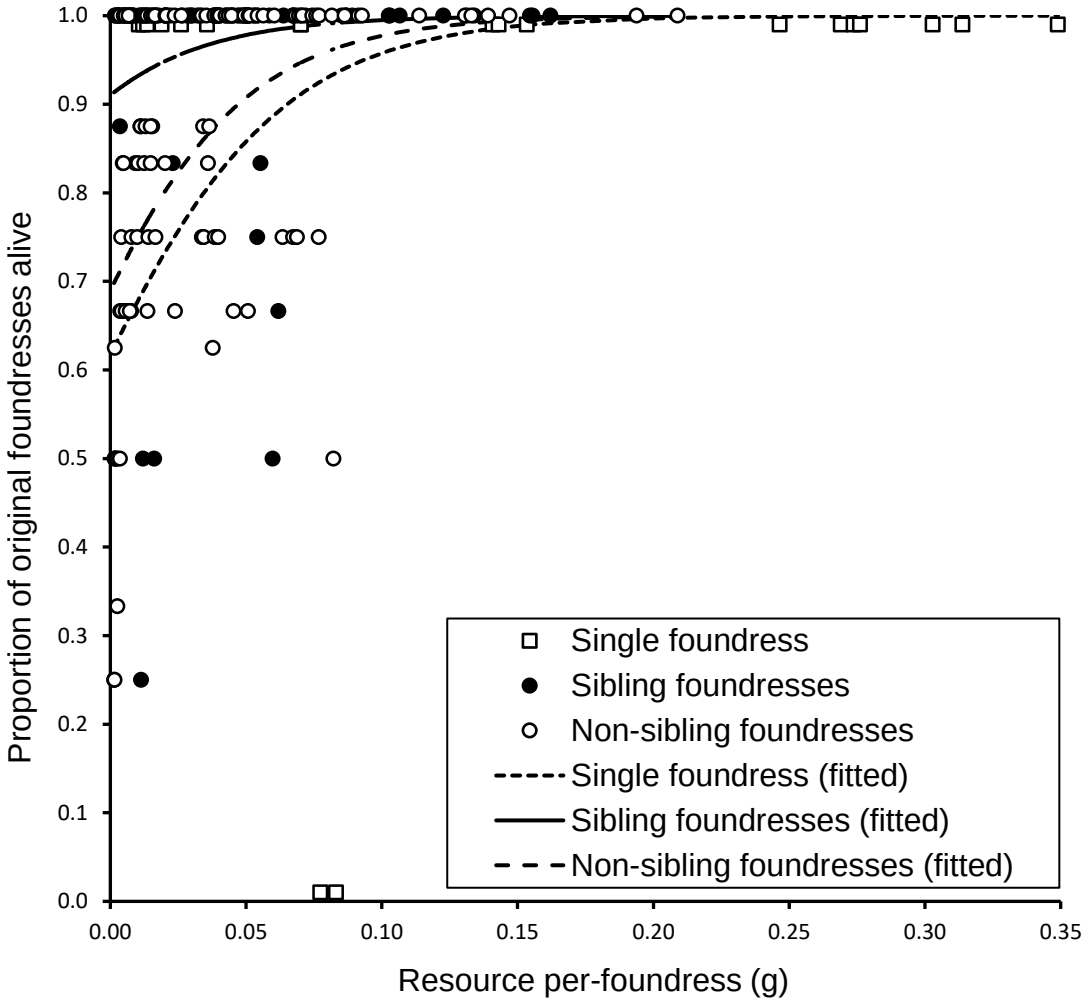


Figure 5.2 Effects of foundress relatedness and resource availability on foundress mortality by the end of the fifth day of confinement. Single foundress group is not significantly different from either sibling or non-sibling groups, but the latter two were different from each other.

5.4.2 Probability of paralysis

Almost all (98.18%) hosts became paralysed and all the hosts that were paralyzed were paralyzed within one day. The probability of paralysis was greater when there were more foundresses (G_5=2.22, P=0.049, %Dev=27.76) and successive aggregation of factor levels showed that host paralysis was similarly likely (overall 93.33%) when there were one or two foundresses, while larger numbers of foundresses always paralysed the host. We observed simultaneous attack of the host by multiple foundresses in both sibling and non-

sibling experimental treatments. The probability of paralysis was not affected by foundresses relatedness (G_1=0.00, P=0.967, %Dev=0.01) or host size (G_1=0.00, P=0.999, %Dev=0.00) or by any interactions between the three main effects.

5.4.3 Position of foundresses

Although contests were observed in all multi-foundress treatments (see above), we also observed multiple females on single hosts, indicating that individual females did not always exclude all other females from the vicinity of the host. The mean number of foundresses in close spatial association (on or next to) with a host was 2.646 (SE = +0.128, -0.122; data analyzed were the per-replicate means from the 5 daily observations). The mean number of foundresses observed on a given host increased with the number of foundresses in the replicate ($F_{5,218}$=100.83, P<0.001, %Dev=67.06, Figure 5.3a) and host size ($F_{1,214}$=47.43, P<0.001, %Dev=6.31) but was not influenced by relatedness ($F_{1,195}$=3.49, P=0.063, %Dev=0.70) or by interactions between these variables (foundress number x relatedness: $F_{4,194}$=1.68, P=0.157, %Dev=1.35, foundress number x host size: $F_{5,113}$=1.68, P=0.141, %Dev=1.12; relatedness x host size: G_1=0.36, P=0.549, %Dev=1.17). The mean proportion of foundresses closely associated with hosts was 0.615 (SE = +0.0324, -0.0334) and increased with an increase of host size (G_1=9.14, P=0.002, %Dev=15.83, Figure 5.3b) but was not influenced by foundress number (G_4=0.34, P=0.848, %Dev=3.26) relatedness (G_1=0.11, P=0.739, %Dev=0.26) or by any interactions (foundress number x relatedness: G_4=0.49, P=0.745, %Dev=4.61; foundress number x host size: G_5=1.00, P=0.415, %Dev=8.67; relatedness x host size: G_1=0.08, P=0.772, %Dev=0.20).

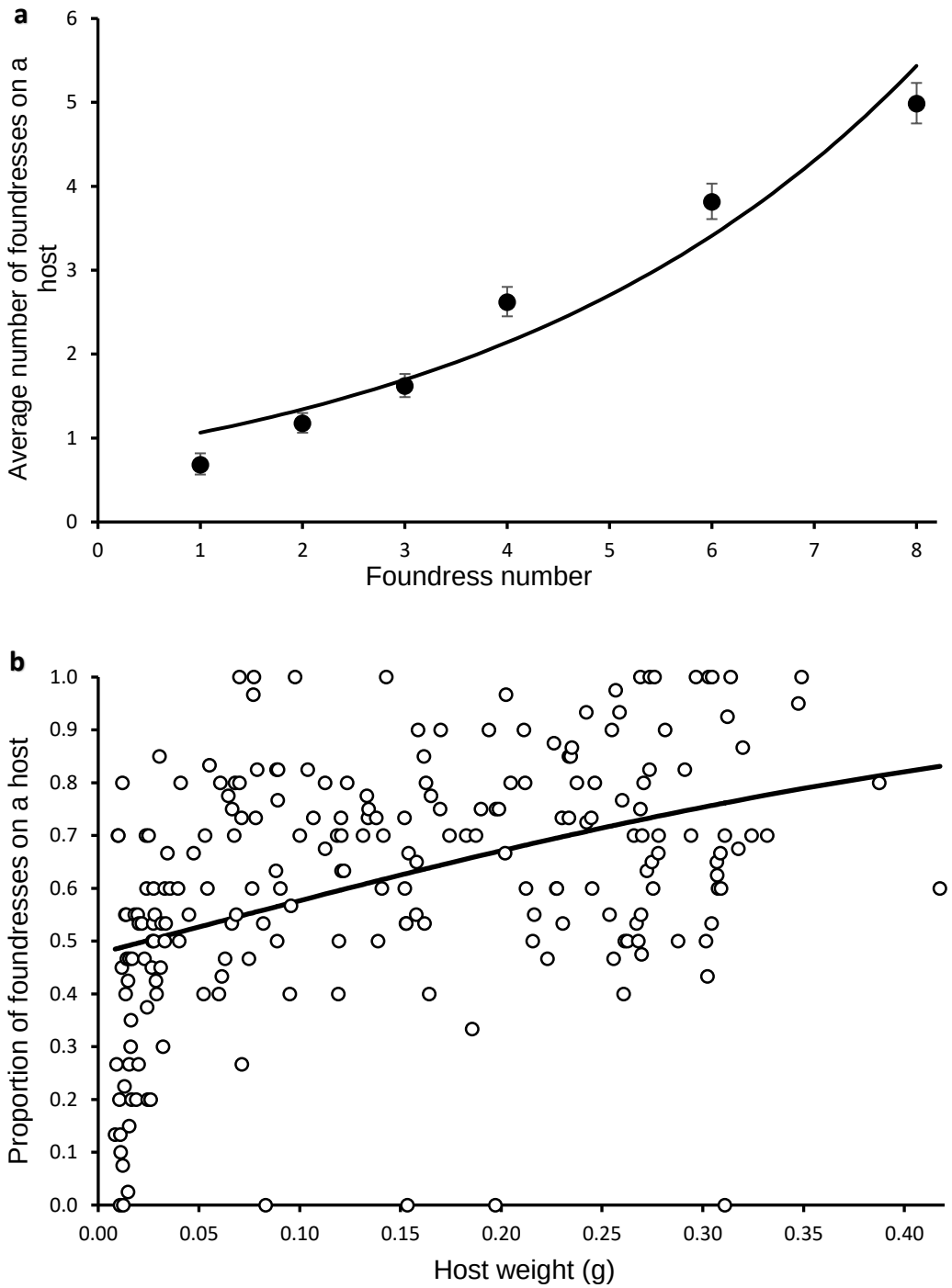


Figure 5.3 Wasps sharing hosts. (a) The relationship between foundress number and the mean number of foundresses observed on or next to the host. The line was fitted by log-linear regression and bars represent standard errors of means and are asymmetrical due to back transformation from the logarithm scale. (b) The relationship between the proportion of foundresses on a host and the size of the host. The line was fitted by logistic regression. In both panels data were analysed as the mean across 5 days for each replicate.

5.4.4 Probability of oviposition

Of the 216 of hosts that were paralyzed, 93.52% were subsequently oviposited on. The probability oviposition was greater when hosts were larger (G_1=20.39, P<0.001, %Dev=19.67) and when there were more foundresses (G_5=3.67, P=0.003, %Dev=17.71) and there was a significant interaction between host size and foundress number (G_1=16.65, P<0.001, %Dev=16.06), as some large groups of foundresses groups did not oviposit when hosts were very small but otherwise always laid eggs, while for smaller groups of foundresses the probability of oviposition was less effected by host weight.

Excluding single-foundress replicates and thus considering foundress relatedness found a significant interaction between foundress number and relatedness (relatedness main effect: G_1=0.31, P=0.579, %Dev=0.31; Interaction: G_4=3.52, P=0.007, %Dev=13.91, n=198), such that the probability of oviposition increased with an increasing foundress number for siblings but decreased for non-siblings (Figure 5.4). There was no significant interaction between foundress relatedness and host size (G_1=1.79, P=0.181, %Dev=1.77).

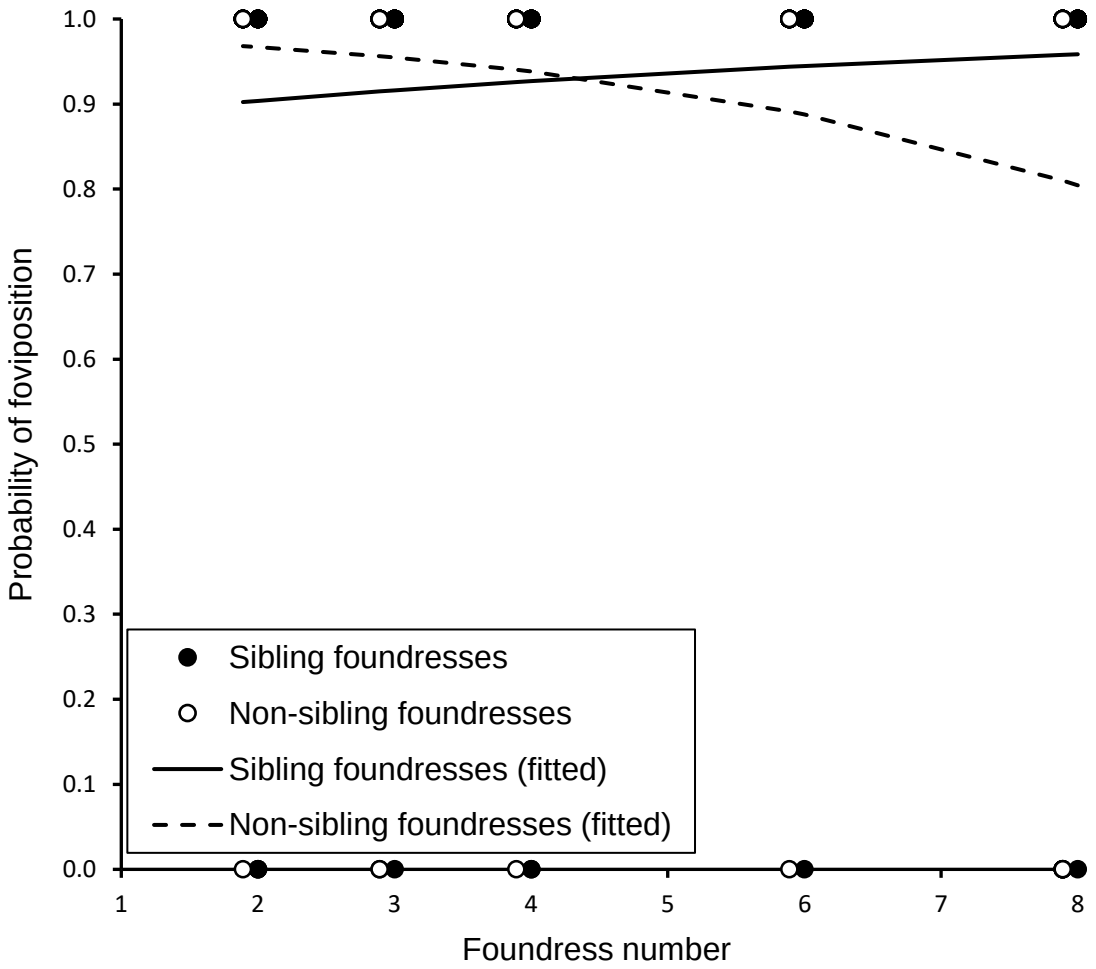


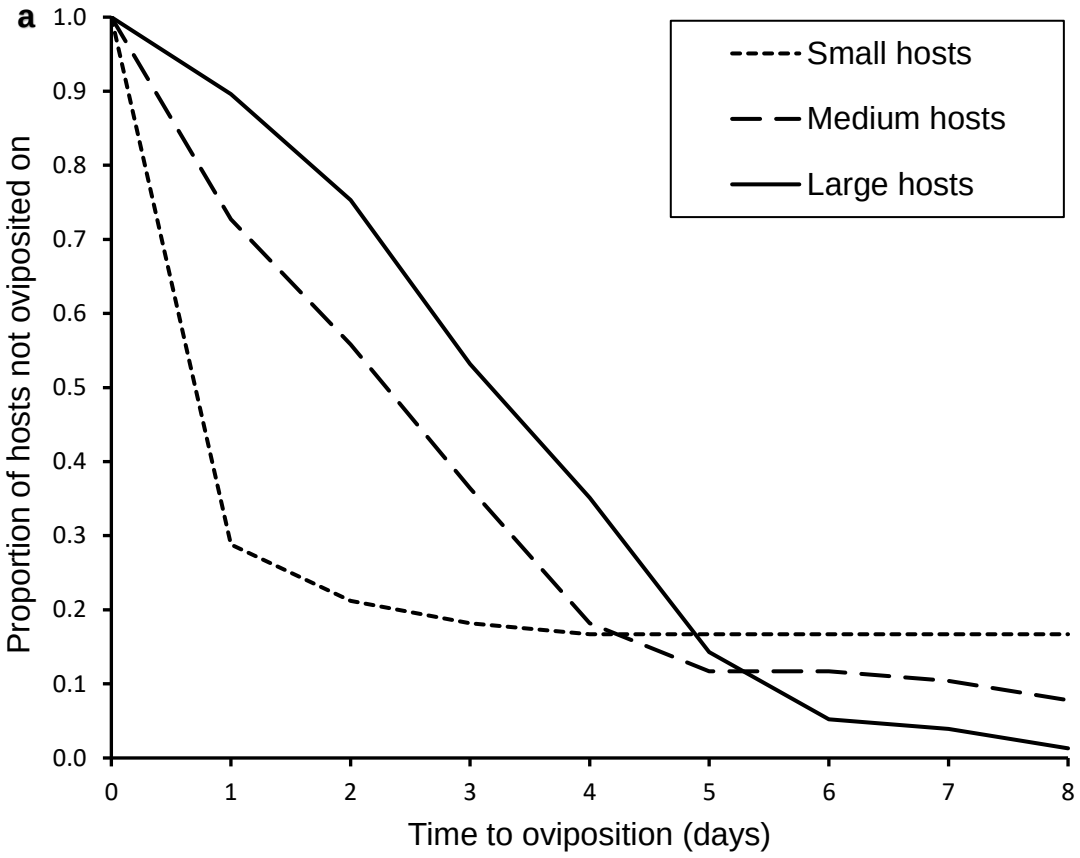
Figure 5.4 The effect of Foundress number and relatedness on the probability of oviposition.

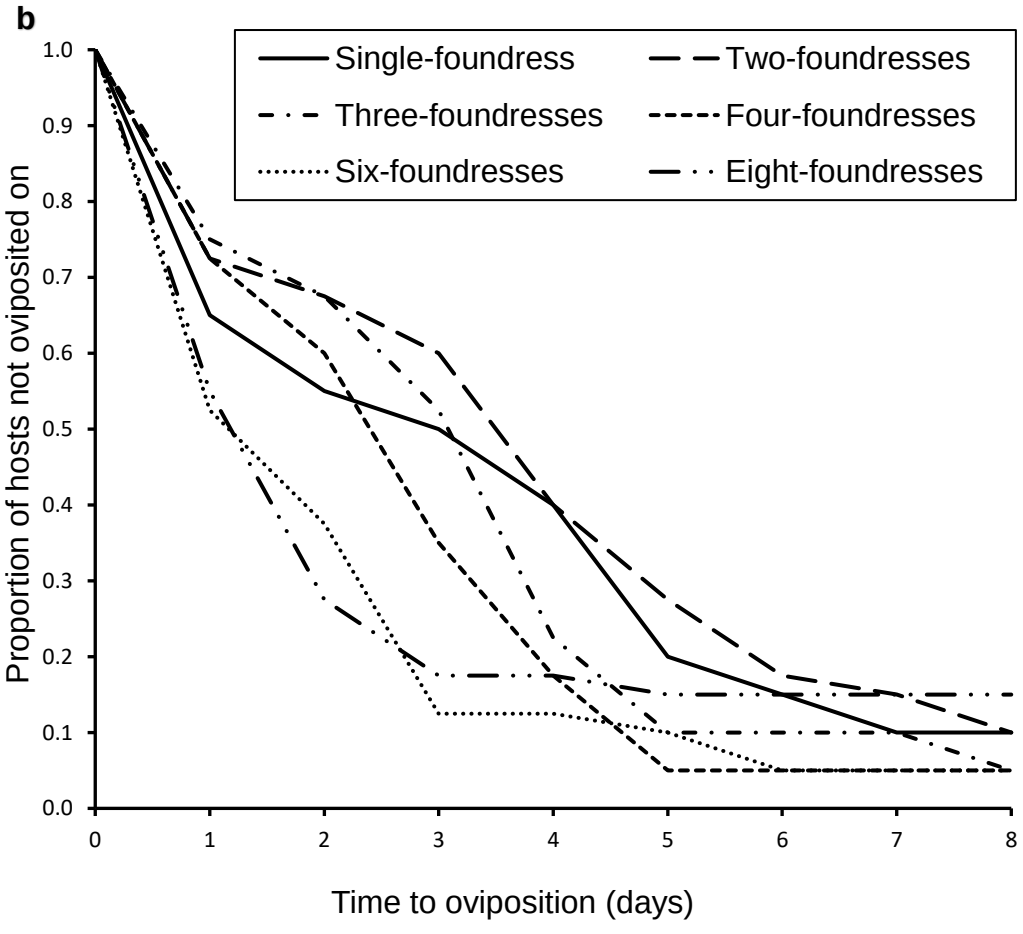
5.4.5 Timing of oviposition

Among replicates in which there was oviposition (n=202), the time from the presentation of a host to the first laying of eggs ranged between 1 to 8 days and was affected by host size, foundress number and relatedness, either directly of via interactions. In some multiple foundress replicates, we observed eggs being laid in temporally separate batches, often with the first batch failing to mature before a second batch was laid. Times to begin oviposition were affected by an interaction between foundress number and host size (From host presentation: G_{10}=109.9, P<0.001, %Dev=33.30; From host paralysis:

G_{10}=107.7, P<0.001, %Dev=33.82; Host size main effect: From presentation: G_2=0.7, P=0.705, %Dev=0.138; From paralysis: G_2=2.4, P=0.301, %Dev=0.596; Foundress number main effect: From presentation: G_5=11.0, P=0.051, %Dev=2.599; From paralysis: G_5=7.5, P=0.186, %Dev=1.813); times were shorter when hosts were smaller and there were more foundresses (Figure 5.5).

Excluding single-foundress replicates and considering foundress relatedness found that times to oviposition were not affected by an interaction between relatedness and host size (from presentation: G_2=4.4, P=0.111, %Dev=1.76; from paralysis: G_2=4.5, P=0.105, %Dev=0.241) but were affected by an interaction between foundress relatedness and number (from presentation: G_4=12.6, P=0.013, %Dev=3.878; from paralysis: G_4=13.0, P=0.011, %Dev=2.761; Relatedness main effect: from presentation: G_1=0.0, P=1.000, %Dev=0.000; from paralysis: G_1=0.1, P=0.752, %Dev=0.000), such that times to egg laying were significantly shorter when foundresses were siblings and were more foundresses (Figure 5.5).


a
Small hosts
Medium hosts
Large hosts
Proportion of hosts not oviposited on
Time to oviposition (days)


b
Single-foundress
Two-foundresses
Three-foundresses
Four-foundresses
Six-foundresses
Eight-foundresses
Proportion of hosts not oviposited on
Time to oviposition (days)

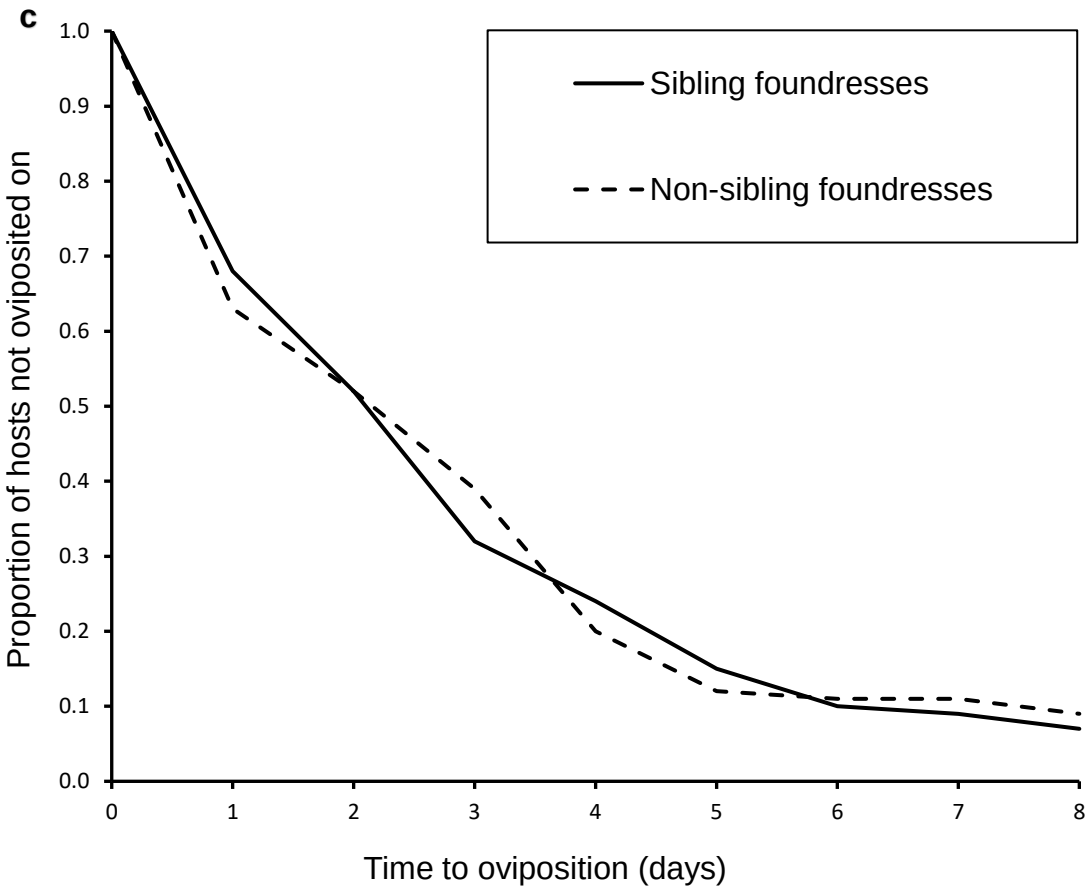


Figure 5.5 Influences on time from host presentation to oviposition. (a) Host size; (b) Foundress number; (c) Foundress relatedness. Panels show main effects separately but host size and foundress number also interacted significantly, as did foundress number and relatedness.

5.4.6 Clutch size

Clutches laid by single foundresses ranged from 6 to 33 eggs and clutches laid by multiple foundress groups ranged up to 72: this maximum was observed from 8 sibling foundresses while clutch size did not exceeded 51 in other replicates. As noted above, several temporally separate, clutches were laid on some hosts. Here we consider clutch size as the total number of eggs laid. Clutch size increased with an increase of both host size ($F_{1,196}$=80.19, P<0.001, %Dev=24.70,) and foundress number ($F_{5,200}$=8.51, P<0.001, %Dev=13.10,), without a significant interaction ($F_{5,195}$=1.90, P=0.097, %Dev=2.92, n=202, Figure 5.6).

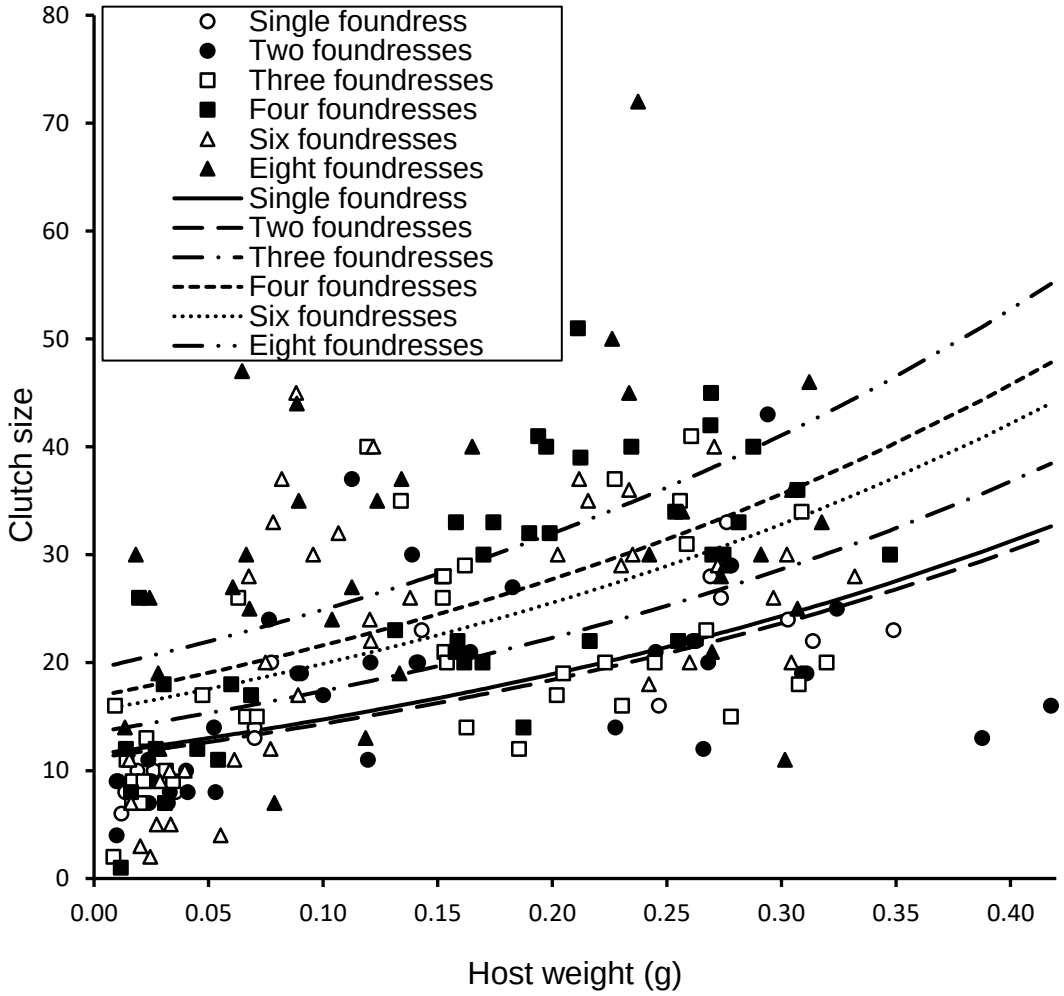


Figure 5.6 Effects of host weight and number of foundresses on the number of eggs laid on to hosts.

Analysis of multiple foundress replicates only (n=184) confirmed that clutch size was positively affected by host size ($F_{1,174}$ =73.46, P<0.001, %Dev=24.87) further found that it was affected by an interaction between foundress number and relatedness (Number: $F_{4,177}$=9.24, P<0.001, %Dev=12.51; Relatedness: $F_{1,174}$ =4.17, P=0.043, %Dev=1.41; Interaction; $F_{4,173}$=2.59, P=0.039, %Dev=3.51); on hosts of a given size, larger clutches were laid by larger groups of foundresses and also by sibling groups (Figure 5.7) but the clutch size response of non-sibling foundresses groups was less than that of sibling groups. The interaction between foundress relatedness and host size was not significant ($F_{1,169}$=0.10, P=0.749, %Dev=0.04).

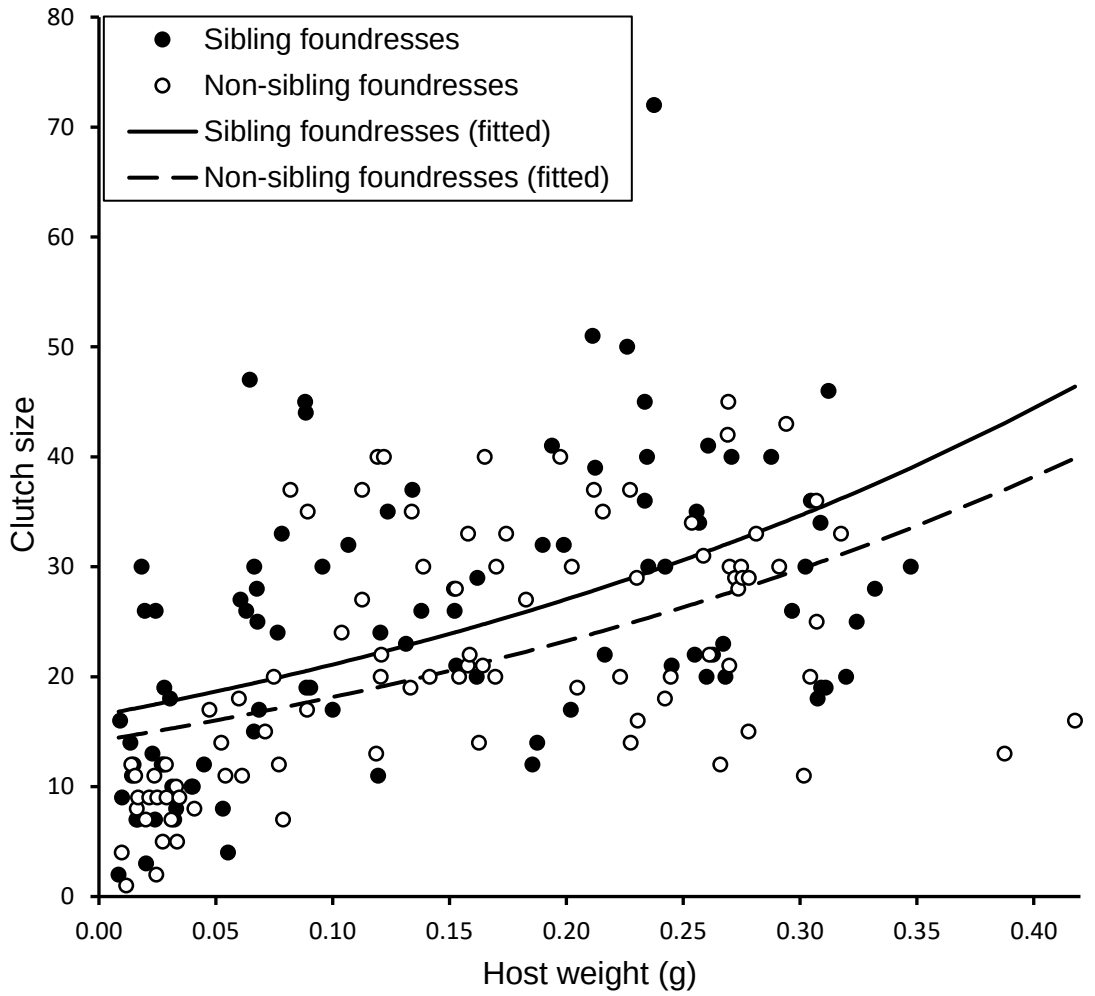


Figure 5.7 Effects of foundress relatedness on clutch size: Sibling foundresses laid collectively larger clutches.

As the clutch produced by siblings in one replicate was exceptionally large (see above) and could have a disproportionate influence on data interpretation, we re-tested the effect of relatedness on clutch size with this replicate removed: this confirmed that larger clutches were produced by sibling foundresses ($F_{1,169}$=4.22, P=0.042, %Dev=1.48, n=183) but the interaction between foundress number and relatedness was no longer significant ($F_{4,176}$=2.07, P=0.086, %Dev=2.90) and nor was the interaction between foundress relatedness and host size ($F_{1,168}$=0.23, P=0.633, %Dev=0.08).

The mean number of eggs laid per foundress (clutch size divided by number of foundresses) was not influenced by relatedness (multiple foundress replicates:

$F_{1,178}$=1.07, P=0.302, %Dev=0.29) or by its interaction with foundress number ($F_{4,177}$=1.84, P=0.124, %Dev=2.01) or by an interaction with host size ($F_{1,169}$=0.02, P=0.890, %Dev=0.01). Across all foundress number treatments, per-foundress clutch size increased with host size ($F_{1,196}$=112.99, P<0.001, %Dev=17.70) and decreased progressively with increase in foundress number ($F_{5,200}$=63.90, P<0.001, %Dev=50.06), without significant interaction between host size and foundress number ($F_{5,195}$=1.64, P=0.152, %Dev=1.28): *per capita* production by foundresses was greatest when females reproduced alone (Figure 5.8).

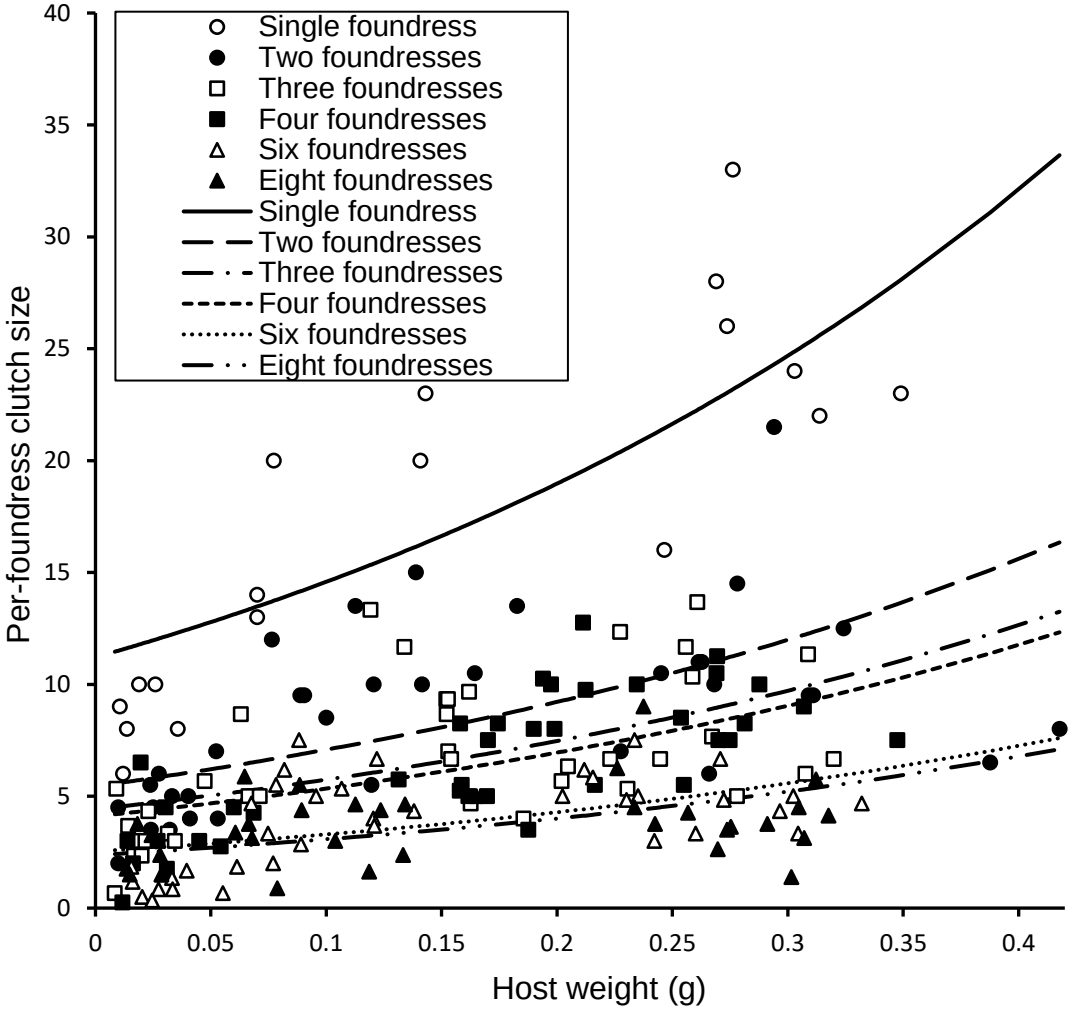


Figure 5.8 Effects of host weight and number of foundresses on the mean per capita offspring production for foundresses.

5.4.7 Developmental time

The time taken for eggs to develop to adulthood time ranged from 11 to 25 days. Development was not influenced by foundress relatedness (analysis of multiple foundress replicates only: G_1=0.7, P=0.403, %Dev=0.25, n=184). There was no significant interaction between foundress relatedness and host size (G_2=0.3, P=0.861, %Dev=0.13). The interaction between foundress relatedness and number was not significant (G_4=9.20, P=0.056, %Dev=2.27). Developmental time was affected by host size, foundress number and their interaction (host size: G_2=49, P<0.001, %Dev=12.72; foundress number: G_5=54.6, P<0.001, %Dev=16.03; interaction: G_{10}=38.5, P<0.001, %Dev=11.06), such that development was faster on medium or larger hosts, and in replicates with progressively fewer foundresses.

5.4.8 Developmental mortality

The overall probability of offspring pre-adult mortality of eggs was 0.6236 (SE = +0.0261, -0.0269). We observed several instance of entire clutches of eggs failing to reach the larval stage, with subsequent oviposition of a further clutch of eggs. Overall, developmental mortality was not affected by relatedness (multiple foundress replicates only, n=184: $F_{1,178}$=1.80, P=0.182, %Dev=0.82) or by its interaction with foundress number ($F_{4,173}$=0.37, P=0.829, %Dev=0.68) or host size ($F_{1,169}$=0.40, P=0.526, %Dev=0.18). Across all replicates (n=202), developmental mortality decreased as host size increased ($F_{1,196}$=11.35, P<0.001, %Dev=4.35) and increased as foundress number increased ($F_{5,200}$=10.81, P<0.001, %Dev=20.72) without significant interaction ($F_{5,195}$=1.54, P=0.178, %Dev=2.96; Figure 5.9).

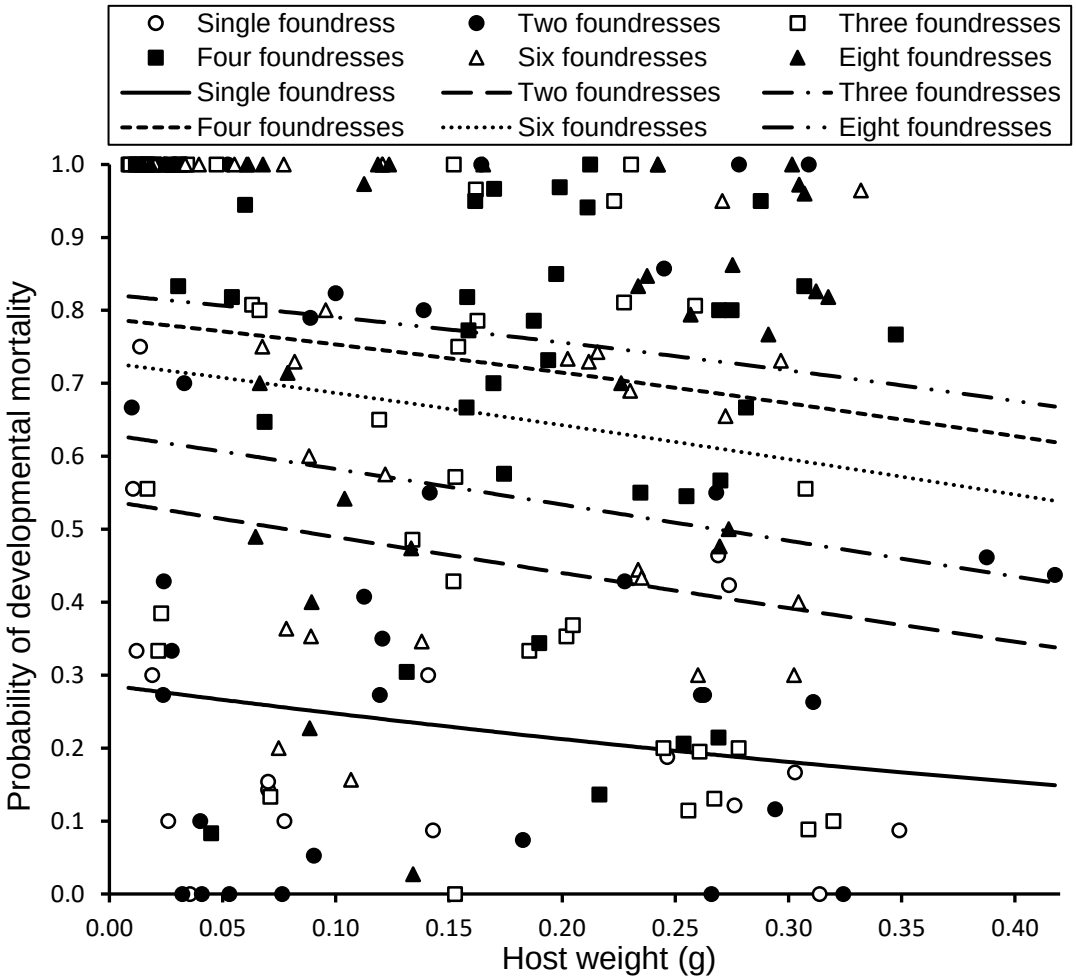


Figure 5.9 Effects of host weight and foundress number on offspring mortality.

5.4.9 Brood size

The total number of adult offspring produced hosts that had been oviposited on ranged between 1 and 38 (mean=11.6023, SE = +0.6818, -0.6439, n=151). Brood size was not influenced by foundress relatedness (multiple foundress replicates only: $F_{1,123}$=1.81, P=0.181, %Dev=1.17, n=133) or by its interaction with foundress number ($F_{4,122}$=0.96, P=0.432, %Dev=2.48) or with host size ($F_{1,118}$=0.02, P=0.881, %Dev=0.01). For groups of 1 to 4 foundresses, brood size increased with host size but for groups of 6 and 8 it declined (Host size: $F_{1,140}$=7.79, P=0.006, %Dev=4.37, n=151; Foundress number: $F_{5,144}$=4.15, P=0.002, %Dev=11.64; Interaction: $F_{5,144}$=5.16, P<0.001, %Dev=14.48, Figure 5.10(a)).

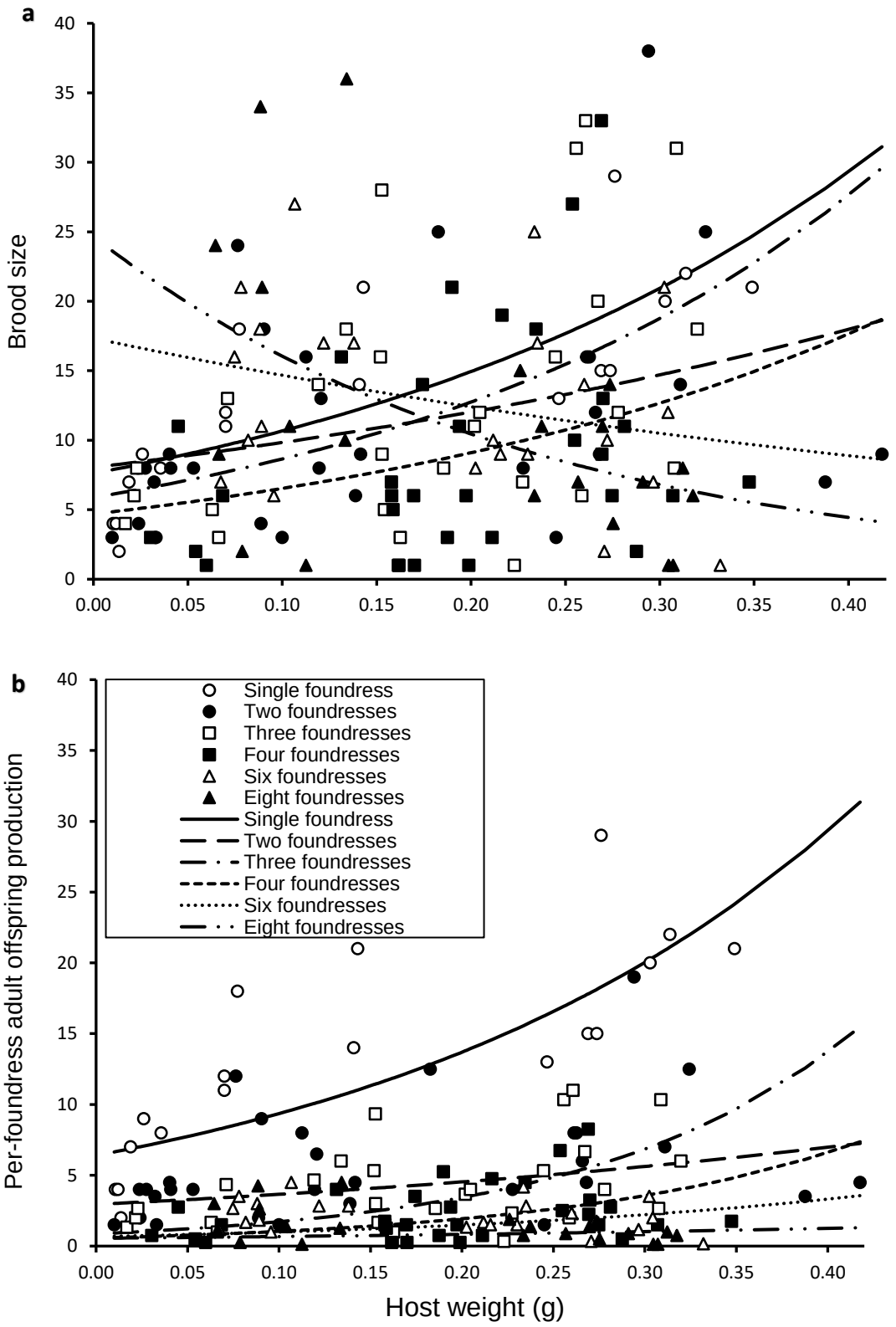


Figure 5.10 Interactive effects of foundress number and host size on the number of adult offspring produced. (a) Overall production from a given host; (b) Per foundress production.

In terms of the number offspring produced per foundress, production was not affected by foundress relatedness (multiple foundress replicates only: $F_{1,123}$=0.41, P=0.522, %Dev=0.19, n=133) or by its interaction with foundress number ($F_{4,122}$=0.71, P=0.589, %Dev=1.33) or with host size ($F_{1,118}$=0.30, P=0.586, %Dev=0.14). For groups of 1 to 4 foundresses, per-foundress offspring production increased with host size and decreased with foundress number while for groups of 6 and 8 production declined with host size (Host size: $F_{1,140}$=33.62, P<0.001, %Dev=7.91, n=151; Foundress number: $F_{5,144}$=6.44, P<0.001, %Dev=7.57; Interaction: $F_{5,144}$=3.07, P=0.012, %Dev=3.62, Fig. 10b).

5.4.10 Adult offspring size

The average (within broods) size of adult female offspring ranged between 0.6 – 1.35 mm (mean thorax length =1.0484, SE = ±0.0125). Size was not affected by foundress relatedness (multiple foundress replicates only: $F_{1,127}$ =0.19, P=0.667, r^2=0.001, n=133) or by its interaction with foundress number ($F_{4,122}$=1.08, P=0.371, r^2=0.03) or with host size ($F_{1,118}$=0.07, P=0.796, r^2=0.00). Female offspring were larger when hosts were larger ($F_{1,150}$=54.19, P<0.001, r^2=0.26, n=151, Figure 5.11 (a)) but size was not affected by foundress number ($F_{5,149}$=1.55, P=0.179, r^2=0.04: Host size × foundress number interaction: $F_{5,144}$=1.02, P=0.410, r^2=0.03). Females offspring were larger when they had emerged from larger broods ($F_{1,149}$=9.73, P=0.002, r^2=0.06, n=151, Figure 5.11 (b)).

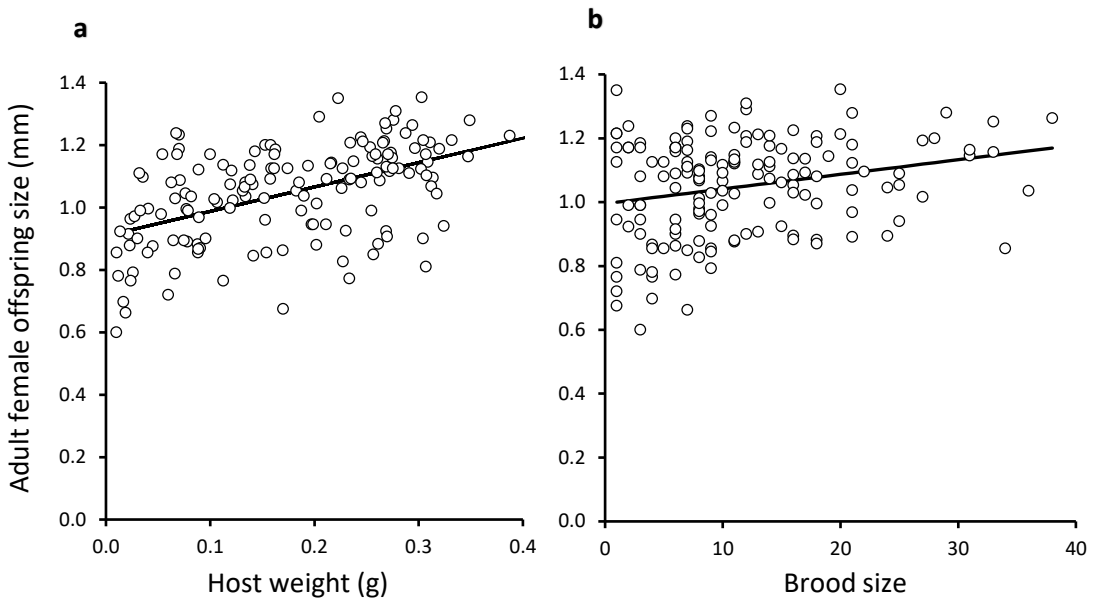


Figure 5.11 Effects of host size (a) and brood size (b) on offspring size.

5.4.11 Adult offspring sex ratio

Of the 151 replicates that produced adult offspring, 28 contained males only, most likely due to the mothers being unmated and constrained to lay haploid eggs (Godfray, 1990): 15 of these replicates were single foundress or sibling foundress replicates in which insemination failure was likely due to individual single males. The remaining non-sibling foundress replicates were mostly either those in which there were small number of foundresses, making it likely that they all had been failed by a small number of different males, or replicates in which offspring production was low (e.g. 8 non-sibling foundresses producing just one adult offspring), making it likely that female eggs were laid but failed to survive. Following procedures adopted by prior studies of *G. nephantidis* sex allocation (Hardy & Cook, 1995), we excluded these all-male broods, leaving 123 replicates for the analysis of brood sex ratios.

The sex ratios of groups of adult offspring (males/total offspring) were female biased (mean proportion male=0.2529, SE= +0.0255, -0.0240). Sex ratios of

offspring produced by sibling foundresses were significantly more female-biased compared to sex ratios of offspring produced by non-siblings (multiple foundress replicates only, n=108: $F_{1,99}$=25.08, P<0.001, %Dev=17.45, Figure **5.12**). There was a significant interaction between foundress relatedness and number ($F_{4,102}$=3.85, P=0.006, %Dev=10.71), such that groups of non-siblings produced higher and relatively invariant sex ratios while sibling foundresses typically produced lower sex ratios except when in the largest foundress groups. However, this interaction effect was not significant when a large outlier brood produced by 8 sibling foundress was excluded from the data ($F_{4,101}$=0.35, P=0.556, %Dev=0.23).

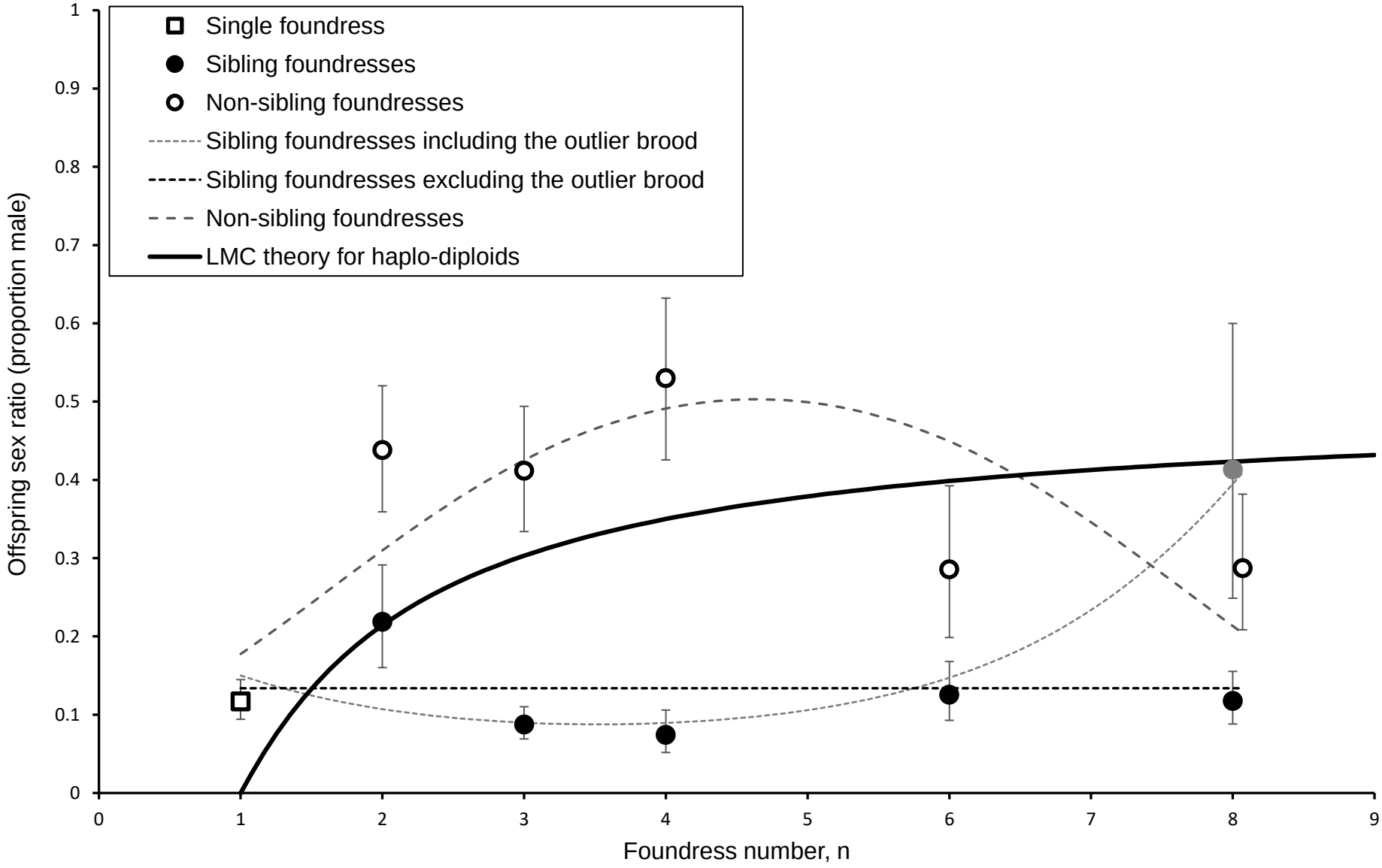

Single foundress
Sibling foundresses
Non-sibling foundresses
Sibling foundresses including the outlier brood
Sibling foundresses excluding the outlier brood
Non-sibling foundresses
LMC theory for haplo-diploids
Offspring sex ratio (proportion male)
Foundress number, n
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5
6
7
8
9

Figure 5.12 The relationship between offspring sex ratio and foundress number (n) and relatedness. Data points show means with errors bars of ±1 SE (some are asymmetric due to the bounded nature of proportional data). Analysis restricted to multiple foundress replicates showed that sex ratio was affected by relatedness and a number × relatedness interaction (see main text). The fitted logistic regression lines illustrate the statistical minimal adequate model sex ratio response of foundresses to increasing foundress number when foundress groups are either siblings or non-siblings; these regressions were carried out separately and included data from the single foundress case. The regression for sibling foundresses was carried out including and excluding the outlying brood (the grey symbol illustrates the mean sex ratio with this brood included). The two lower lines are the fitted logistic regression for sex ratio for sibling foundresses when data exclude the outlier brood [thicker line: $y=0.1338$)] and the fitted quadratic logistic regression for sex ratio among sibling foundresses when the outlier brood is included [the thinner line: $y=1/(1+(1/(EXP(-(0.673n)+(0.0955n^2)-1.156))))$]. The dashed line shows the fitted logistic regression for non-sibling foundresses sex ratio [$y=1/(1+(1/(EXP((1.082n)-(0.1166n^2)-2.498))))$]. Also illustrated is the predicted evolutionarily stable sex ratio according to the number of foundresses whose offspring will mate prior to dispersal, for species with haplo-diploid genetics [(n–1)(2n– 1)]/[n(4n–1)] (Hamilton 1979).

Sex ratios were significantly affected by foundress number ($F_{5,122}$=2.63, P=0.028, %Dev=9.90), but not influenced by host size ($F_{1,117}$=0.10, P=0.749, %Dev=0.08) or by an interaction between foundress number and host size ($F_{5,116}$=1.70, P=0.141, %Dev=6.39). Similarly, sex ratios were unaffected by the amount of resource available per foundress ($F_{1,106}$=0.02, P=0.897, %Dev=0.01) of by an interaction between relatedness and resource-per-foundress ($F_{1,105}$=3.08, P=0.082, %Dev=2.37).

We explored sex ratio patterns among multi-foundress replicates further by analyzing male and female offspring production separately. The number of males per brood was not affected by foundress number ($F_{5,117}$=1.04, P=0.397, %Dev=4.26, n=123) but was higher when relatedness was lower ($F_{1,99}$=11.72, P<0.001, %Dev=9.22, n=108, Figure 5.13a). There an interaction between these main effects ($F_{4,102}$ =3.88, P=0.006, %Dev=12.23) but this was not significant when the outlier brood for foundress number 8 was removed ($F_{4,101}$ =1.10, P=0.360, %Dev=3.37). The mean number of males produced per-foundress in each replicate was lower among sibling foundress groups ($F_{1,103}$=17.62, P<0.001, %Dev=11.89, n=108) and declined with an increase of foundress number ($F_{5,117}$=4.75, P<0.001, %Dev=16.86, n=123) without interaction ($F_{4,102}$=2.15, P=0.080, %Dev=5.81) (Figure 5.13c).

The number of females per brood was greater among broods produced by sibling foundresses than among non-sibling foundresses ($F_{1,103}$=7.38, P=0.008, %Dev=6.41, n=108, Figure 5.13b) and was not affected by foundress number ($F_{5,117}$=2.01, P=0.082, %Dev=7.91, n=123) or an interaction between number and relatedness ($F_{4,102}$ =0.68, P=0.611, %Dev=2.35). Similarly, mean female production per foundress was greater among sibling foundress groups

($F_{1,103}$=6.56, P=0.012, %Dev=4.37, n=108) and declined with foundress number ($F_{5,117}$=32.33, P<0.001, %Dev=58.01, n=123), without significant interaction between numbers and relatedness ($F_{4,102}$=1.10, P=0.362, %Dev=2.93) (Figure 5.13(d)).

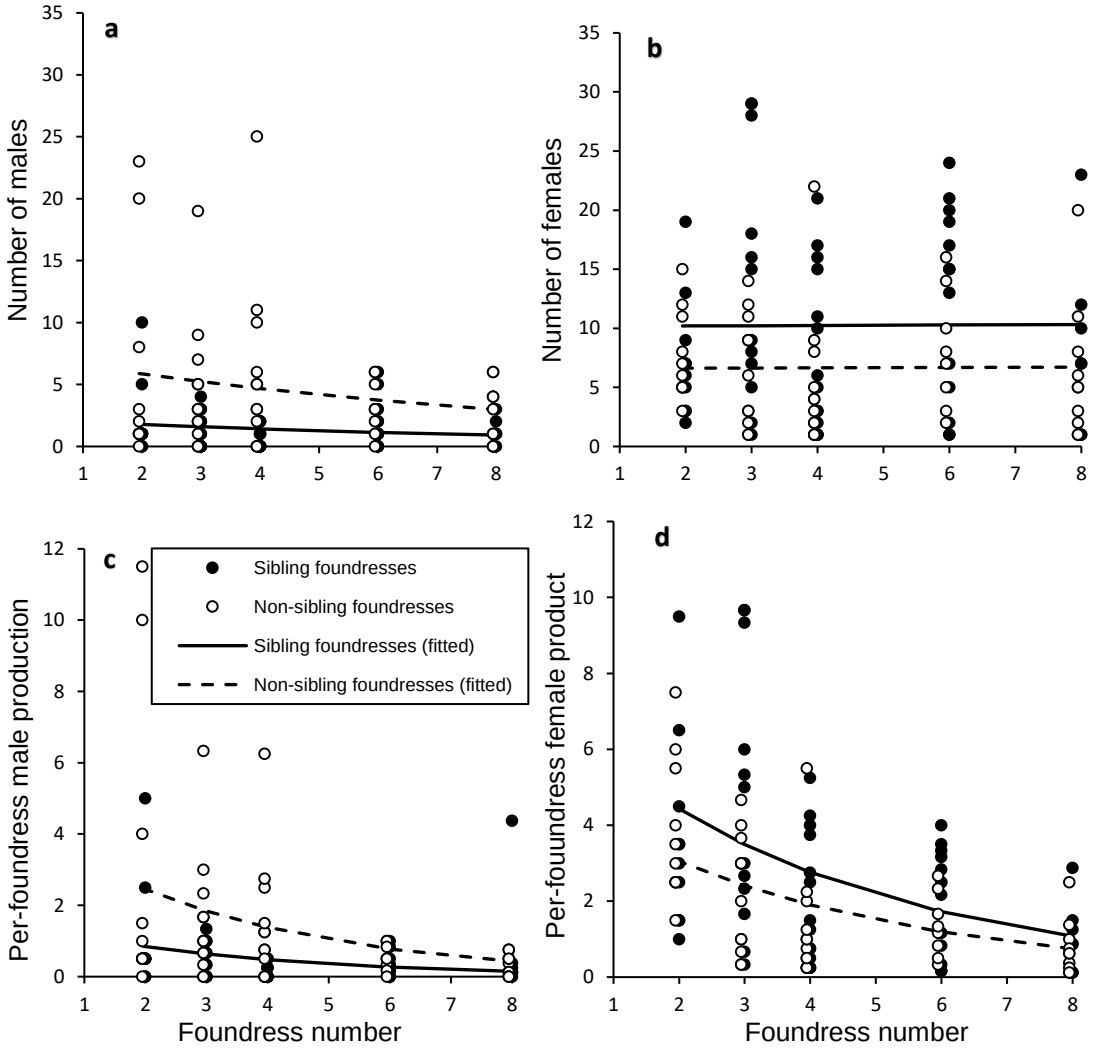


Figure 5.13 Effects of foundress number and relatedness on the sexual composition of multi-foundress broods.

5.5 Discussion

Effects of kinship on parasitoid life-histories have been relatively little explored, while there is substantial literature on effects of host size and of foundress number (Salt, 1938; Charnov *et al.*, 1981; Heinz & Parrella, 1989; Godfray, 1994; Zaviezo & Mills, 2000; Ode & Hardy, 2008; West, 2009; Tang *et al.*, 2014). For *Goniozus legneri*, Lizé *et al.* (2012) found that aggression during short-term contests for paralysed hosts was reduced when females were recognized as siblings. Despite being aggressive, short-term contests between females of *Goniozus* species, including *G. nephantidis*, are rarely fatal (Humphries *et al.*, 2006; Hardy *et al.*, 2013). Our current results, however, suggest that interactions over longer-time scales can lead to the death of females (see also Venkatesan *et al.* (2009b)). Some of the observed foundress mortality may have been caused by host death or other factors, but the effect of relatedness is likely to be due to direct agonistic interactions or, indirectly, to females being excluded from access to the host (and thus haemolymph feeding). In accord with prior studies on *G. nephantidis* showing that both contest outcomes and aggression within contests are correlated with the value of the contested resource (Humphries *et al.*, 2006; Stockermans & Hardy, 2013), we found that foundress mortality only occurred when resources were the most limiting (i.e. when hosts were small and/or when foundress numbers were large). Current evidence thus indicates that the intensity of intraspecific competition in *G. nephantidis* depends simultaneously on both kinship and resource availability (West *et al.* (2002) provide a general review of the associated theory); in some other Hymenopteran systems, one or the other of these selective factors appears to dominate (West *et al.*, 2001; Giron *et al.*, 2004).

Host exploitation by multiple foundress is genrerally considered disadvantegeous to individual mothers because it increases ressouce competition amongst their progeny (Van Alphen & Visser, 1990; Godfray, 1994; Dorn & Beckage, 2007; Harvey *et al.*, 2013). Under natural conditions, *G. nephantidis* is not thought to share host resouces with conspecifics but using experimental conditions of enforced close contact (Legner & Warkentin, 1988; Venkatesan *et al.*, 2009b) shows that it is within the behavioural repetoire of females to tolerate the presence conspecifics on a host (see also (Sreenivas & Hardy, 2016; Velasco-Hernandez *et al.*, unpublished). Beyond competition for access to respources, we found further effects of kinship on the the probablity and timing of reproduction, clutch size and the sexual composition of broods on maturity.

Foundress number and relatedness combined to influence the probablity of reproduction, such that when foudress groups were large, oviposition was more likely when foundresses were siblngs. The time taken to start laying eggs was also less when foundresses were siblings and also, similar to findings for other bethylids (Gao *et al.*, 2016a), when there were more foundresses. Once oviposition commenced, we found, as with prior studies of *G. nephantidis* (Hardy *et al.*, 1992; Humphries *et al.*, 2006), that single foundresses laid larger clutches on larger hosts. Further, we presented females with a larger range of host sizes and observed clutch size maxima around twice the previously reported value (33 vs. 18 eggs; Hardy *et al.* (1992)). We also found that larger groups of foundresses produced larger clutches than single foundresses, although the average per-foundress contribution of eggs was reduced (see also Venkatesan *et al.* (2009b)). Females may have choosen to reduce the number

of eggs they laid in anticipation of future competition to be experienced by their offspring (Petersen & Hardy, 1996; Mesterton-Gibbons & Hardy, 2004; Goubault *et al.*, 2007) or their intended oviposition may have been disturbed by other females (Sreenivas & Hardy, 2016) or their deposited eggs may have been eaten by other foundresses before being experimentally observed (Hardy & Blackburn, 1991; Hardy *et al.*, 1999; Goubault *et al.*, 2007; Venkatesan *et al.*, 2009b).

The finding that groups of sibling foundresses ultimately produced larger groups of offspring, is consistent with the notion that the observed forms of competition and interference are reduced by close kinship. While kinship may promote the evolution of cooperative reproduction in some parasitoids and other hymenopterans (Abbot *et al.*, 2011; Abdi *et al.*, Chapter 3; Abdi *et al.*, Chapter 4), in *G. nephantidis* there appears to be little advantage for individual females to reproduce in multi-foundress groups, as single foundresses had the highest *per capita* production of adult offspring, once a host was paralyzed. Similarly, individual *G. nephantidis* females were able to suppress even the largest hosts they were presented with and, as such, they are unlikely to gain substantial direct fitness benefits from communal host attack in the manner reported for quasi-social species of *Sclerodermus* (Tang *et al.*, 2014). Given that female *G. nephantidis* are mutually aggressive but tolerate other foundresses most when they are kin or when hosts are large, they may be bordering on a degree of sociality that (facultatively) includes a shared breeding site, somewhat similar to species of *Melittobia* (Hymenoptera: Eulophidae) (Matthews & Deyrup, 2007; Matthews *et al.*, 2009). Present evidence suggests that *Melittobia, Copidosoma* and *Sclerodermus* are the most socially advanced genera of parasitoids; all of

them typically attack hosts which are large relative to the size of the adult wasps and effects of kinship have also been reported (Abdi *et al.*, Chapter 3; Abdi *et al.*, Chapter 4) or suggested (Matthews *et al.*, 2009) in these genera. We consider that if there are *Goniozus* species that naturally attack very large hosts, these are the most likely members of the genus to exhibit more socially advanced behaviour than *G. nephantidis*.

The sex ratios of broods produced by individual *G. nephantidis* females are typically female biased (around 9% of offspring are males) (Hardy & Cook, 1995; Hardy *et al.*, 1999) and largely conform to expectations from the classical LMC theory for the single foundress case (Hamilton, 1967; Hamilton, 1979; Godfray, 1994; West, 2009). Effects of foundress number and relatedness have not previously been explored (except in the context of optimising foundress number for mass-rearing efficiency, (Venkatesan *et al.*, 2009b), where relatedness was not considered), largely because multiple-foundress clutches are not thought to occur in nature. Nontheless, there was a sex ratio response to an increase in foundress number among non-sibling groups that broadly followed expectation from classical LMC theory attuned to the haplo-diploid genetics of hymenopterans (Hamilton, 1979) and is also similar to responses observed in many other parasitoid and non-parasitoid species (Werren, 1983; Godfray, 1994; Burton-Chellew *et al.*, 2008; Ode & Hardy, 2008; Hu *et al.*, 2010). Current evidence for *G. nephantidis* does not, thus, fit with the notion that natural selection for adaptations to uncommon situations might be too weak to lead to facultative sex ratio adjustment (Herre, 1985; Shuker *et al.*, 2004a; Shuker *et al.*, 2004b; Innocent *et al.*, 2007; Burton-Chellew *et al.*, 2008; West, 2009).

In contrast to sex ratios produced by non-sibling foundresses, the sex ratios of broods produced by groups of siblings tended to be similar to those produced by single foundresses, irrespecitive of foundress number. Classical LMC theory implicity assumes that the sexual compositions of offspring groups at oviposition and at maturity are equivalent whereas this will not be the case when there is developmental mortality (Nagelkerke & Hardy, 1994; Hardy & Cook, 1995; Khidr *et al.*, 2012; Wilkinson *et al.*, 2016). We found that mortality became substantially more common as foundness numbers increased, thus reducing our ability to evaluate correspondence between observed and predicted sex ratios. Nonetheless, as we detected no differece in developmental mortality between broods produced by siblings and non-siblings, mortality patterns are not a likely cause of the observed effect of kinship on the sex ratios of adult offspring that developed successfully: we thus consider that the observed difference is most likely due to sex allocation responses by ovipositing foundresses.

Sex ratio theory has developed from Hamilton's original models to consider further the influence of relatedness; a typical result is that increased interactions between kin (sibmating or closely related foundress females) select for greater female bias (Frank, 1985; Taylor & Crespi, 1994; Shuker *et al.*, 2004b; Innocent *et al.*, 2007; Burton-Chellew *et al.*, 2008; West, 2009; Hu *et al.*, 2010). The patterns we observe among *G. nephantidis* broods are thus consistent with predictions of models considering co-foundress relatedness [and we note that sex ratios of the congener *G. legneri* respond to the relatedness between a female and her mate in the predicted direction (Du *et al.*, Chapter 6)]. There have been very few evaluations of the sex ratio response of parastoids to

foundress relatedness, or indeed among other species, and prior evidence has not collectively supported theory (Shuker *et al.*, 2004a; Burton-Chellew *et al.*, 2008). Studies by Shuker *et al.* (2004a) and Burton-Chellew *et al.* (2008) found that *Nasonia vitripennis* co-foundresses do not adjust their offspring sex ratio according to whether they were sisters or nonrelatives. They considered that *N. vitripennis* may not able to assess their relatedness to other foundresses in the vicinity, possibly because they so rarely oviposit on the same patch as close relatives that there will be limited selection for kin discrimination and thus facultative sex ratio adjustment. In contrast, although the known biology of *G. nephantidis* also suggests that multi-foundress offspring group production will be rare in nature, kin recognition in *Goniozus* has been demonstrated (Lizé *et al.*, 2012), most likely based on variation in cuticular hydrocarbons (Khidr *et al.*, 2013), and foundresses apparently adjust sex allocation according to kinship in the direction that theory predicts.

5.6 Conclusion

The potential for the evolution of sociality among bethylids, and what this might imply about hymenopteran sociality more generally, has been of longstanding interest (Wheeler, 1928; Malyshev, 1968; Evans, 1978; Gorokhovskaya, 2015). The present study was designed to explore whether prolonged female-female contact would elicit behavioural and reproductive responses that might reveal incipient higher-level sociality in a naturally sub-social species, *Goniozus nephantidis*. We conclude that under many circumstances, females will not be selected to share the resources of a paralysed host with other females. However, selection against sharing appears to be less stringent when either relatedness or resource availability are higher. Despite being presented with

what is thought to be an unnatural reproductive environment, females in multi-foundress groups apparently attune sex allocation according to both foundress number and foundress relatedness. *Goniozus nephantidis* appears to be the only studied parasitoid in which sex ratios conform to predictions of both classical and extended Local Mate Competition theory.

Chapter 6: Parental relatedness and parasitoid sex ratios under local mate competition

6.1 Abstract

Sex ratio theory predicts that, under local mate competition, a more female-biased offspring sex ratio will be produced by females when their relatedness to their mates is closer. We assess how the sexual composition of broods responds to mother-mate relatedness using *Goniozus legneri*, a bethylid wasp that has biased sex ratios due to local mate competition. Relatedness was varied by providing virgin females with a mate which was either a sibling, a non-sibling from the same strain, from a strain with geographically similar origin or from a strain with geographically distant origin. The sex ratios among broods produced by mothers that had mated with a sibling or a same-strain male were similar and were more biased than broods produced by females mated with males from a different strain. Brood sex variances were higher after mating with different-strain males. Previous evaluations of parasitoid sex ratio responses to mate relatedness have not found the patterns predicted, possibly because the species investigated can control progeny sex ratio but cannot discriminate kin. In contrast, female *G. legneri* appear to possess both these abilities and respond to mate relatedness in qualitative agreement with theoretical predictions.

6.2 Introduction

Evolutionary theory predicts that the optimal sex ratio for a mother to produce should depend on the number of mothers contributing offspring to a future mating group. When there are one or a few mothers (termed foundresses) strong female-bias is selected for as a result of the advantages of reducing competition for local mating opportunities between sons and increasing the number of mates for each son; the theory is known as 'local mate competition' (LMC) (Hamilton, 1967; Green *et al.*, 1982; Herre, 1985; Nunney & Luck, 1988; Orzack *et al.*, 1991; Greeff, 1996; Hardy *et al.*, 1998; Reece *et al.*, 2004; Shuker *et al.*, 2004b; Shuker *et al.*, 2006; Burton-Chellew *et al.*, 2008; Hong *et al.*, 2008; West, 2009; Khidr *et al.*, 2012; Chung *et al.*, 2019). Extending theory for strict LMC (all mating occurs prior to dispersal from natal groups Hamilton (1967)) to consider different degrees of pre-mating dispersal (partial LMC) predicts that non-local mating selects for less biased sex ratios among broods produced by multiple foundresses (Nunney & Luck, 1988; West, 2009). Under LMC, selection also favours low sex ratio variance, although subsequent developmental mortality may act to increase variance across broods and select for higher brood sex ratios (proportion of offspring that are male) (Green *et al.*, 1982; Hardy *et al.*, 1998; West, 2009; Khidr *et al.*, 2012; Wilkinson *et al.*, 2016; Chung *et al.*, 2019).

Inbreeding is an additional predicted influence on the sex ratios of haplo-diploids (females develop from fertilized, diploid, eggs; males develop from unfertilized, haploid, eggs): it increases mother-daughter relatedness more than mother-son relatedness and favours more female-biased sex ratios under multi-foundress LMC (Herre, 1985; West, 2009). Females could in principle adjust

their offspring sex ratios in facultative response to mating with a sibling or a non-sibling male, to the average levels of inbreeding or utilize fixed sex allocation sequences (Greeff, 1996; Reece *et al.*, 2004; Chung *et al.*, 2019). Tests for a facultative response to mate-relatedness using the gregarious parasitoid wasp *Nasonia vitripennis* have found no effects on sex ratios of broods produced by 1-7 foundresses, seemingly because females are unable to assess their relatedness to mating partners (Reece *et al.*, 2004; Shuker *et al.*, 2004b; Burton-Chellew *et al.*, 2008). Here we test for sex ratio effects of mate relatedness using a gregarious parasitoid species in which females have been shown to attune aspects of behaviour according to kinship and that has biased sex ratios due to single foundress LMC. Under single-foundress strict LMC, classical models predict that sex ratio optima are maximally biased under any degree of relatedness (Herre, 1985; West, 2009) but under partial LMC non-sibling mating is predicted to select for greatly reduced sex ratio bias among single foundress broods (Nunney & Luck, 1988; West, 2009).

Goniozus legneri is an ectoparasitoid of lepidopteran larvae that has a life-cycle that largely complies to the assumptions of LMC theory and its female-biased sex ratios with low variance comply with its predictions (Gordh *et al.*, 1983; Hardy *et al.*, 1998; Hardy *et al.*, 2000; Wilkinson *et al.*, 2016). Due to aggressive host and brood guarding by mothers for several days prior to and after oviposition (Bentley *et al.*, 2009), broods are produced by a single foundress. Clutches typically range from 4 to 18 eggs but broods may be smaller at maturity due to developmental mortality (Khidr *et al.*, 2012). Males are protandrous, typically mating with their sisters as these mature but males also disperse from natal patches so some non-sibling mating is likely (partial LMC Hardy *et al.*

(2000)), especially as broods often contain female offspring only (chiefly due to male developmental mortality) (Hardy *et al.*, 1998; Khidr *et al.*, 2012). Although *G. legneri* females engage in aggressive interactions with conspecific females during the host and brood guarding periods, aggression is reduced when competitor females are siblings or perceive each other as familiar (Lizé *et al.*, 2012), possibly based on the hydrocarbon composition of their integuments (Khidr *et al.*, 2013): adult females thus have some kin discrimination abilities.

6.3 Materials and Methods

6.3.1 Host and insect cultures and origins

Four mating relatedness treatments were established using three strains of *G. legneri* Gordh (Hymenoptera: Bethylidae). All strains were reared on larvae of the moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Experiments and cultures were kept at 26°C, 60-80% relative humidity and a 18:6h light: dark cycle. All females used in experiments belonged to a strain originally collected from Santiago, Chile, in 2003 (Zaviezo *et al.*, 2007), maintained in laboratory culture in Chile and subsequently cultured in our laboratory since 2009; we refer to this strain as 'Chile Lab' (CL) (Khidr *et al.*, 2012; Lizé *et al.*, 2012; Khidr *et al.*, 2014). Males used in experiments were from the CL strain or one of two further strains. The 'Chile Field' (CF) strain was field-collected in Santiago in May 2009 and subsequently cultured in our laboratory (Khidr *et al.*, 2012; Khidr *et al.*, 2014). The 'U' strain was obtained in 2003 from a commercial insectary in the USA, with the original material believed to have been collected from southern Uruguay in 1978 (Gordh, 1982; Gordh *et al.*, 1983), around 1,800km east of Santiago, and subsequently cultured in our laboratory (Khidr *et al.*, 2012; Lizé *et al.*, 2012; Khidr *et al.*, 2014). Analysis of DNA microsatellite

polymorphisms indicates a lack of variation within each of these strains but consistent between-strain differences (Khidr *et al.*, 2012; Khidr *et al.*, 2014). CL and CF strains are estimated to be more closely related to each other than either is to the U strain (Khidr *et al.*, 2014). These strains can readily interbreed (see below and (Khidr *et al.*, 2012)) and produce offspring with very similar patterns of developmental mortality to those observed in naturally produced U strain broods(Gordh *et al.*, 1983; Hardy *et al.*, 1998; Khidr *et al.*, 2012).

6.3.2 Experiment

Relatedness between mates was varied experimentally by placing 5-6 virgin CL females in a vial with 1-2 males from a given strain and allowing them to mate for 2-3 days. When males were from the CL strain they were either siblings of the females (from the same developmental brood) or non-siblings (from a different brood). In terms of closeness of relatedness between mates, the treatments were thus ranked as ‘CL female plus sibling CL male’ > ‘CL female plus non-sibling CL male’ > ‘CL female plus CF male’ > ‘CL female plus U male’. Females (n=225) were then individually provided with a host larva of known weight (range 31mg-39mg) in a stoppered glass vial and the resultant broods were allowed to develop to adulthood, whereupon numbers of male and female progeny were recorded. Broods containing only males on emergence (n=14) were likely produced by unmated females that cannot control sex allocation and were excluded from further analysis (Hardy *et al.*, 1998; Reece *et al.*, 2004; Shuker *et al.*, 2006; Burton-Chellew *et al.*, 2008; Khidr *et al.*, 2012).

6.3.3 Statistical analyses

Data from the remaining 211 broods (CL sib, n=70; CL non-sib, n=66; CF, n=34; U, n=41) were analyzed using generalized linear models (*GenStat* v. 19, VSN International Ltd.). Log-linear analyses with quasi-Poisson error distributions were used to explore effects in terms of numbers of males per brood (Hardy *et al.*, 1998; Khidr *et al.*, 2012) and to evaluate relationships between brood size and host size. Logistic analysis with quasi-binomial errors was used to explore the effect of mate relatedness treatment on secondary sex ratios (proportion of male offspring within broods) (Orzack *et al.*, 1991; Hardy *et al.*, 1998; Shuker *et al.*, 2006; Khidr *et al.*, 2012). Brood sex ratio variance was estimated using Heterogeneity Factors (HF) and variance ratios (R), deviation from binomial variance was assessed using Meelis tests (Hardy *et al.*, 1998; Khidr *et al.*, 2012; Wilkinson *et al.*, 2016) and differences between treatments were evaluated using Bartlett's test for homogeneity of variances.

6.4 Results

Females produced larger broods on larger hosts ($F_{1,203}$=221.81, P<0.001, %Dev=48.31). The brood size response to host size interacted weakly with mate relatedness ($F_{3,203}$=5.36, P<0.001, %Dev=3.55) such that the rate of brood size increase was greatest when females were mated with sibling males. There was no significant difference in the brood size response to host size between females mated with males from either CF or U (aggregation of factors levels including interaction term: $F_{2,205}$=0.31, P=0.732, %Dev=0.133) but the responses by CL females mated with sibling or by non-sibling CL males were different from each other ($F_{2,207}$=5.39, P=0.005, %Dev=2.28).

The numbers of males per brood increased with brood size ($F_{1,207}$ =43.86, P<0.001, %Dev=14.23) and were also affected by mate relatedness ($F_{3,209}$=14.25, P<0.001, %Dev=13.87) without significant interaction (relatedness×brood size: $F_{3,206}$=1.72, P=0.165, %Dev=1.67): there were more males produced for a given brood size when females had mated with males from a different strain (irrespective of whether this was CF or U: $F_{1,207}$=0.04, P=0.845, %Dev=0.012) than when they had mated with same-strain males (irrespective of sibship: $F_{1,208}$=0.49, P=0.483, %Dev=0.185) (Figure 6.1 (a)).

The mean brood sex ratio across all replicates was 0.1763 (+SE=0.0098, -SE=0.0094) with variance that was not significantly different to binomial (HF=1.29, R=1.246; Meelis test: U=1.98, P>0.05). Sex ratios were significantly affected by mate relatedness ($F_{3,207}$=13.96, P<0.001, %Dev=16.51) but not by brood size ($F_{1,206}$=1.92, P<0.168, %Dev=0.75) or by an interaction between brood size and relatedness ($F_{3,203}$=2.30, P=0.078, %Dev=2.72). There was no significant difference in sex ratios produced following mating with CF or U strain

males $F_{1,208}$=0.01, P=0.936, %Dev=0.002%) or following mating with sibling or non-sibling CL strain males ($F_{1,209}$=0.09, P=0.759, %Dev=0.04). The mean sex ratio of broods produced by females mated with CL strain males was 0.1321 (+SE=0.0090, -SE=0.0085) and had significantly less than binomial variance (HF=0.852, R=0.692; Meelis test: U= -2.19, P<0.05) while the mean sex ratio produced by females mated with males from a different strain was 0.2499 (+SE=0.0196, -SE=0.0186) and had significantly greater than binomial variance (HF=1.49, R=1.44; Meelis test: U=2.49, P<0.05). Sex ratio variance was significantly lower among broods produced by females mated with males from the same strain than among broods mated to males from a different strain (Bartlett's test: χ^2 =35.88, d.f.=1, P<0.001). We conclude that females that had mated with males from the same strain produced more female-biased offspring sex ratios than did females that mated with males from another strain (Figure 6.1(b)) and that they also produced sex ratios with lower variance.

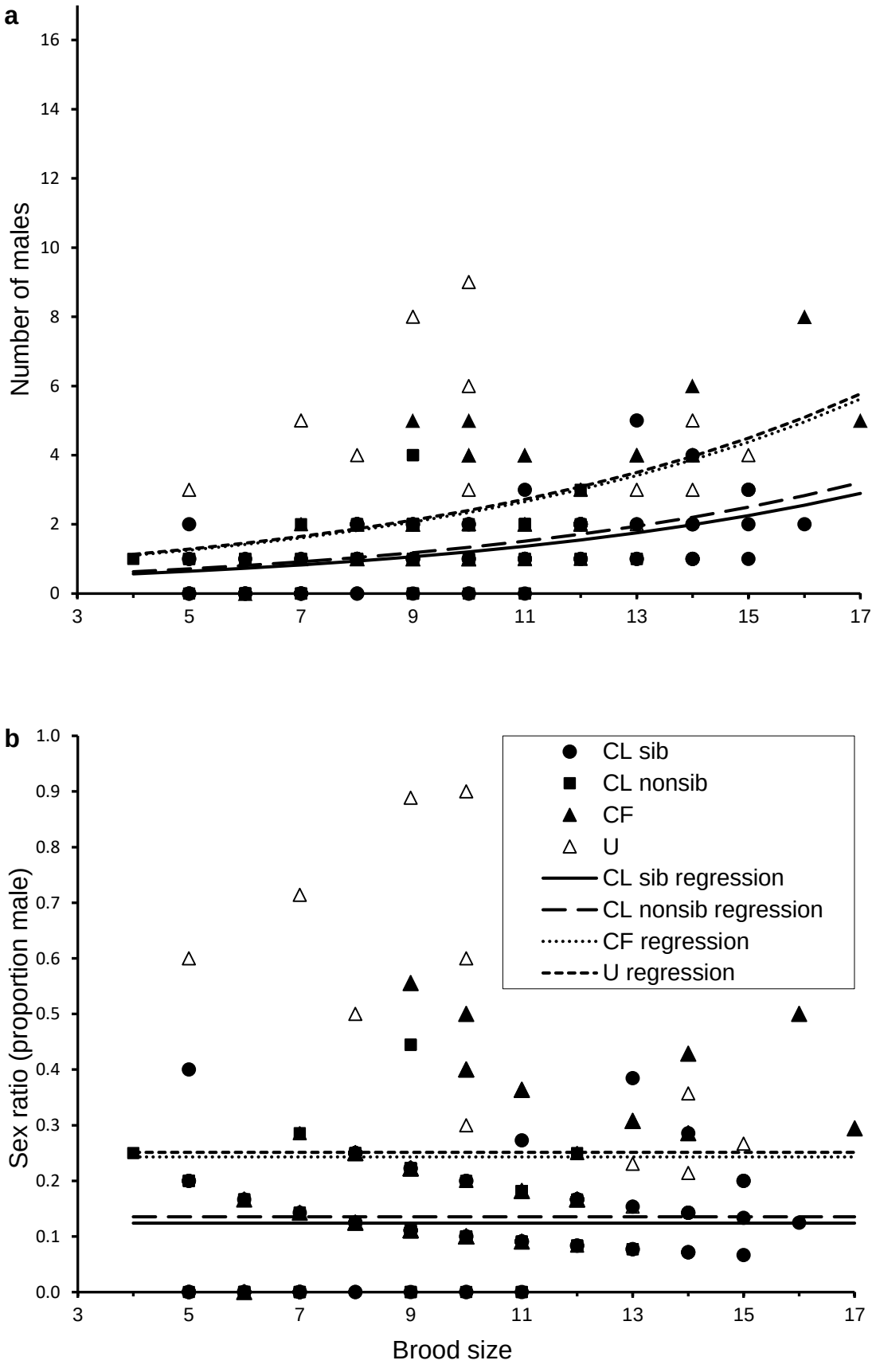

a
Number of males
0
2
4
6
8
10
12
14
16
3
5
7
9
11
13
15
17
b
Sex ratio (proportion male)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Brood size
CL sib
CL nonsib
CF
U
CL sib regression
CL nonsib regression
CF regression
U regression

Figure 6.1 Effect of mate relatedness on brood sexual composition. Panel (a) shows the data in terms of the number of males per brood ratios produced by females following the different mate relatedness treatments. Numbers of males increased with brood size and were also significantly higher when females had mated with a male from another strain (irrespective of strain identity) comparted to a same-strain male (irrespective of sibling status). Separate log-linear regression lines are shown for each mating treatment to illustrate the similarities and differences in responses. Panel (b) shows the same data in terms of brood sex ratios. Sex ratios did not differ significantly when mates were either sibling or non-sibling CL males and also did not differ when males belonged to either of the other strains, but were more biased when mates were from the same strain rather than a different strain. Sex ratios did not vary significantly according to brood size: the fitted logistic regression lines thus have no slope and represent the means for each mating treatment. The key is common to both panels.

6.5 Discussion

The sexual composition ratios of *G. legneri* broods complied broadly with those observed in prior studies that did not manipulate mate relatedness (Gordh, 1982; Hardy *et al.*, 1998; Hardy *et al.*, 2000) and were additionally the most female biased when mothers had mated with closely related males. Encountering a male from another strain may thus influence a female's estimation of the probability of inbreeding among her offspring and favour increased investment in males, as predicted under several LMC scenarios (Herre, 1985; Nunney & Luck, 1988; Greeff, 1996; Reece *et al.*, 2004; West, 2009). Less biased sex ratio optima are, under some scenarios, also expected to reduce selection for low sex ratio variance (West, 2009; Wilkinson *et al.*, 2016), offering explanation for the higher variances observed among broods produced by females that had mated with males from a different strain. Candidate mechanistic cues for the required kin discrimination are cuticular hydrocarbon profiles, which are known to differ between CL and U strain males

(Khidr *et al.*, 2012). However, that similar sex ratios produced by females mated with sibling or non-sibling males from the same strain suggests females lack the ability to distinguish between relatively close male kin, even though they can distinguish within-strain differences between other females (Lizé *et al.*, 2012).

While a straightforward interpretation of our results is that females directly assess their relatedness to the male they mate with and adjust subsequent sex allocation accordingly, there are several further factors that could potentially influence the sex ratio patterns observed. First, developmental mortality can alter brood sexual composition between offspring production and maturity, diminishing maternal influence on secondary sex ratios and increasing sex ratio variance (Green *et al.*, 1982; Khidr *et al.*, 2012; Wilkinson *et al.*, 2016). Sex ratios at oviposition were not assessed because such evaluation in *G. legneri* currently necessitates cross-strain mating (Khidr *et al.*, 2012), confounding the purpose of our experiment. Second, strain-specific effects beyond merely 'closeness of relatedness' may operate. The weak developmental mortality interaction of female-male strain with brood size observed by Khidr *et al.* (2012) is compatible with the weak interaction between brood size and mate relatedness we observed. In *N. vitripennis* strain identity may affect females' sex ratio responses to LMC cues (Orzack *et al.*, 1991), and, in particular, males from different strains may exert differing degrees of influence over a female's progeny sex ratio, via sexual conflict or genetic incompatibilities (Shuker *et al.*, 2006; Geuverink *et al.*, 2009). Although, further work is required to fully separate possible confounds from kin recognition effects in *G. legneri*, it is noteworthy that Khidr *et al.* (2012) found no primary or secondary sex ratio differences

between the offspring of CL females mated with U males and those of U females mated with CL males.

6.6 Conclusion

Predictions for adaptive sex ratios responses to relatedness assume that mothers are able to both control sex allocation and discriminate kin. *Goniozus legneri* is known to have both abilities (Khidr *et al.*, 2012; Lizé *et al.*, 2012) and we have found that it appears to utilize them in combination to adjust sex ratios as predicted.

Chapter 7: General Discussion

This project aimed to develop the potential of *Sclerodermus brevicornis* as an agent of biological control of invasive wood boring beetles. This development included relatively straightforward considerations, such as finding suitable alternative hosts for its efficient mass rearing, but because *Sclerodermus* are quasi-social, it also included gaining an understanding of the reproductive behaviours that make up this unusual parasitoid life-history. This was done by investigating the potentially interacting roles of host size, foundress number and co-foundress kinship as evolutionary influences on cooperative reproduction in *Sclerodermus*. On the basis that quasi-sociality probably evolved from less complex social systems, the project also included an investigation of host size, parasitoid number and kinship effects in members of a related parasitoid genus, *Goniozus*, that is naturally sub-social (exhibiting maternal care not cooperative reproduction) with the aspiration of generating insights into transitions between 'levels' of sociality. Effects of female-female kinship were found in both study systems: these included sex ratios effects in *Goniozus* but not in *Sclerodermus*, and the final part of the project found further kinship effects (mating partner relatedness) on *Goniozus* sex ratio. The project has furthered understanding of the reproductive biology of *Sclerodermus* and of *Goniozus*, both are genera of agro-economic importance. In this chapter, I summarize the key results and then discuss each empirical chapter, emphasizing possibilities for future investigations and biocontrol applications.

7.1 Summary of results

The main results of these studies can be summarised into the following main points:

- The larvae of the rice moth, *Corcyra cephalonica,* can be utilized as factitious hosts for *Sclerodermus brevicornis* that normally develop on coleopterans, with around 75% of hosts becoming paralysed and around 60% of all hosts being oviposited on, with overall reproductive success of around 40% and a strongly (**~** 92% females) female-biased offspring sex ratio (Chapter Two).
- The use of *Goniozus legneri* females to pre-paralyse hosts can enhance *S. brevicornis* production but also adversely affect some stages of the immature parasitoid life-cycle (Chapter Two).
- Mean per-foundress offspring production is maximised when hosts were pre-paralysed and when presented to single foundresses, whereas mean brood size is maximized when there were six-foundresses per host (Chapter Two).
- Generally, larger groups of *S. brevicornis* foundresses are more successful in terms of host attack and timing (Chapter Three).
- The time taken to paralyse standard-sized hosts is shorter when the relatedness between *S. brevicornis* co-foundress is higher (Chapter Three).
- Per-foundress adult offspring produced by *S. brevicornis* brood-mate foundresses is greater than by non-siblings but also led to offspring being smaller (Chapter Three).

- Using a public goods model framework (Hamilton's rule), and data from prior studies that experimentally varied host size for other *Sclerodermus* species, generates a prediction that host size and inter-foundress kinship will interactively influence host attack behaviour (Chapter Three).
- Effects of both host size and kinship operate during several of the stages of *S. brevicornis* host attack and reproduction and, as predicted, they interact to influence the timing of host attack (Chapter Four).
- A public goods model of host attack using parameters estimated for *S. brevicornis* gives broadly similar results to models using data from congeners, again suggesting that selection for an individual foundresses to attack and then, if successful, share their hosts will depend on both size of the host and the foundresses' relatedness to any co-foundresses present (Chapter Four).
- Confinement of foundresses suggests that resource availability and relatedness both affect the intensity of agonistic interactions. Foundresses will reproduce together on a host, but they produce most offspring *per capita* when reproducing alone (Chapter Five).
- The sex ratios among broods produced by *Goniozus nephantidis* females that are forced to share a host with sibling foundresses are as biased as when females reproduce alone, whereas broods produced by groups of non-sibling foundresses are considerably less biased and conform broadly to patterns predicted by both classical and extended models of Local Mate Competition (Chapter 5).
- The sex ratios among broods produced by *Goniozus legneri* mothers that have mated with a sibling or a same-strain male are similar and are more

biased than broods produced by females mated with males from a different strain. Brood sex variances are also higher after mating with different-strain males (Chapter Six).

7.2 Performance of *Sclerodermus brevicornis,* a parasitoid of invasive longhorn beetles, when reared on rice moth larvae

Rearing parasitoid species of *Sclerodermus brevicornis* on longhorn beetles is entirely possible, but the process of rearing the hosts is time-consuming and physically demanding (Lupi *et al.*, 2013; Lupi *et al.*, 2015a; Lupi *et al.*, 2017). Moreover, *S. brevicornis* foundresses are best left undisturbed for at least four days to successfully paralyse its host, a process that increases the overall duration of parasitoid rearing. Hence, this study was designed to evaluate the potential use of larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), a commonly used factitious host for rearing around 75 species of natural enemies (Chaudhuri & Senapati, 2017), as hosts for this parasitoid. This study explored the performance of *S. brevicornis* when presented with *C. cephalonica* larvae by quantifying the probability and timing of host attack and parasitism as well as reproductive success, offspring production and characteristics. In addition, this study explored the effects of varying the number of females to which individual hosts are presented in order to determine the optimal female-to-host ratio (Wei *et al.*, 2017). Further, females of the bethylid wasp, *Goniozus legneri* were used to paralyse hosts prior to their presentation to *Sclerodermus* females as the time to paralysis can be a limiting factor in rearing protocols of this parasitoid.

The study found that larvae of the rice moth, *Corcyra cephalonica,* can be utilized as factitious hosts for *Sclerodermus brevicornis*. This is in contrast to a

previous attempt to rear *S. brevicornis* on lepidopteran larvae (*Galleria mellonella*) and it is noteworthy that *Sclerodermus* species normally develop on coleopterans (Gordh & Móczár, 1990). Around 75% of hosts were paralysed (similar to what was found by estimates obtained from *S. brevicornis* attack on equivalently sized long-horn beetle, Chapter 4), and around 60% of all hosts presented had eggs laid on them (compared to 73% when provided with beetle larvae, see chapter four) and an overall reproductive success of around 40% with strongly female-biased adult offspring (around 92% females).

The use of *G. legneri* to pre-paralyse hosts adversely affected some stages of the life-cycle such as probability and timing of oviposition, timing of larval hatching and reproductive success, but positively affected the time from pupae to adult eclosion and the size of adult offspring production.

On the other hand, probabilities of paralysis and oviposition increased with foundress number, as did the probability of a host yielding at least one parasitoid offspring foundress as reported by (Tang *et al.*, 2014). The total adult offspring per-host production increased with an increase of foundress numbers whereas production of per-foundress brood size per-host increased as foundress groups decreased.

The maximum per-foundress adult offspring per-host was produced by hosts that were pre-paralysed by females of *G. legneri* when presented to single foundress groups, whereas the maximum total adult offspring per-host was recorded when foundress number was six.

Further study is recommended to be conducted to evaluate the subsequent performance and control efficiency of *S. brevicornis* females that were reared on this alternative host, larvae of rice moth after their release into the field.

7.3 Co-foundresses relatedness in a quasi-social parasitoid mediates suppression of dangerous hosts

The unusual cooperative reproductive behaviour (quasi-sociality), exhibited by females of *Sclerodermus*, a parasitoid genus of hymenopterans, was previously explained in terms of direct fitness benefits accruing to females when hosts are too large to be likely to be suppressed by an individual foundress, irrespective of kinship (Tang *et al.*, 2014). However, the explanation did not preclude that effects of kinship between foundresses may also operate. This study evaluated potential effects of co-foundress relatedness and number on aspects of cooperative reproduction, throughout the offspring-production cycle of the quasi-social parasitoid *Sclerodermus brevicornis*. Several studies have experimentally assessed the variation in *Sclerodermus* foundress numbers (Tang *et al.*, 2014; Gao *et al.*, 2016b; Wang *et al.*, 2016; Wei *et al.*, 2017; Abdi *et al.*, Chapter 2), whereas variation in their relatedness had not been assessed. It was found that larger foundress groups were more successful in terms of host attack and timing. This is consistent with what has been found in several prior studies on *Sclerodermus* species (Li & Sun, 2011; Tang *et al.*, 2014; Gao *et al.*, 2016b; Abdi *et al.*, Chapter 2). Despite that successful performance by larger foundress groups, similar brood size was produced by smaller foundress groups from paralysed hosts and thus the per-foundress reproductive output of larger foundresses was lower. Tang *et al.* (2014) have previously shown that the costs and benefits to *Sclerodermus* females of sharing a host with other foundresses are host-size dependent, essentially mediated by the lower probability of success for individuals or small groups of foundresses when faced with larger, and likely more dangerous hosts (Liu *et al.*, 2011; Wei *et al.*, 2017).

While a previous study by Wu *et al.* (2017) has shown that foundress-offspring relatedness had negligible effects on offspring development in *S. guani*, this study found that the time taken to paralyse standard-sized hosts is shorter when co-foundress relatedness is higher. The shorter latency to host paralysis observed among groups of prior brood-mates indicates that foundresses are more prone to take the risk of attacking and paralysing a host when the beneficiaries of a successful attack include their kin. In addition, the per-foundress adult offspring produced by brood-mate foundresses was (marginally significant) greater compared to non-siblings, but that greater number of broods produced by brood-mates on a given host size led to offspring with smaller body size, which is in agreement with results for *S. harmandi* (Liu *et al.*, 2011) and other bethylids (Mayhew, 1998). The small body size may constitute a fitness disadvantage (Gao *et al.*, 2016a).

Hamilton's rule and data from prior studies (Liu *et al.*, 2011; Tang *et al.*, 2014; Wei *et al.*, 2017) that experimentally varied host size (which was held constant in the experiment reported in Chapter 3) were used to explore how the greater risks and greater benefits of attacking larger hosts and co-foundress relatedness could combine to determine the size of hosts that a female is selected to attack as a public good. The public goods model framework suggested that both host size and inter-foundress kinship will interactively influence host attack behaviour; an assertion that was then tested in a following empirical study (Chapter 4).

The natural dispersal pattern (i.e., dispersal from the natal patch individually or as a group) and the likelihood of *Sclerodermus* co-foundresses being kin in the field is unknown. Hence, further studies will need to be undertaken to discover

natural patterns of *Sclerodermus* dispersal in the field. Moreover, since *Sclerodermus* co-foundresses in the current study have shown that host attack and per-foundress fitness gains are kin mediated behaviours, future studies are suggested to explore in detail that *Sclerodermus* co-foundresses may attune their behaviour at other life-history stages according to inter-foundress relatedness. For instance, the existence of selfish exploitation that leads to reproductive skew after a successful suppression of a dangerous host. Finally, future studies should be conducted to explore further information on the natural life-histories of *Sclerodermus* species that will help to assess and develop the key assumptions of a simplistic use of a public goods model framework to capture the essence of a female's decision to attack and share a host according to her relatedness to co-foundresses.

7.4 Co-foundress relatedness and host dangerousness interactively affect host exploitation in a quasi-social parasitoid

The work in Chapter 3 on the quasi-social parasitoid species, *Sclerodermus brevicornis* showed that the time lag to paralysis of small sized hosts is shorter when co-foundress relatedness is higher. A public goods model, using data from congeners attacking a range of host sizes, predicted that the greater risks and greater benefits of attacking larger hosts would combine with co-foundress relatedness to determine the limits to the size of a host that a female is selected to attack to provide essential reproductive resources to co-foundresses as well as to herself. It was also predicted that the time to host attack would, in consequence, be affected by an interaction between host size and relatedness. This work in Chapter 4 was is designed to test empirically the combined effects of host size and *S. brevicornis* co-foundress relatedness holding foundress

number constant at two females on the timing and success of host attack behaviour and other aspects of reproductive success.

In addition, attacking a host is a challenging step for *Sclerodermus* females and would result in approximately 25-40% of *S. brevicornis* foundresses die prior to host suppression depending on the size of the attacked host (Liu *et al.*, 2011; Wei *et al.*, 2017). It was also observed during the single-foundresses assay study that many of the dead wasps were severed into two pieces and that indicates that hosts engage in active, and often successful, behavioural defence against parasitism, as also observed by Li and Sun (2011) and Liu *et al.* (2011). Hence, the probability of a single foundress mortality during attacking hosts of different sizes was also assayed to be used as a cost parameter estimate for the tested prediction of the public goods model.

It was shown that effects of both host size and kinship operate during several of the stages of *S. brevicornis* host attack and reproduction and, as predicted, that they interact to influence the timing of host attack. Further, a public goods model of host attack using parameters estimated for *S. brevicornis* gives broadly similar results to the previous models, again suggesting that selection for an individual foundresses to attack and then, if successful, share their hosts will depend on both size of the host and the foundresses' relatedness to any co-foundresses present. Females will not be selected to bear the individual cost of a public good when hosts are large and dangerous nor when their relatedness to co-foundress relatedness is low.

To develop a full picture, future studies will be required to reveal further influences of co-foundress relatedness on some reproductive behaviours following host suppression, such as the apparently cooperative nature of post-

paralysis behaviour and brood production in *Sclerodermus* (e.g. to test for disproportionate reproduction by some foundresses [reproductive skew, Hammond *et al.* (2006)]) and any mediating effects of co-foundress kinship.

7.5 Co-foundress confinement elicits kinship effects in a naturally sub-social parasitoid

Parasitoids in the bethylid genus *Goniozus* are sub-social and engage in contests (that are not normally fatal) to aggressively defend their host and their brood against conspecific females. However, aggression is reduced when conspecifics are kin (Lizé *et al.*, 2012). Prior studies have evaluated factors affecting the outcomes of competitive interactions between pairs of *Goniozus* females (e.g. a brood guarding host 'owner' and an 'intruder'), in the context of testing game-theoretic models of contest behaviour by examining relatively only short-term interactions (<3h) and considering initial contest outcomes to be decisive, such that losers would be permanently excluded (reviewed by Hardy *et al.* (2013)). While the findings of these studies were largely compatible with model predications, the possibility of longer-term, or repeated, interactions between competing females remains. It was also observed that prolonged confinement of multiple females and multiple hosts together during the entire period of offspring development (Sreenivas & Hardy, 2016; Velasco-Hernandez *et al.*, unpublished), could result in females tolerating each other's presence on a host, even though overall offspring production can be reduced (Sreenivas & Hardy, 2016).

Females of the naturally sub-social *Goniozus nephantidis* were used to explore the life-history effects of factors that may have influenced the evolution of sociality in the Bethylidae: host size (which seems to be correlated with

sociality), foundress number (that could lead to either cooperation [e.g., *Sclerodermus*] or competition over resources and f foundress relatedness (which plays a substantial role in the evolution of social behaviour). *Goniozus nephantidis* females (experimentally varying number and relatedness) were confined together with one host (experimentally varying size) to explore whether prolonged female-female contact would elicit behavioural and reproductive responses (such as foundress mortality, host attack, offspring production and sex ratio) that might reveal incipient higher-level sociality in a naturally sub-social species, *Goniozus nephantidis*.

This indicated that the intensity of intraspecific competition in *G. nephantidis* depends simultaneously on both kinship and resource availability, such that co-foundresses confinement led to the death of some females, suggesting that interactions over longer-time scales can lead to the death of females, although the mortality of foundresses was less common when foundresses were siblings. In addition, foundress mortality occurred only when host resources were most limiting. This is consistent with prior studies on *G. nephantidis* showing that both contest outcomes and aggression within contests are correlated with the value of the contested resource (Humphries *et al.*, 2006; Stockermans & Hardy, 2013).

While kinship may promote the evolution of cooperative reproduction in some parasitoids and other hymenopterans (Abbot *et al.*, 2011; Boomsma *et al.*, 2011; Abdi *et al.*, Chapter 3; Abdi *et al.*, Chapter 4), there was little advantage for individual females to reproduce in multi-foundress groups: single foundresses suppressed even the largest hosts presented and had the highest *per capita* production of adult offspring. Sibling foundresses produced larger

offspring which is consistent with the notion that competitive interactions among *Goniozus* females are reduced when conspecifics are kin.

The sex ratio of *G. nephantidis* is typically female biased and largely conforms to expectations from the classical LMC theory for the single foundress case. This study showed that, despite single-foundress reproduction being the norm, *G. nephantidis* females in multi-foundress groups appear to attune sex allocation according to both foundress number and foundress relatedness: broods produced by sibling foundresses had similar sex ratios to broods produced by single foundresses whereas the sex ratios of broods produced by non-sibling females were substantially higher and broadly increased with foundress number.

It is concluded that, under most circumstances, females will not be selected to share the resources of a paralysed host with other females. However, selection against sharing appears to be less stringent when either relatedness or resource availability are higher. In addition, females in multi-foundress groups apparently adjust sex allocation according to both foundress number and relatedness, despite being presented with what is thought to be an unnatural reproductive environment. In fact, *G. nephantidis* appears to be the only studied parasitoid in which sex ratios conform to predictions of both classical and extended Local Mate Competition theory. Finally, since *G. nephantidis* co-foundresses in the current study have shown that clutch size in multi-foundress groups is a kin mediated behaviour, future studies are suggested to discover in detail the existence or absence of reproductive skew: for instance, it will be interesting to discover whether females that tolerate each other's presence on

a host, then contribute the same or different numbers of offspring to the broods that develops on that host.

7.6 Parental relatedness and parasitoid sex ratios under local mate competition

The theory of sex ratio, under local mate competition (LMC) predicts that a more female-biased adult offspring sex ratio will be produced by foundresses when relatedness to their mates is closer. The existing body of research on testing for a facultative response to mate-relatedness using the gregarious parasitoid wasp *Nasonia vitripennis* has found no effects on sex ratios of broods produced by 1-7 foundresses, seemingly because females are unable to assess their relatedness to mating partners (Reece *et al.*, 2004; Shuker *et al.*, 2004b; Burton-Chellew *et al.*, 2008).

This study assessed how the sexual composition of adult offspring produced responds to mother-mate relatedness using the gregarious parasitoid wasp *Goniozus legneri*, a bethylid wasp that has biased sex ratios due to local mate competition (Gordh *et al.*, 1983; Hardy *et al.*, 1998; Hardy *et al.*, 2000; Wilkinson *et al.*, 2016) and has been shown to attune some behavioural aspects according to kinship (Lizé *et al.*, 2012). Cuticular hydrocarbon profiles are candidate mechanistic cues for the required kin discrimination between strains (Khidr *et al.*, 2012). Relatedness was varied by providing virgin females with a mate that was either a sibling, a non-sibling from the same strain, from a strain with geographically similar origin or from a strain with geographically distant origin.

The sexual composition ratios of *G. legneri* broods complied broadly with those observed in prior studies that did not manipulate mate relatedness (Gordh, 1982; Hardy *et al.*, 1998; Hardy *et al.*, 2000), but additionally this study showed

that the sex ratios among broods produced by mothers that had mated with a sibling or a same-strain male were similar and were more biased than broods produced by females mated with males from a different strain. This additional finding shows that encountering a male from another strain may have an influence on a female's estimation of the probability of inbreeding among her offspring and, consequently, favours increased investment in males, as predicted under several LMC scenarios (Herre, 1985; Nunney & Luck, 1988; Greeff, 1996; Reece *et al.*, 2004; West, 2009). However, the similar sex ratios produced by females mated with sibling or non-sibling males from the same strain suggests females lack the ability to distinguish between relatively close male kin, even though they can distinguish within-strain differences between other females (Lizé *et al.*, 2012). Moreover, the current study showed that brood sex variances were higher after mating with different-strain males, and this could be attributed to the less biased sex ratio optima that are, under some scenarios, expected to reduce selection for low sex ratio variance (West, 2009; Wilkinson *et al.*, 2016).

There are several further factors that could potentially influence the sex ratio patterns observed such as developmental mortality, which diminishes maternal influence on secondary sex ratios and increasing sex ratio variance (Green *et al.*, 1982; Khidr *et al.*, 2012; Wilkinson *et al.*, 2016). The reason primary sex ratios were not assessed in this study is that the method of assessment involves cross-strain mating (Khidr *et al.*, 2012), which could have confounded the purpose of this experiment. Another factor to be taken into account is the strain-specific effects beyond merely 'closeness of relatedness'. Although, further work is required to fully separate possible confounds from kin recognition effects

in *G. legneri*, it is noteworthy that Khidr *et al.* (2012) found no primary or secondary sex ratio differences between different mating strain combinations.

Conclusion

Parasitoid wasps have been key organisms in the development of a number of areas of evolutionary and applied ecology. Fundamental and applied research programmes have benefitted from the reciprocal exchange of facts and theories that leads to an improved understanding of the natural world and improved abilities to manage crop production systems, although numerous challenges remain. This project has included elements of both fundamental and applied research. Two immediate outcomes are practical recommendations for mass rearing programmes and a body of new evidence for kinship effects on parasitoid behaviour and reproduction, a previously underexplored research area. The longer-term outcome is a greater potential to utilize parasitoids to tackle pests of agro-economic importance.

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