Evolution and Maintenance of Sociality in Crab Spiders (Thomisidae)

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Summary

In this thesis I investigated factors that may explain the evolution and maintenance of sociality in crab spiders (Thomisidae). Group-living crab spiders differ from most other social spiders because they lack a capture web, a factor considered to be very important for the evolution of sociality in spiders. Colonies of subsocial and social spiders are usually comprised of family members, and thus another unusual trait of subsocial crab spiders is the regular acceptance of unrelated conspecifics into their groups. Previous work on the subsocial crab spider *Diaea ergandros* has demonstrated that maternal care as well as the construction and occupation of nests as protective retreats are important factors that may explain group-living in this species. Furthermore, it has been shown that these spiders are able to recognize kin, which offers an excellent opportunity to study group dynamics between relatives and immigrants. Building up on this knowledge, I examined potential costs and benefits of group living with a focus on the effect of unrelated spiderlings. Moreover, I estimated the genetic relatedness within family groups, and studied the broader natural history as well as phylogenetic relationships of subsocial and social crab spiders.

With a detailed natural history description of the crab spider *Xysticus bimaculatus* I have demonstrated that lifestyle and demographics are very similar to the subsocial *Diaea ergandros* (chapter 2). This new discovery of subsocial behaviour outside the genus *Diaea* indicated that subsociality may have evolved multiple times independently within Thomisidae. Testing this hypothesis, I investigated the evolutionary history of social behaviour in crab spiders in a molecular-phylogenetic context. The results suggest that subsociality has at least two independent origins confirming that *X. bimaculatus* is not closely related to any of the other group-living thomisid species (chapter 3).

The evolution of sociality in spiders is accompanied by a switch from outbreeding to inbreeding. *D. ergandros* has been suggested to be at a particularly advanced transitory stage from subsocial to social behaviour, and a previous study has shown that populations are inbred. Considering that low mating rates and inbreeding favour offspring cooperation of highly related individuals, females may benefit from monogamous or even incestuous mating. I studied the mating behaviour of *D. ergandros* and investigated natural mating rates with microsatellite markers (chapter 4). However, mating trials did not provide evidence for female choice. Accordingly, the genetic analyses did not support the existence of a monogamous mating system but rather supported relatively low mating rates, which may still sufficiently secure offspring cooperation while simultaneously providing some degree of outbreeding.

Genetic relatedness has been identified as an important factor promoting cooperation in many subsocial spiders and other organisms studied to date. With two laboratory experiments (chapter 5 & 6) I confirmed that siblings of *Diaea ergandros* had an advantage over mixed groups that included immigrants. The latter were generally accepted but negatively affected female-offspring and offspring-offspring foraging interactions. Nevertheless, accepting immigrants may have benefits

as well. In another experiment, larger groups of *D. ergandros* outperformed small groups in that they built larger protective retreats and had a lower mortality as well as higher individual growth (chapter 7). Group size varies considerably under natural conditions and small groups may thus benefit from accepting immigrants.

Zusammenfassung

In meiner Doktorarbeit habe ich Faktoren untersucht, die die Evolution und den Erhalt von Sozialverhalten bei Krabbenspinnen (Thomisidae) erklären könnten. Die meisten subsozialen und sozialen Spinnen bauen große Fangnetze, mit denen sie große Beutetiere überwältigen können und der Bau dieser Netze als gemeinsame Jagdinvestition gilt als ein wichtiger Faktor, der den Erhalt von Sozialitat bei Spinnen erklärt. Anders als die meisten sozialen Spinnen bauen Krabbenspinnen aber keine Netze zum Beutefang. Ein weiterer wichtiger Unterschied zwischen gruppenlebenden Thomisiden und anderen sozialen Spinnen ist in den Verwandtschaftsverhältnissen innerhalb der Gruppen zu finden. In der Regel sind bei subsozialen und sozialen Spinnen die Gruppenmitglieder nah miteinander verwandt und Kooperation zwischen den Individuen wird mit indirekten Fitnessvorteilen erklärt. Subsoziale Krabbenspinnen akzeptieren aber auch nicht-verwandte Artgenossen in ihren Gruppen und unterscheiden sich somit auch in dieser Hinsicht von den meisten anderen subsozialen und sozialen Spinnen.

Frühere Studien an der subsozialen Krabbenspinne *Diaea ergandros* haben gezeigt, dass mütterliche Fürsorge und das Konstruieren und Bewohnen von Blattnestern, die als Zufluchtsort dienen, wichtige Faktoren sind, die das Gruppenleben in dieser Art erklären könnten. Des weiteren wurde gezeigt, dass Individuen dieser Art in der Lage sind, Verwandte von Nicht-Verwandten zu unterscheiden. Daher eignet sich diese Art besonders gut, um die Auswirkung von nicht-verwandten Einwanderern auf die Gruppendynamik zu erforschen. Den Fokus auf die Auswirkung von nicht-verwandten Einwanderern legend, konnte ich somit auf den Ergebnissen vorheriger Studien aufbauen und mögliche Kosten und Nutzen des Lebens in Gruppen bei Krabbenspinnen untersuchen. Darüber hinaus habe ich die genetische Verwandtschaft innerhalb natürlicher Familiengruppen erforscht, die allgemeine Naturkunde dieser Spinnen untersucht sowie die phylogenetischen Verwandtschaftsverhaltnisse von subsozialen und sozialen Krabbenspinnen rekonstruiert.

Mit der detaillierten Beschreibung der Naturgeschichte der Krabbenspinne *Xysticus bimaculatus* konnte ich zeigen, dass diese Art in Bezug auf ihren Lebenszyklus und ihre Demographie der subsozialen Krabbenspinne *Diaea ergandros* sehr ähnelt (Kapitel 2). Diese neue Entdeckung von subsozialem Verhalten außerhalb der Gattung *Diaea* deutete an, dass Subsozialität bei Krabbenspinnen mehrfach unabhangig entstanden sein könnte. Daher habe ich die Wurzeln des Sozialverhaltens bei Krabbenspinnen in einem molekular-stammesgeschichtlichen Kontext untersucht. Diese Studie hat bestätigt, dass *X. bimaculatus* nicht näher mit den anderen gruppenlebenden Krabbenspinnen verwandt ist (Kapitel 3) und ich konnte zeigen, dass Subsozialitat mindestens zweimal unabhängig innerhalb der Thomisidae entstanden ist.

Die Evolution von Sozialverhalten bei Spinnen wird von der Verschiebung eines ursprünglich ausgezüchteten Paarungssystems hin zu einem ingezüchteten Paarungssystem begleitet. Bei *D. ergandros* scheint es sich um eine Art zu handeln, deren subsoziales Verhalten besonders weit entwickelt und daher nah am sozialen Verhalten angesiedelt ist. Diese Schlussfolgerung drückt sich

unter anderem darin aus, dass sie ein ingezüchtetes Paarungssystem aufweist. Geringe Paarungsraten oder sogar Inzucht können aufgrund der daraus resultierenden hohen Verwandtschaft die Kooperation zwischen Nachkommen erhöhen, und somit könnten Weibchen ein Interesse an wenigen Verpaarungen oder sogar der Verpaarung mit Geschwistern haben. Daher war die Erforschung der Paarungsraten dieser Art ein weiteres Ziel meiner Arbeit. Ich habe das Paarungsverhalten im Laborexperiment beobachtet, sowie die natürlichen Paarungsraten von *D. ergandros* genetisch mit Hilfe von Mikosatellitenmarkern untersucht (Kapitel 4). In den Paarungsexperimenten habe ich keine Hinweise dafür gefunden, dass Weibchen wählerisch sind und die genetischen Analysen haben keine Hinweise darauf geliefert, dass es sich bei *D. ergandros* um ein streng monogames Paarungssystem handelt. Trotzdem waren die natürlichen Paarungsraten relativ gering, möglicherweise gering genug um hohe Kooperation zwischen Nachkommen zu gewährleisten.

Genetische Verwandtschaft wurde als ein wichtiger Faktor für die Förderung von Kooperation bei vielen subsozialen Spinnen und anderen sozialen Organismen identifiziert. In zwei Laborstudien (Kapitel 5 & 6) konnte ich zeigen, dass Geschwister der Art *Diaea ergandros* Vorteile gegenüber gemischten Gruppen hatten, die aus nicht-verwandten Individuen bestanden. Nicht-verwandte Individuen haben sich zwar gegenseitig akzeptiert, aber sie haben sowohl die Interaktionen zwischen Weibchen und Nachkommen, als auch die Interaktionen innerhalb der Nachkommenschaft im Hinblick auf die Gruppendynamik während gemeinsamer Nahrungsaufnahme negativ beeinträchtigt. Gemischte Gruppen wiesen geringere Wachstumsraten und teilweise höhere Sterblichkeit auf. Obwohl nicht-verwandte Einwanderer die Kooperation innerhalb der Gruppe im Hinblick auf Jagen und Fressen beeinträchtigen, scheinen sie nicht generell von Nachteil für die Gruppe zu sein. In einem weiteren Experimement konnte gezeigt werden, dass bei *D. ergandros* die größe schützender Nester mit der Gruppengröße steigt. Große Spinnengruppen hatten dadurch einen Überlebensvorteil und ein größeres individuelles Wachstum (Kapitel 7). Da die Gruppengröße von Nestern unter natürlichen Bedingungen erheblich schwankt, könnte es sein, dass es insbesondere für kleine Gruppen vorteilhaft ist, wenn sie nicht-verwandte Einwanderer akzeptieren.

Certificate of originality

I declare that the work presented in this thesis is entirely my own except where indicated otherwise in the text. It has not been submitted to any other university or institution for a higher degree.

Approvals

An ethics approval was not necessary for working on invertebrates. A *Licence to Take Fauna for Scientific Purposes* has been obtained (No. 008968) from the Government of Western Australia, Department of Environment and Conservation (for chapter 3).

Statement of Contribution

Chapter 1: General Introduction

I wrote the introduction, which was proof-read by my supervisors Jutta M. Schneider and Marie E. Herberstein.

Chapter 2: Re-description of *Xysticus bimaculatus* Koch, 1867 (Araneae, Thomisidae) and characterization of its subsocial lifestyle

The chapter was conceived by myself. Melanie Gralow and Torben Riehl assisted with spider collection and collecting field data during several fieldtrips to Brisbane (Australia) and surroundings. Data analyses and interpretation were carried out by myself. I compared specimens with collection material located at the Australian Museum (Sydney, Australia), the Queensland Museum (Brisbane, Australia) and the Zoological Museum Hamburg (Germany), and discussed the findings with Torben Riehl. The micro-computed tomography was carried out by Peter Michalik at the Ernst-Moritz-Arndt-University of Greifswald (Germany). Photographs of type material were taken by myself. I wrote the paper, Torben Riehl and Peter Michalik contributed by commenting on the manuscript.

Chapter 3: Multiple origins of subsociality in crab spiders (Thomisidae)

I conceived the chapter and carried out fieldwork with assistance from Torben Riehl, Patricio Lagos, Marie E. Herberstein, Stefanie Kaiser and Catherine Lacey (several fieldtrips to Queensland, Western Australia and New South Wales (Australia)). Felipe Gawryszewski donated spiders that he collected in 2008 in Australia. DNA extractions were carried out by myself at Macquarie University laboratories. Further lab work (PCR, Sequencing) was by performed by Ingi Agnarsson and Laura J. May-Collado at the University of Vermont (USA). Sequence alignments were performed by Ingi Agnarsson, Torben Riehl and myself. Data analyses and interpretation were carried out by myself, Ingi Agnarsson and Torben Riehl. Phylogenetic trees based on the alignments were constructed by Ingi Agnarsson and Torben Riehl. The paper was written by myself; Torben Riehl, Ingi Agnarsson and Laura May-Collado contributed with helpful comments on the manuscript.

Chapter 4: Mating behaviour and natural mating rates in a subsocial spider

The chapter was conceived by myself and Jutta M. Schneider and discussed with Theodore A. Evans. I collected the spiders with assistance from Jutta M. Schneider and Marie E. Herberstein. DNA extraction was carried out by myself. I designed the primers and performed the genotyping at the Max-Planck-Institute in Plön (Germany), with assistance from Henrik Krehenwinkel. Data were analysed by myself. Mating observations in 2010 and 2012 were carried out by myself. The paper was written by myself. Jutta M. Schneider, Theodore A. Evans and Henrik Krehenwinkel contributed by commenting on the manuscript.

Chapter 5: Offspring dynamics affect food provisioning, growth and mortality in a brood-caring spider

The project was conceived by myself and Jutta M. Schneider. Marie E. Herberstein contributed to the study design with additional ideas. Theodore A. Evans provided helpful discussions prior to the project. I collected and analysed all data and wrote the paper. Marie E. Herberstein and Jutta M. Schneider contributed by commenting on the manuscript. The manuscript was moreover improved thanks to valuable comments from Torben Riehl, Theodore A. Evans, Stefanie Zimmer, Jannis Liedtke, Wiebke Schuett and Rainer Neumann as well as from Per Smiseth and an anonymous reviewer.

Chapter 6: Families hunt more successfully: effect of group composition on hunting and communal feeding in a subsocial crab spider

The project was conceived by myself, Jutta M. Schneider and Marie E. Herberstein. Theodore A. Evans provided helpful discussions prior to the project. I performed the experiment, analysed data and wrote the paper. Marie E. Herberstein and Jutta M. Schneider provided helpful feedback on structure and expression of the manuscript. Statistical analyses were discussed with Stano Pekar prior to analyses. Redouan Bshary and three anonymous reviewers commented on the manuscript.

Chapter 7: Hunted hunters? Effect of group size on predation risk and growth in the Australian subsocial crab spider *Diaea ergandros.*

This project was conceived by myself and Jutta M. Schneider; the MSc-Student Bianca Unglaub and Marie E. Herberstein contributed to the concept of the study with additional ideas. Theodore A. Evans provided helpful discussions prior to and during the project. Spider- and data collection was shared equally between Bianca Unglaub and myself. Raelene Giffney helped with weighing the spiders at Macquarie University. Data analysis was performed in equal shares by myself and Bianca Unglaub. Statistical analyses were discussed with Andrew Allen and Stano Pekar. The paper was written by myself and Bianca Unglaub. Marie E. Herberstein and Jutta M. Schneider made helpful suggestions on the structure of the manuscript. The manuscript was moreover improved thanks to valuable comments from two anonymous reviewers.

Chapter 8: General Discussion

I wrote the discussion. Jutta M. Schneider and Marie E. Herberstein commented on it.

Note: The use of American or British English depends on the individual journal requirements and thus varies between data chapters.

Jasmin Ruch, July 2014. Revised in November 2014

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I would moreover like to thank Theodore A. Evans, who introduced me to social crab spiders. My work benefited from sharing his detailed knowledge on the biology of these fascinating creatures. Thank you so much for your patience and answering all my questions!

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Chapter 1

General Introduction

Evolution of social behaviour

The evolution of social behaviour and processes favouring its maintenance are major themes in behavioural ecology and sociobiology (Choe and Crespi, 1997; Darwin, 1859; Hamilton, 1964). A social lifestyle is associated with costs, such as high risk of disease transmission (Pie et al., 2005), resource competition (Blumstein et al., 2002; Grand and Dill, 1999; Grove, 2012) or competition over reproduction (Bilde et al., 2007). In order for a social lifestyle to be maintained, these costs must be outweighed by significant fitness benefits (Alexander, 1974).

Understanding causes and consequences of social behaviour are a rich field of research (Szekely et al., 2010) and have for example been discussed in the context of interactions between mating partners (sexual behaviour as a social trait (Pizzari and Bonduriansky, 2010)), parental care (McGraw et al., 2010), cooperation between kin and non-kin (Griffin and West, 2003; Hamilton, 1964) as well as ecological factors (Bailey et al., 2013; Émlen, 1982; Émlen, 1991).

A theoretical explanation for the evolution of social behaviour was proposed by Hamilton (1964) who concluded that individuals gain inclusive fitness by cooperating with genetic relatives, termed kin selection by Maynard Smith (1964). An individual's inclusive fitness comprises two components: direct and indirect fitness (Brown and Brown, 1981; West et al., 2007). Individuals have direct benefits when reproducing themselves, while they gain indirect fitness when related individuals reproduce (West et al., 2007). Social behaviours have fitness consequences for both performing and receiving individuals (Hamilton, 1964; West et al., 2007). They can be costly for the actor but beneficial for the recipient (altruism), beneficial for both individuals (mutual benefit), beneficial for the actor but costly for the recipient (selfishness) or costly for both (spite) (Hamilton, 1964; West et al., 2007).

'Hamilton's rule' states that genes for altruistic behaviour are selected for as long as the benefits for a genetically related recipient of this behaviour exceed the direct costs of the actor (Bourke, 2014; Hamilton, 1964). Hamilton moreover suggested that the mechanisms favouring kin selection are kin recognition and limited dispersal (Hamilton, 1964). On the other hand, such limited dispersal can increase competition between genetically related individuals, which may reduce kin-selected benefits in some cases (West et al., 2002).

Parental care and a delayed dispersal of juveniles prolong social behaviour in family groups (Hamilton, 1964). In taxa with parental care, parents and offspring are faced with conflicts over resource distribution (Parker and Macnair, 1979; Trivers, 1974). Parental care is

limited, especially when parents need to retain resources for future broods and offspring often compete heavily with each other over sharing this resource (Mock and Parker, 1997; Roulin and Dreiss, 2012). However, siblings may also cooperate to increase the overall amount of parental care (Roulin and Dreiss, 2012). According to Hamilton's inclusive fitness model, cooperation between offspring will be favoured when inclusive fitness gains exceed the direct costs of cooperation. Thus, offspring should be less competitive towards closely related nestmates but become more competitive with decreasing relatedness, for instance as a result of multiply mating (polyandrous) females (Cornwallis et al., 2010; Godfray, 1995; Royle et al., 1999).

Decreased relatedness due to high rates of polyandry can lead to a loss of cooperation. It has been suggested that the transition from a solitary to a social lifestyle is facilitated by low levels of polyandry (monogamy hypothesis) (Boomsma, 2009; Boomsma et al., 2011; Cornwallis et al., 2010). When females mate with only one male, all her offspring share half of the genes with their siblings, which increases cooperation. Studies on eusocial insects − highly social insects with a division of labour (Wilson, 1971) − and cooperatively breeding birds provide empirical evidence for the monogamy hypothesis (Boomsma, 2009; Cornwallis et al., 2010).

Many theoretical and empirical studies have shown the importance of kin selection, for example in understanding the evolution of eusociality in various insect groups (Boomsma, 1991; Boomsma et al., 2011; Foster et al., 2006; Ratnieks and Helantera, 2009) and naked mole rats (Jarvis et al., 1994). However, kin selection is not the only theoretical framework to understanding the evolution of sociality. Trivers (1971) demonstrated that individuals may cooperate in the absence of genetic relatedness and take turns in helping each other, which he termed reciprocal altruism. Cooperation between unrelated individuals can, however, only be evolutionarily stable when the actor gains direct benefits from the cooperative act as well (Bshary and Bronstein, 2011; West et al., 2007).

Apart from the genetic approach to understanding the evolution of sociality, ecological factors can promote the latter (Alexander, 1974). Individuals in groups may benefit from improved detection of food resources and group hunting (Alexander, 1974; Packer and Ruttan, 1988). Another advantage of group living is a reduction of the individual predation risk (Hamilton, 1971; Inman and Krebs, 1987). While the examples mentioned above summarise ecological benefits arising from group living, dispersal may also be restricted due to ecological factors. Ecological constraint models assume that offspring remain in their natal group, for example due to a lack of available breeding sites, although a solitary lifestyle would be more beneficial in terms of direct fitness (Émlen, 1982; Émlen, 1991). Thus, social groups are formed by delayed (or no) dispersal of family members (Émlen, 1991; Lubin and Bilde, 2007).

Evolution of sociality in spiders

Sociality in spiders is a rare and particularly fascinating phenomenon, considering that spiders are usually highly aggressive towards conspecifics (Avilés, 1997; Lubin and Bilde, 2007). Permanently social spiders live their

entire life in communal nests and cooperate in webbuilding, foraging and parental care (Avilés, 1997; Lubin and Bilde, 2007). Less than 25 of the 44500 spider species described to date are considered to be permanently social (Platnick, 2014). Although rare, permanent sociality has evolved independently across eight spider families (Agnarsson et al., 2006). Social spiders differ from eusocial insects because they do not show a division of labour and all individuals of the colony are able to reproduce. This leads to very high levels of inbreeding (Agnarsson et al., 2013; Avilés and Purcell, 2012; Bilde et al., 2007; Lubin, 1995; Lubin et al., 2009; Riechert and Roeloffs, 1993).

Within two spider families, the cobweb spiders (Theridiidae) and the velvet spiders (Eresidae) there is phylogenetic evidence for multiple origins of sociality. In the cobweb spiders, nine independent origins of sociality in three genera are documented (Agnarsson et al., 2006; Avilés, 1997; Avilés and Bukowski, 2006) and most social lineages comprise only a single species. The latter indicates a young evolutionary age and consequently a lack of diversification but probably also high rates of extinction (Agnarsson et al., 2006), likely due to the high levels of inbreeding (Agnarsson et al., 2013). In the velvet spiders, three independent origins of sociality gave rise to exclusively single-species social lineages as well, all of them assigned to the genus *Stegodyphus* (Johannesen et al., 2007).

Permanently social spiders most likely evolved from a subsocial state (Wickler and Seibt, 1993), where females provide extended care and spiderlings cooperate for a certain period but disperse prior to maturity (Avilés, 1997; Lubin and Bilde, 2007; Yip and Rayor, 2013). Maternal care enhances offspring survival and in subsocial spiders, several forms of care can be found (Lubin and Bilde, 2007; Yip and Rayor, 2013). For example, females capture prey and share it with the young in subsocial huntsman spiders (Sparassidae) and crab spiders (Thomisidae) (Evans, 1998b; Yip and Rayor, 2011). In velvet spiders (Eresidae), females regurgitate food and are finally consumed by their offspring (matriphagy; Salomon et al., 2005; Schneider, 2002; Schneider et al., 2003).

Sociality in spiders may also have evolved due to foraging benefits (Whitehouse and Lubin, 2005). It has been suggested that offspring in subsocial spiders can be described as 'foraging societies', because offspring obtain food more efficiently when staying in the group (Whitehouse and Lubin, 2005).

Subsociality has evolved at least 18 times independently across several spider families (Yip and Rayor, 2013). In both subsocial and social spiders, ecological factors (Avilés and Harwood, 2012; Corcobado et al., 2012) as well as kin selected benefits (Ruch et al., 2009b; Schneider and Bilde, 2008) likely facilitated the evolution of sociality. However, detailed knowledge on the natural history, taxonomy and evolutionary relationships of solitary, subsocial and social species that allow detecting these factors is often lacking (Agnarsson, 2012).

Subsocial spiders are usually outbred but limited male dispersal and the lack of precopulatory inbreeding-avoidance mechanisms indicate that inbred mating is not uncommon in subsocial spiders (Avilés and Bukowski, 2006; Bilde et al., 2005; Ruch et al., 2009a; Tuni et al., 2012). These spiders seem to have a relatively high tolerance towards inbred matings, which

could represent the key preadaptation required to facilitate the evolution of permanent sociality (Bilde et al., 2005), but detailed knowledge on the mating systems of many subsocial spiders is still rare (Lubin and Bilde, 2007).

The majority of subsocial and social spiders communally build large capture webs, which enable them to prey upon large food items that are consumed as a group (Avilés, 1997; Lubin and Bilde, 2007). It has been suggested that the large capture webs are an important component for understanding the evolution of sociality in spiders (Avilés, 1997). Nevertheless, sociality has evolved in two families that lack capture webs: Sparassidae and Thomisidae (Avilés, 1997; Evans, 1995; Yip and Rayor, 2011; Yip and Rayor, 2013; Yip et al., 2012). Hence, benefits of group living for spiders that mutually share a capture web cannot apply in these families (Yip and Rayor, 2011).

Sociality in crab spiders (Thomisidae)

More than 2,000 thomisid species in 174 genera are described worldwide (Platnick, 2014). Only three of them are group-living, with similar biology but varying social complexity and all can be exclusively found in Australia (Evans, 1997). All group-living crab spiders described to date belong to the genus *Diaea* (Evans, 1995). Only one permanently social crab spider is described (*D. socialis* Main, 1988 (Rowell and Main, 1992)). The other two (*D. ergandros* Evans, 1995 and *D. inornata* (synonym *D. megagyna*, Evans 1995 (Szymkowiak and Dymek, 2012)) are subsocial.

Subsocial and social crab spiders construct nests from *Eucalyptus* leaves, which serve as foraging areas (Evans, 1998a; Main, 1988). In *D. ergandros,* a relatively well studied species, spiders seem to be vulnerable to predation from other spiders (Clubionidae) and it has been suggested that the leaf-nests serve as protective retreats (Evans, 1998a).

In *Diaea ergandros*, groups originate as the offspring of a single female that migrated from her natal colony after mating. The female constructs a nest from several *Eucalyptus* leaves and only produces a single clutch consisting of 10-80 eggs (Evans, 1995). After hatching, the female continues to expand the brood chamber and catches prey to provide food for her young. Finally, the female develops trophic eggs that are never laid and is consumed by her offspring (matriphagy) (Evans et al., 1995). After the female's death, offspring stay in the natal nest and communally inhabit the latter until maturity. Younger spiderlings may however migrate into foreign nests when the conditions in their natal nest are unsuitable, for example when the mother is not present anymore (Evans, 1998a). *D. ergandros* is able to recognize kin but mothers generally accept immigrating spiderlings (Evans, 1998b, 1999). Immigrating spiderlings could benefit other group members because larger groups seem less vulnerable to predation (Evans, 1998a). However, immigrating (unrelated) spiderlings could negatively affect group activities as well. For example, nest construction activity is low in groups of unrelated juvenile *D. ergandros* (Evans, 1999). So far, behavioural observations focusing on the effect of immigrating spiderlings on group activities like communal hunting and foraging remain to be studied.

Aims

In my thesis, I use an interdisciplinary approach to detect factors facilitating the evolution and maintenance of sociality in crab spiders (Thomisidae). Specifically, I investigate the broader natural history and phylogenetic relationship of group-living crab spiders, estimate the genetic relatedness within family groups of a subsocial species and study potential costs and benefits of group living with a focus on the effect of immigrating unrelated spiderlings.

Natural history & phylogenetic relationships of subsocial crab spiders

Identification of selective agents facilitating the evolution of sociality in spiders is often difficult due to a lack of detailed knowledge on natural history and taxonomy of solitary, subsocial and social species (Agnarsson, 2012). In **chapter 2**, I describe the natural history and subsocial lifestyle of *Xysticus bimaculatus*, a crab spider inhabiting sclerophyll forests of Southern Queensland. This study enables future work, for example studies on communal activities that build upon the natural-history observations and allow a comparison with other solitary and group-living crab spiders.

The discovery of group living in a crab spider species outside the genus *Diaea* suggests that subsociality may have evolved independently in Thomisidae. In **chapter 3**, I investigate the phylogenetic relationship of the four Australian group-living thomisids using a molecular approach.

Genetic relatedness of family groups

Inbreeding is a key characteristic of permanently social spiders, while subsocial spiders usually have an outbred mating system (Lubin and Bilde, 2007). However, detailed studies on the natural mating rates of many subsocial spiders are still rare (Lubin and Bilde, 2007). In **chapter 4**, I study the natural mating rates of *Diaea ergandros* with microsatellite markers, asking whether females mate multiply to secure outbreeding or whether females are monandrous, thereby increasing cooperation between offspring. I furthermore describe the mating behaviour and ask whether individuals pre-copulatorily chose between related or unrelated mating partners.

Female-offspring interactions with varying within-brood relatedness

A previous study has demonstrated that female *Diaea ergandros* discriminate own from foster offspring, but that foster offspring are accepted (Evans, 1998b). The acceptance of few foreign individuals likely provokes conflicts between female and brood, but also within the brood, which may have consequences for the maintenance of sociality. To test whether the presence of unrelated spiderlings affects female-brood as well as within-brood foraging dynamics, the composition of *D. ergandros* groups is experimentally manipulated in **chapter 5**.

In this experiment, spiderlings are too small as to capture prey items and thus depend on female hunting success and food sharing. Assuming that females have control over food allocation and base the amount of care on the presence of kin-recognition cues, I predict that female

food provisioning should gradually decrease with increasing proportions of foster offspring. If offspring mostly control food distribution and siblings cooperate more than non-siblings, broods consisting of siblings should grow better and have a lower mortality compared to broods of mixed offspring.

Hunting and foraging in groups with varying within-brood relatedness

The presence of unrelated spiderlings may affect group activities such as communal hunting and feeding when the caring female is not present anymore. I study spiderling dynamics in the absence of a caring female in **chapter 6**. I compare hunting success and growth between three group compositions each representing a distinct ratio of relatedness between individuals.

Benefits of group living: reduced predation risk

Apart from kin-selected benefits, ecological factors such as protection from predators can promote the evolution of a social lifestyle. I examine the relationship between group size and predation risk in *Diaea ergandros* in **chapter 7**. Survival probability, nest construction activity and feeding behaviour in differently sized groups with a predator either present or absent is studied in a laboratory experiment.

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Chapter 2

Re-description of *Xysticus bimaculatus* Koch, 1867 (Araneae, Thomisidae) and characterization of its subsocial lifestyle

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Abstract

Spiders have become an important model to study the evolution of sociality, but a lack of their detailed natural history and taxonomy hinders broader comparative studies. Group-living crab spiders (Thomisidae) provide an excellent contrast to other social spiders since they lack a communal capture web, which was thought to be a critical factor in the evolution of sociality. Only three non-webbuilding crab-spider species are known to be subsocial or social, all of which belong to the genus *Diaea* Thorell, 1869. The aim of this study is to describe the social lifestyle of *Xysticus bimaculatus* Koch, 1867 for the first time. Furthermore, we present a detailed re-description of this species and discuss its taxonomic implications. Like other subsocial crab spiders, *X. bimaculatus* builds nests from tree leaves. Nests contain up to 38 spiders and sometimes several adult females, indicating the species may be at a transitory stage between subsociality and permanent sociality.

Keywords: Social spider, cooperation, female care, micro-CT, palp, taxonomy

Introduction

The evolution of sociality is puzzling and determining factors that promote the transition towards a social lifestyle is a major challenge in evolutionary biology. Animals living in social groups benefit from cooperation in foraging, brood care and protection from predators (Brockmann, 1997; Brown, 1983; Choe and Crespi, 1997; Creel, 2001; Dechmann et al., 2010; Unglaub et al., 2013), but group living also entails costs such as competition for mating partners (Huchard and Cowlishaw, 2011). In the last 20 years, the social lifestyle of "non-traditional" social taxa such as clonal aphids (Abbot, 2009) or spiders (Avilés, 1997; Lubin and Bilde, 2007) has become of increasing interest. Spiders are recognized as important model organism to study the evolution of sociality (Agnarsson, 2012; Agnarsson et al., 2006; Avilés, 1997; Evans, 1998a; Johannesen et al., 2005; Lubin and Bilde, 2007; Ruch et al., 2009; Schneider and Bilde, 2008; Yip and Rayor, 2013). They are generally very aggressive and sociality in spiders is extremely rare (Agnarsson et al., 2006; Bilde and Lubin, 2011). Nevertheless, sociality has evolved several times independently across eight families (Agnarsson et al., 2006), suggesting strong selective benefits from living in groups. However, identification of the selective agents is difficult due to a lack of detailed natural history and taxonomy of solitary, subsocial and social species (Agnarsson, 2012). Such knowledge facilitates comparisons of factors promoting social behavior in general, for instance ecological factors (Avilés and Harwood, 2012; Corcobado et al., 2012) and/or kin selection (Schneider and Bilde, 2008).

The generally accepted hypothesis is that sociality in spiders evolved via the 'subsocial route', meaning that permanent sociality derived from ancestors with extended maternal care (Lubin and Bilde, 2007; Wickler and Seibt, 1993). This hypothesis is corroborated by the phylogenetic reconstruction of social spider lineages (Agnarsson et al., 2006; Johannesen et al., 2007). Subsocial spiders differ from permanently social spiders in that they disperse prior to mating and thus have an outbred mating system (Agnarsson et al., 2006; Avilés, 1997; Lubin and Bilde, 2007). In both, subsocial and social spiders, females care intensively for offspring and the latter cooperate, for instance, in hunting, foraging, webbuilding and predator defence (Avilés, 1997; Lubin and Bilde, 2007; Ruch et al., 2014a; Yip and Rayor, 2013). A major characteristic explaining the evolution and maintenance of sociality in spiders is the construction of a communal capture web, which allows capturing large prey items (Avilés, 1997; Lubin and Bilde, 2007). Non-webbuilding subsocial and social lineages are documented in only two families, huntsman spiders (Sparassidae Bertkau, 1872) as well as crab spiders (Thomisidae Sundevall, 1833) The social lineages of both taxa can be exclusively found in Australia (Agnarsson and Rayor, 2013; Evans, 1995).

To date, all subsocial and social crab spiders are described in the genus *Diaea* Thorell, 1869 (Evans, 1995). Three species are known to be subsocial or social: *D. socialis* Main, 1988 from Western Australia, *D. ergandros* Evans, 1995 and *D. megagyna* Evans, 1995 (= *D. inornata* (Szymkowiak and Dymek, 2012)) from Southeastern Australia (Evans, 1997). Subsocial/social *Diaea* mainly build nests in

small-leaved *Eucalyptus* trees. The climatic conditions in their habitats seem to be relatively similar across the range of their distribution from Southern Queensland to Tasmania as well as in Western Australia (Evans, 1997). Nest inhabitants are usually related, however, groups accept immigrating spiders from other nests in *D. ergandros* (Evans, 1998a; Evans and Goodisman, 2002). The presence of immigrating spiderlings seems to affect group dynamics and female care in *D. ergandros* (Ruch et al., 2014b) and female care is very important for offspring survival (Evans, 1998a, b; Unglaub et al., 2013).

We have recently identified another case of subsociality in crab spiders: *Xysticus bimaculatus* Koch, 1867. The discovery of social behavior in a species outside the *Diaea* genus suggests a possible independent evolutionary event and thus the potential to identify common drivers in the evolution of sociality in spiders. Here, we describe the natural history and subsocial behaviour for the first time (Koch, 1867; Koch, 1876) and present a re-description of the species.

Methods

We initially discovered nests inhabited by several individuals of *Xysticus bimaculatus* Koch, 1867 in July 2011 on trees along the Enoggera Reservoir, Queensland, Australia (27°26'27.69"S, 152°55'29.03"E). We later surveyed spider nests in November 2011, April 2012 and November 2012 (N = 166) at four locations around Brisbane (Brisbane Forest Park, Toohey Forest, Mt Coot-tha, Mt Tibrogargan). During these surveys, we measured the nests and identified the trees these were built in. We determined the group composition (number, developmental stage and sex) of spiders inhabiting the nests. We used these data to pinpoint the dispersal stage of spiderlings, which is an indicator of the degree of sociality (Avilés and Harwood, 2012; Lubin and Bilde, 2007). All immature individuals are referred to as 'spiderlings'. We moreover recorded prey items as well as commensals and potential predators in active nests that were inhabited by at least one spider (N = 131).

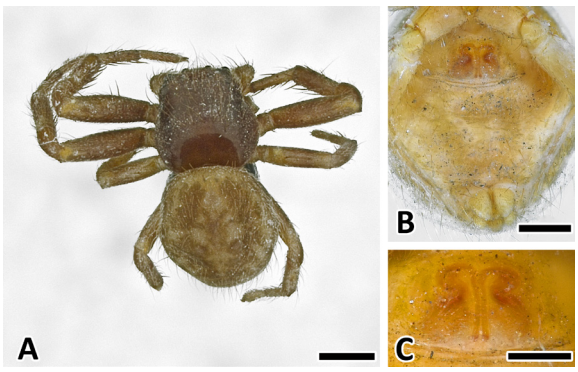

Figure 1: Female holotype of *Xysticus bimaculatus*, (MG 2260, now ZMH). A) Habitus, scale bar 1 mm. B) Ventral, scale bar = 0.5 mm. C) Epigyne, scale bar = 0.25 mm.

For the species re-description, specimens were compared with collection material located at the Australian Museum, Sydney, the Queensland Museum, Brisbane and the Zoological Museum Hamburg and included species from the genera *Cymbacha*, *Diaea, Tharpyna* and *Xysticus* (see material examined, type *X. bimaculatus* see Figure 1). The description of the seta pattern was performed using the format described by Ramirez (2003).

Since the type locality has not been accurately specified in the original description, the species was re-described from specimens collected in the Enoggera Reservoir in April 2012. Specimens were stored in 70% EtOH and examined using a Zeiss Discovery V20 stereo microscope and imaged with a Zeiss MCr camera and a Leica

M205A with a Leica 290 camera as well as with a Keyence Digital Microscope VHX-500 F. The images were edited and plates arranged using Adobe Photoshop CS4.

Female copulatory organs were dissected and macerated using pancreatin (Alvarez-Padilla and Hormiga, 2007) and imaged with a Zeiss MCr camera mounted on a Olympus BX60 light microscope.

The left male palpus (sperm transfer organ) was stained with a 1.0% iodine solution overnight and critical point dried for the micro-tomographic analyses. The dry palp was mounted onto an insect pin and scanned with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss X-ray Microscopy Inc., Pleasanton, USA) at 30 KV and 6 W (20.0 scintillator-objective lens unit, 6 seconds exposure time, 1.18 µm pixel size). The data were processed using the 3D analysis software AMIRA v. 5.4.2 (Visage Imaging, Berlin, Germany). Selected parts of the palp were reconstructed by delineation of the contours in each section and surfaces were computed using the surface editor.

Analyses

Statistical analyses on spider group composition were performed using JMP 9.0 (SAS Institute Inc., USA). Figures were prepared with R version 2.15.3 (R Development Core Team 2013). Continuous data were tested for normal distribution (Shapiro-Wilk-Test) as well as for equal variance. Since data were not normally distributed we used non-parametric tests. Descriptive statistics are given as mean ± standard error (SE).

All measurements in the description are presented in mm unless stated otherwise.

Results

Natural History

Nest characteristics and host trees

The nests of *Xysticus bimaculatus* Koch, 1867 were constructed from 7.77 ± 0.49 leaves (range = 2 – 48 leaves, *N* = 149). The inside of the nests usually consisted of older, brown leaves and spiders subsequently and repeatedly attached fresh green leaves on the outside. The most common host tree across all study sites was Blackwood (*Acacia melanoxylon,* 68%, Figure 2 D). However, the spiders were not restricted to these trees and could also be found on other species, for example Brisbane Golden Wattle (*Acacia fimbriata,* 7%, Figure 2 E) and Soap Trees (*Alphitonia excelsa,* 20%, Figure 2 C).

Group composition

X. bimaculatus has an annual life cycle. Living spiderlings were found in 120 of the 166 surveyed nests. 27 of the 166 nests were old and no longer inhabited by *X. bimaculatus*. Adult living females were found in 71 nests. Ten of these adult females were found with an egg sac and the others with living spiderlings. On average, we found 10.5 ± 0.3 spiderlings per nest and group size ranged between one and 38 spiderlings (*N* = 120 nests). We found five size classes of spiderlings and all of these were found with caring adult females present in both seasons of examination (April and November). Usually, all spiderlings within a nest were of approximately the same size. We

Figure 2: A) Male and female *Xysticus bimaculatus*. B) Spiders attach leaves with silk to construct a typical nest. C) Nest constructed from *Alphitonia excelsa*. D) Nest constructed from *Acacia melanoxylon*. E) Nest constructed from *Acacia fimbriata.* Scale bars = 1 cm.

tested whether there was a certain size class after which group size decreases and found that there was no significant difference between size class (as a factor) and number of spiders inhabiting the nests (Wilcoxon Rank Sums: $\chi^2_4 = 3.59$, $P = 0.46$, $N = 116$, Figure 3), although the largest size class was found in smaller groups. This finding indicates that spiders disperse only shortly before maturation. While adult females were alive and present in 85.71% of nests containing small

spiderlings (size class 1, N_{nests} = 14), the presence of an adult female significantly declined when spiderlings were larger (Pearson: χ^2= 9.8, P = 0.04, N = 116). However, the likelihood of an adult female present did not differ between size class 2 with 43.75% (N_{nests} = 32 nests), size class 3 with 56% (N_{nests} = 25), size class 4 with 40.74% (N_{nests} = 27), and size class 5 with 38.89% (N_{nests} = 18) of the nests containing an adult living female. Subadult and adult males were exclusively found in November with a maximum of six adult males in a single nest.

In four nests we found multiple adult females caring for a brood and in four other cases we found two distinct broods within one nest (these were excluded from the analyses of age and number of spiders). The presence of multiple adult females did not overlap with the presence of two distinct broods within one nest. Living adult females were found in April (56.57%) as well as in November (26.79%), meaning that the presence of an adult living female inside the nest was significantly more likely in April (Pearson: χ^2= 12.78, P = 0.0004). The number of spiderlings per nest was significantly higher when an adult female was present (Wilcoxon: Z = - 4.31, P < 0.0001, N = 120, Figure 4).

Prey, commensals and potential predators

On average, nests contained 2.3 ± 0.25 prey items (N = 131 nests). Main prey types were beetles (Coleoptera, 50%) and ants (Hymenoptera, 36%). In addition, we found wasps (Hymenoptera, 2%), caterpillars (Lepidoptera, 6%) and flies (Diptera, 1%). Most abundant commensals were woolly scale insects (Hemiptera, Coccoidea, 13%) and cockroaches (Blattodea, < 5%). Potential predators present in the nest were other spiders, for example Clubionidae (4%) and Salticidae (1%).

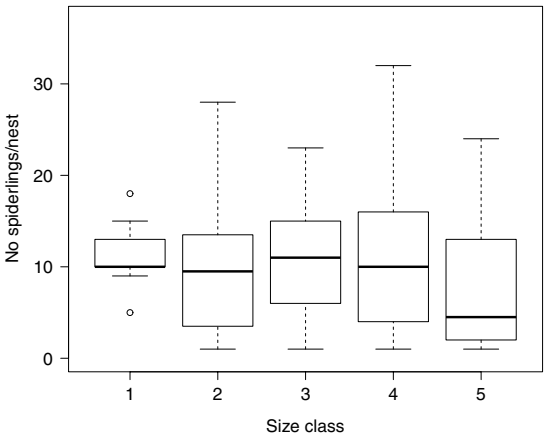

Figure 3: Average number of spiderlings per nest depending on spiderling size class (which reflects age). We found no significant decline in group size with increasing size class, indicating that spiderlings disperse shortly before maturation. The upper and lower whiskers show 1.5 times interquartile range, the box shows median and upper and lower quartile. Individual dots indicate outliers.

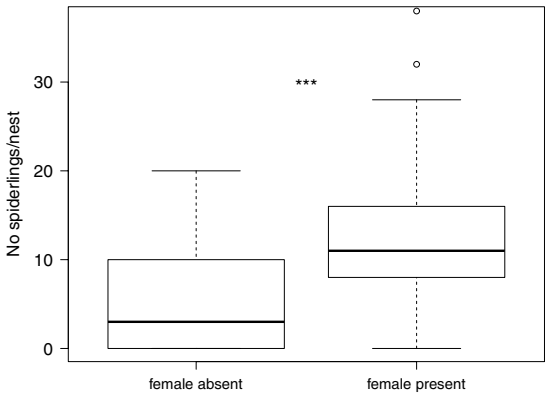

Figure 4: Number of spiderlings per nest is positively affected by the presence of a caring female. The upper and lower whiskers show 1.5 times interquartile range, the box shows median and upper and lower quartile. Individual dots indicate outliers. *** P < 0.0001 indicates a statistically significant difference

Species Re-description

Abbreviations

AM = Australian Museum, Sydney, Australia
MG = Museum Godefroy (now Zoological Museum Hamburg)
ZMH = Zoological Museum Hamburg, Germany
ALE = anterior lateral eyes
AME = anterior median eyes
PLE = posterior lateral eyes
PME = posterior median eyes
RTA = retrolateral tibial apophysis

Female

Based on paratype female KS120583 (AM)

Measurements

Body length: 4.36, carapace length: 1.83, carapace width: 1.83, carapace height: 1.21, carapace length/width ratio: 1, abdomen length: 2.53, abdomen width: 2.34, abdomen height: 2.03, abdomen length/width ratio: 1.08

Coloration and markings

Carapace and chelicerae colored evenly black-brown. Sternum brown-yellowish with a darker outer frame. Labium and maxillae dark brown with white tips (Figure 5 D).

The first two legs (Leg I & II) black-brown with faint orange annulations. Femur of leg I and II black-brown, patella anterior orange and posterior black, tibia anterior black with orange annulation and posterior black-brown, metatarsus and tarsus anterior orange and posterior black-brown.

Leg III and IV with distinct white annulations. Femur of leg III and IV anterior white and posterior black, patella anterior white and posterior black, tibia anterior black with white annulation and posterior black, metatarsus and tarsus anterior more white than black.

Abdomen dark brownish with a dark indented cranial spot and two white spots dorsally in the middle (Figure 5 A). Sides of the abdomen with black-brown vertical stripes. Ventral side of the abdomen lighter than the dorsal side with a dark brown section between epigyne and spinnerets (Figure 5 E). Surroundings of the epigyne dark, spinnerets brown-yellowish.

Carapace

Carapace shape slightly convex and as long as wide.

Eyes

Lenses in order of diameter: ALE >PLE >AME> PME.
Distance between eyes: AME—AME = 0.45, ALE—ALE = 1.1, AME—ALE = 0.29, ALE—PLE = 0.29, PLE—PLE = 1.39, AME—PME = 0.33, PME—PME = 0.59, PME—ALE = 0.34, PME—PLE = 0.39.
Clypeus width 1.1, height 0.37, surface smooth. One long lateral seta (0.26) next to ALEs.

Chelicerae, maxillae and labium

Chelicerae oval and bulky, length 0.65 and width 1.09, wrinkled surface (Figure 5 C). Fangs short (0.17).

Maxillae rounded, arched around labium, length 0.51. Labium shorter (0.36) than maxillae.

Sternum

Shield-shaped and convex, narrower towards leg III and IV, 0.84 long and 0.74 wide. Covered with fine setae (Figure 5 D).

Legs

Legs I and II longer than legs III and IV. Surface of the legs evenly covered with setae.
Leg setation: I: femur d 0-0-1, p 0-2-2-0; tibia p 0-0-1-0, v 2-2-2; metatarsus r 1(ap), v 2-2-0-2-p1; II: femur d 1-1; tibia v 0-2-0-2-2; metatarsus v 0-2-0-2-2; III: femur d 1-1; tibia v 2(ap); metatarsus p d1, v 2; IV: femur d 0-1-1-0; tibia v 2(ap); metatarsus p 2
Leg I. Fe: 1.73, Pa: 0.71, Ti: 1.15, Me: 0.90, Ta: 0.85, Total: 5.34
Leg II. Fe: 1.69, Pa: 0.77, Ti: 1.19, Me: 0.85, Ta: 0.85, Total: 5.34
Leg III. Fe: 1.21, Pa: 0.49, Ti: 0.76, Me: 0.53, Ta: 0.53, Total: 3.53
Leg IV. Fe: 1.36, Pa: 0.48, Ti: 0.84, Me: 0.59, Ta: 0.56, Total: 3.84

Leg formula: I = II > III < IV

Abdomen

Oval, covering the posterior part of the cephalothorax. Covered with evenly arranged setae. Five obvious indents.

Genitalia

Epigyne slightly wider than long (Figure 5 F). Copulatory openings in upper part of epigyne medially to broad heart-shaped sclerotized central hood. Copulatory ducts curved, leading to large ovoid and bipartite spermathecae (Figure 5 G).

Male

Based on paratype male KS120583 (AM)

Measurements

Body length: 3.3, carapace length: 1.43, carapace width: 1.50, carapace height: 1.01, carapace length/width ratio: 0.95 abdomen length: 1.87, abdomen width: 1.49, abdomen height: 1.23, abdomen length/width ratio: 1.25

Coloration and markings

Carapace and chelicerae black-brown, sternum brown. Labium and maxillae dark brown with white tips. Palps dark brown.

Leg I & II black-brown with posterior annulations. Femur, patella and tibia of leg I and II black-brown, metatarsus and tarsus anterior white and posterior black-brown.

Leg III and IV with distinct white annulations. Femur and patella of leg III and IV anterior white and posterior black, tibia anterior black with white annulation and posterior black, metatarsus and tarsus anterior more white than black.

Abdomen black with a white anterior frame, an anterior dark indented spot and four median dark indented spots (Figure 5 B). Sides of the

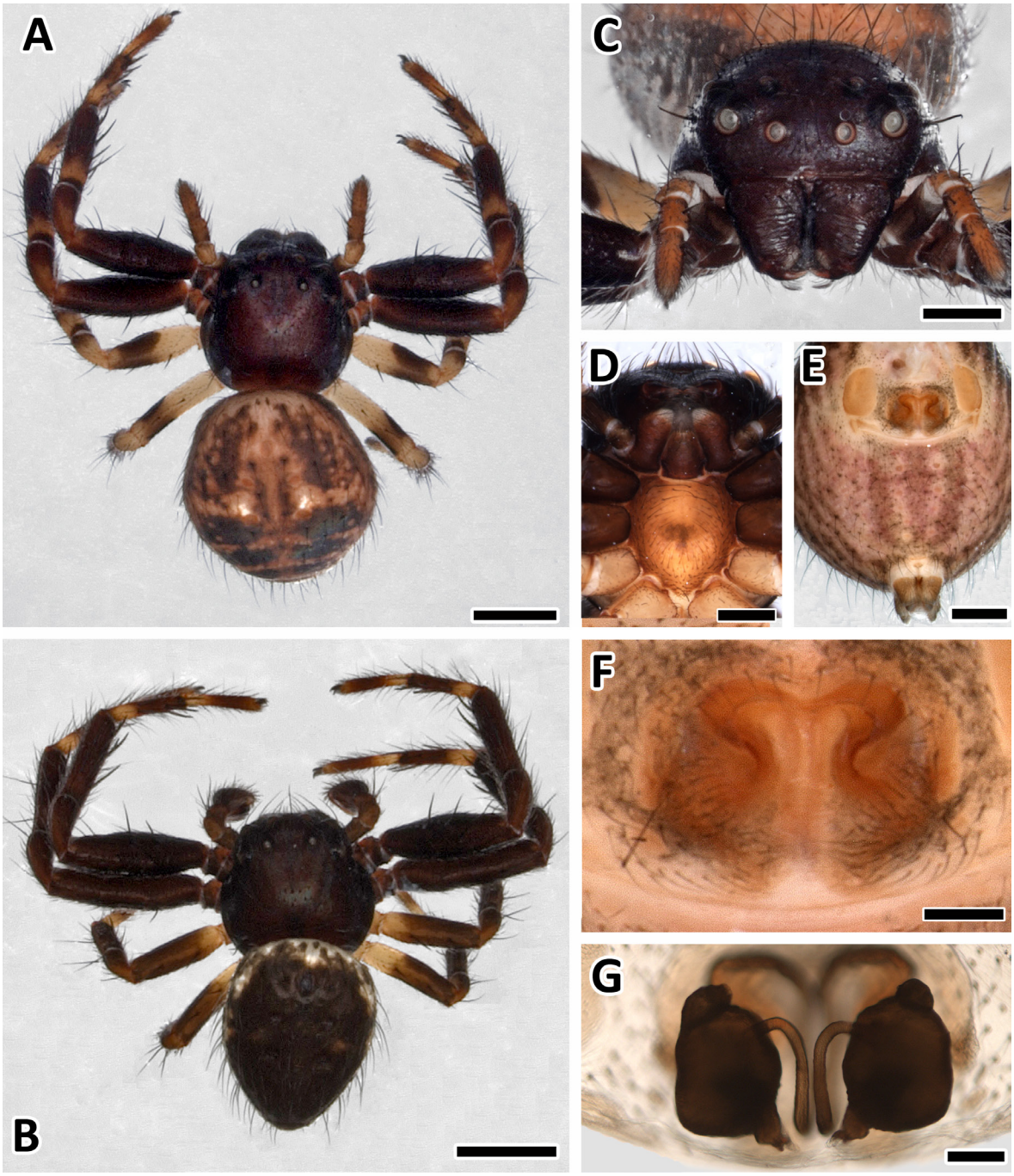

Figure 5: A) Female *Xysticus bimaculatus* (AM, KS120583), habitus, scale bar = 1 mm. B) Male (AM, KS120583), habitus, scale bar = 1 mm. C) Female (AM, KS120583), frontal view, scale bar = 0.5 mm. D) Female (AM, KS120583), sternum and maxillae, scale bar = 0.4 mm. E) Female (AM, KS120583), ventral view, scale bar = 0.5 mm. F) Female (AM, KS120583), epigyne, scale bar = 0.25 mm, G) Female (AM, KS120583), vulva, scale bar = 0.1 mm.

abdomen black. Ventral side of the abdomen dark brown, spinnerets brown.

Carapace

Carapace slightly convex and as long as wide.

Eyes

Distance between eyes: AME—AME = 0.38, ALE—ALE = 0.90, AME—ALE = 0.29, ALE—PLE = 0.30, PLE—PLE = 0.95, AME—PME = 0.24, PME—PME = 0.46, PME—ALE = 0.27, PME—PLE = 0.31.

Clypeus width 1.14, height 0.39, surface smooth. One long lateral seta (0.31) next to ALEs.

Chelicerae, maxillae and labium

Chelicerae oval and bulky 0.41 long, 0.70 wide, wrinkled surface. Fangs 0.17 long.

Maxillae rounded, arched around labium, 0.43 long. Labium shorter (0.28) than maxillae.

Sternum

Shield-shaped and convex, narrower towards leg III and IV, covered with fine setae. 0.80 long and 0.68 wide.

Legs

Setation of legs: I: femur d 1-1, p 1-1; tibia p 1-1, r 1-1, v 2-2-2; metatarsus p 0-1-1, r 0-1-1, v 2-2; II: femur d 1-1; tibia p1-1, r 1-1, v 0-2-0-2-2; metatarsus p 2-1(ap), r 1-1(ap), v 0-r1; III: femur d 1-1; tibia p 0-1, r 1, v p1-2(ap); metatarsus p 0-2, r 0-1; IV: femur d 1-0-1; tibia r 0-1, v p1-2(ap); metatarsus r 1, v 0-0-p1-p1

Leg I. Fe: 1.47, Pa: 0.61, Ti: 1.02, Me: 0.88, Ta: 0.94, Total: 4.91

Leg II. Fe: 1.47, Pa: 0.53, Ti: 0.92, Me: 0.81, Ta: 0.76, Total: 4.49

Leg III. Fe: 1.01, Pa: 0.41, Ti: 0.56, Me: 0.49, Ta: 0.43, Total: 2.90

Leg IV. Fe: 0.99, Pa: 0.40, Ti: 0.60, Me: 0.57, Ta: 0.44, Total: 3.00

Leg formula: I > II > III < IV

Abdomen

Egg-shaped, covered with evenly arranged setae. Five obvious indents.

Genitalia

Male pedipalps small with convex cymbium (Figure 6). Embolus short. Tibial apophyses strongly sclerotized. Ventral and intermediate tibial apophyses of similar length and half the size of RTA, RTA curved towards dorsal. No bulbar muscles, well-developed basal hematodocha. Large apodeme in distal part of tibia as attachment for two tibial muscles.

Distribution

Probably widespread in sclerophyll forests around Brisbane, Queensland (Australia).

Discussion

We report the demographics of *Xysticus bimaculatus*, a non-webbuilding subsocial crab spider from southern Queensland. Its lifestyle appears to be very similar to the subsocial crab spider *Diaea ergandros* (Evans, 1995). Like in other subsocial crab spiders, the presence of a caring female seems to be important for offspring survival in *X. bimaculatus*. We found higher numbers of spiderlings in nests with a caring adult female present and a similar pattern was found in *D. ergandros* (Unglaub et al., 2013). The presence of an adult female is beneficial in *D. ergandros*, but also in the subsocial huntsman spider *Delena cancerides*, since adult spiders are able to capture prey more efficiently (Evans, 1998a, b; Yip and Rayor, 2011). We found that the likelihood of an adult living *X. bimaculatus* female being present in the

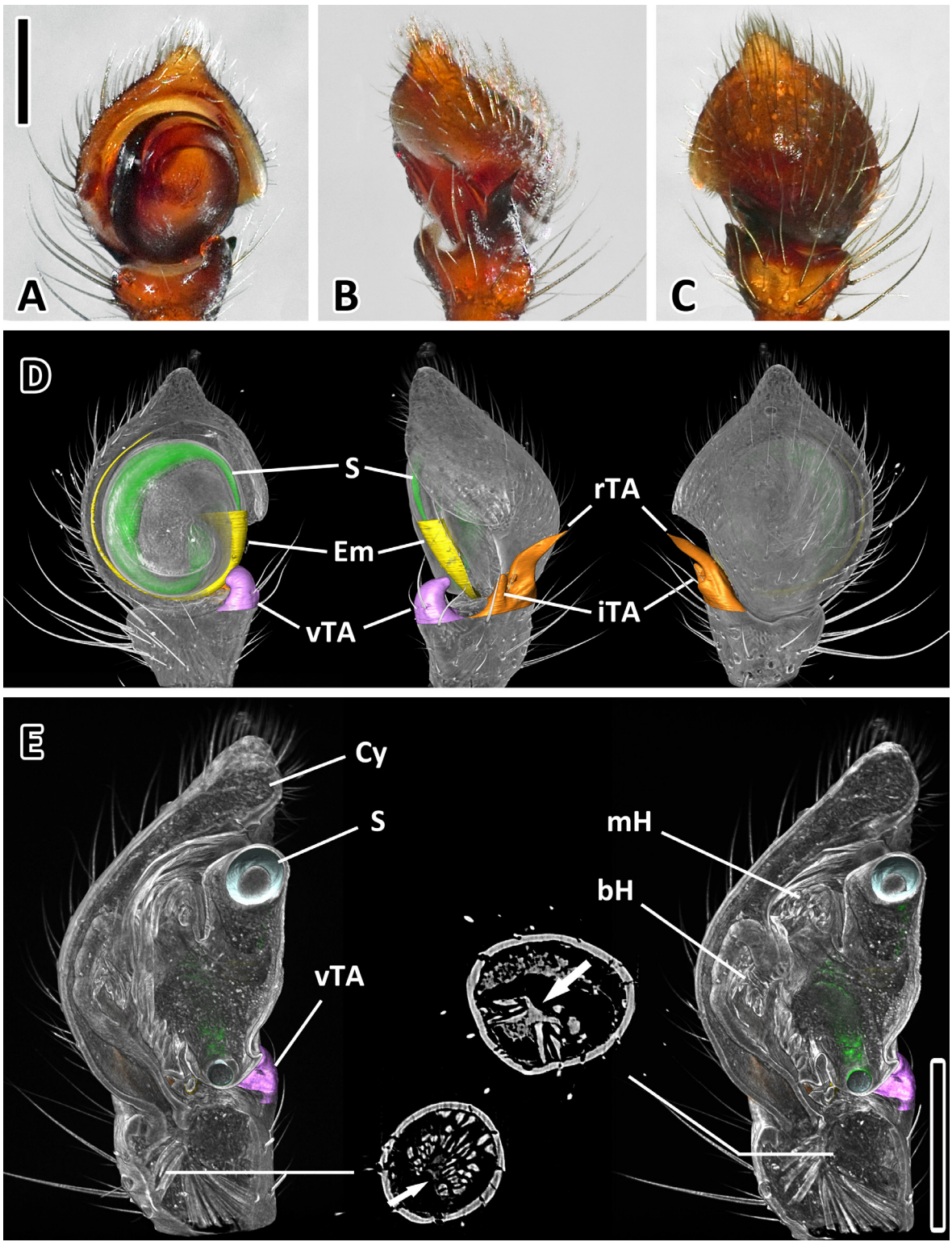

Figure 6: Left male palp of *Xysticus bimaculatus* (AM, KS120583) A), Ventral view. B) Retro lateral view. C) Dorsal view, D) Coloured surface models of different parts of the male superimposed on the volume rendering of the male palp (ventral, retrolateral, dorsal). E) Longitudinal sections of the volume rendered male palp showing the two prominent hematodochae. Muscles are only present in tibia and attached to a large apodeme (see arrows in cross-sections).

Abbreviations: bH, basal hematodocha; Cy, cymbium; Em, embolus; iTA, intermediate tibial apophysis; mH, median hematodocha; rTA, retrolateral tibial apophysis; S, spermophor; vTA, ventral tibial apophysis. Scale bars = 0.25 mm.

nest was high when spiderlings were very young but declined when spiderlings were older. In *D. ergandros* some females are consumed by their offspring (matriphagy) (Evans et al., 1995) and it remains to be studied whether matriphagy occurs in *X. bimaculatus* as well and could explain the reported pattern.

Unlike subsocial *Diaea*, *X. bimaculatus* builds its nests mostly from *Acacia* and not from *Eucalyptus* leaves. This may favor the occurrence of the species in areas that are dominated by *Acacia melanoxylon*, which is however widely distributed and common along the Australian east coast. We only recorded those trees that were used for nest construction and did not quantify potentially available host trees, but both *Acacia* and *Eucalyptus* trees were present in all of our study sites. We never found *D. ergandros* and *X. bimaculatus* occurring sympatrically. *D. ergandros* seems to be absent along the northern coast of New South Wales and southern coast of Queensland (Evans, 1997) and so far we did not detect *X. bimaculatus* nests south of Queensland.

Similar to *D. ergandros*, nests of *X. bimaculatus* serve as foraging areas and major prey types are beetles (Coleoptera), but also wasps and ants (Hymenoptera) (Evans, 1998a). In contrast to *D. ergandros* nests (Unglaub et al., 2013), we only found very few potential predators inside nests of *X. bimaculatus* however, the nest may still protect spiders from predators that we did not detect.

We found that nests contain on average 10 spiderlings in *X. bimaculatus*, which is fewer than in *D. ergandros,* where nests contain on average 45 inhabitants (Evans, 1995). However, spiderling numbers in *X. bimaculatus* did not significantly decrease with increasing age, indicating that spiders have a relatively long period of communal activities. The finding that spiders disperse only shortly before maturation suggests a transitory stage between subsocial and permanently social (Lubin and Bilde, 2007). In almost all social spiders studied to date, a transition from subsociality to sociality is accompanied by a switch from outbreeding to inbreeding, which has major consequences for speciation processes (Agnarsson, 2012; Agnarsson et al., 2006; Agnarsson et al., 2013; Bilde et al., 2005; Johannesen et al., 2007). An exception can be found in social spiders of the genus *Tapinillus* (Oxyopidae), which is thought to be outbred because it does not have a female-biased sex ratio (Avilés, 1994). It would be highly interesting to investigate the mating system and sex-ratio of *X. bimaculatus* and to compare it with other subsocial and social crab spiders.

The taxonomy of Thomisidae is challenging and a revision of most genera is needed (Benjamin et al., 2008; Szymkowiak, 2007). Similarly, a recent molecular phylogeny of Sparassidae showed that two genera with subsocial species previously described as *Eodelena* are synonymous with *Delena* and all three known group-living *Delena* are closely related (Agnarsson and Rayor, 2013). A molecular phylogeny of the group-living Thomisidae may thus help to understand whether sociality has evolved multiple times in this family or whether the species, albeit being assigned into different genera, are closely related as well. Since thomisid genera often lack a clear definition and diagnosis, species were assigned (especially in Australia) to the most common and cosmopolitan genera *Diaea, Misumena, Thomisus* and *Xysticus* (Lethinen, 2002; Szymkowiak, 2007). However, the taxonomic

status of these genera is highly problematic. For example, Jantscher (2002) studied various thomisid genera of central Europe with a focus on the genus *Xysticus* and found at least three different groups within this genus characterized by the organization of the male palp (further previous suggestions of subgroups within *Xysticus* s.l. are reviewed in Jantscher (2002) and not addressed here). Since *X. bimaculatus* lacks tegular structures it does not belong to the group "*Xysticus* s. str." sensu Jantscher (2002), which is characterized by a complex tegular structure and at least two distinct tegular apophyses. Based on the apomorphies proposed by Jantscher (2002), *X. bimaculatus* might be part of the "*Proxysticus*" group characterized by the three distinct tibial apophyses. Nevertheless, these suggestions are only based on data of European material and comprehensive studies of *Xysticus* s.l., a group which is likely paraphyletic (Jantscher, 2002) are still lacking.

Although crab spiders have a worldwide distribution (Platnick, 2014) group-living crab spiders can be exclusively found in Australia. This continent has a history of long isolation and is renowned for its harsh environmental conditions (Herberstein et al., 2014). It has been suggested that certain evolutionary phenomena are more pervasive in Australia, such as cooperative breeding or deception (Herberstein et al., 2014). Some solitary Australian crab spiders, for example, use their body UV reflection as deceptive signal to attract and hunt naïve pollinators (Heiling et al., 2004). The harsh environmental conditions prevalent in Australia may as well have played a role in the evolution of sociality in two spider families (Thomisidae and Sparassidae). The multiple independent origins across spider families provide the opportunity for comparative investigations aiming to unravel selective forces being responsible for the evolution of this lifestyle. Since both Thomisidae and Sparassidae do not build capture webs, alternative perspectives on key factors for the evolution of sociality need to be considered (Evans, 1998a). Ecologically rather similar, the subsocial *Xysticus* and *Diaea* are a very suitable model to study their behaviour and its drivers on comparative grounds.

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Author Contributions

JR and TR collected the field data, compared specimens in museum collections, measured specimens and took photographs of collection material. JR analyzed and interpreted the natural history data. TR took the in situ photographs. PM scanned and reconstructed the male palp and described the seta pattern. Figure plates were prepared by JR, TR and PM. JR wrote the

manuscript, with contributions from TR and PM.

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Table 1: List of species examined.

Museum and ID	Species	Sex	Type material
ZMH (MG 1467)	*Cymbacha cerea*	female	y
ZMH (MG 9932), Type catal. Araneae 142	*Cymbacha festiva*	female	y
AM KS77337	*Cymbacha ocellata*	female	n
AM KS107220	*Cymbacha ocellata*	spiderling	n
ZMH (MG 1477)	*Cymbacha ocellata*	female	y
ZMH (MG 9896)	*Cymbacha ocellata*	female	y
QLM	*Cymbacha saucia*	female	n
ZMH, Type catal. Araneae 144	*Cymbacha saucia*	female	y
ZMH (MG 6526), Type catal. Araneae 145	*Cymbacha setosa*	female (subadult)	y
ZMH, Type catal. Araneae 146	*Cymbacha similis*	female	y
ZMH (MG) 3754	*Cymbacha stratipes*	female	y
AM KS099848	*Diaea adusta*	female	n
AM KS099823	*Diaea cruentata*	female	n
AM KS108075	*Diaea decempunctata*	female	n
AM KS099817	*Diaea dimidiata*	female	n
ZMH (MG) 2268	*Diaea dimidiata*	female	y
AM KS9265	*Diaea evanida*	female	n
AM KS099828	*Diaea olecempunctata*	female	n
AM KS107968	*Diaea pilula*	female	n
ZMH (MG) 9924	*Diaea pilula*	female	n
AM KS14220	*Diaea praetaxa*	female	n
AM KS099847	*Diaea praetaxa*	female	n
AM KS099846	*Diaea praetaxa*	female	n
ZMH (No illegible)	*Diaea prasina*	female	n
AM KS107225	*Diaea punctata*	female	n
AM KS108086	*Diaea punctata*	female	n
ZMH (MG) 14593	*Diaea punctata 2*	female	y
ZMH (MG) 9900, 14593	*Diaea punctata1*	female	y
AM KS099819	*Diaea punctipes*	female	n
AM KS099825	*Diaea rosea*	spiderling	n
AM KS099826	*Diaea rosea*	females, male	n
AM KS107983	*Diaea rosea*	female	n
AM KS43188	*Diaea* sp.	female	n
ZMH (MG) 14586	*Diaea tumefacta*	female	n
AM KS107986	*Diaea variabilis*	female	n
ZMH (MG) 6511	*Diaea variabilis*	female	y
QLMS67516	***Tharpyna (= Xysticus bimaculatus)***	female	n
QLM	*Tharpyna albo-signata*	female	n
ZMH (No illegible)	*Tharpyna albo-signata*	female	y
AM KS10547	*Tharpyna campestrata*	female	n
ZMH, Expedition Dr. Michalsen 1905	*Tharpyna campestrata*	female	n
ZMB 1909	*Tharpyna decorata*	female, male	y
AM KS83226	*Tharpyna diademata*	female	n
AM KS107214	*Tharpyna diademata*	spiderling	n
ZMH (MG 9926)	*Tharpyna diademata*	female	y

Museum and ID	Species	Sex	Type material
AM KS109023	*Tharpyna hirsuta*	female	n
AM KS109026	*Tharpyna munda*	female	n
AM KS6695	*Tharpyna simpsonii*	female	y
QLM S65425	***Tharpyna sp. (= Xysticus bimaculatus)***	female	n
AM KS107969	*Tharpyna* sp.	female	n
AM KS83207	*Tharpyna speciosa*	female	n
AM KS88728	*Tharpyna speciosa*	female	n
AM KS109028	*Tharpyna venusta*	female	n
ZMH (MG) 9911	*Tharpyna venusta*	male	y
AM KS107984	*Xysticus bilimbatus*	female	n
ZMH (MG) 2260	*Xysticus bimaculatus*	female	y
AM KS108111	*Xysticus crsitatus*	female	n
ZMH (MG) 9922	*Xysticus cruentatus*	female	y
ZMH (MG) 9923	*Xysticus daemelii*	male	y
AM KS109060	*Xysticus elegans*	male	n
ZMH, Type catal. Araneae 771	*Xysticus evanidus (= Diaea evanida)*	male	y
AM KS45647	*Xysticus geometres*	female	n
ZMH (MG) 9925	*Xysticus geometres*	female	y
ZMH (MG) 4604	*Xysticus inornatus (=Diaea inornata)*	female	y
ZMH (MG) 22676	*Xysticus pustulosis (=Thomisus spectiabilis)*	female	y
AM KS31410	*Xysticus socialis = Diaea inornata*	female	n
AM KS45644	*Xysticus triguttatus*	female	n

QLM = Queensland Museum, Brisbane, Australia; AM = Australian Museum, Sydney, Australia; ZMH = Zoological Museum, Hamburg, Germany, MG = Museum Godefroy (now Zoological Museum Hamburg), ZMB = Zoological Museum Berlin, Germany

Chapter 3

Multiple origins of subsociality in crab spiders (Thomisidae)

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Abstract

Determining factors that facilitate the transition from a solitary to a social lifestyle is a major challenge in evolutionary biology, especially in taxa that are usually aggressive towards conspecifics. Most spiders live solitarily and few species are known to be social. Nevertheless, sociality has evolved multiple times across several families and nearly all studied social lineages have originated from a periodically social (subsocial) ancestor. Group-living crab spiders (Thomisidae) are exclusively found in Australia and differ from most other social spiders because they lack a communal capture web. Three of the group-living species were placed in the genus *Diaea* and another in the genus *Xysticus*. Most Australian thomisids are, however, difficult to identify as most descriptions are old and of poor quality, and the genera *Diaea* and *Xysticus* may not correspond to monophyletic groups. Here, we clarify the phylogenetic relationships of the four group-living Australian thomisids and conclude that amongst these subsociality has evolved two to three times independently. The subsocial *Xysticus bimaculatus* is not closely related to any of the social *Diaea* and an independent origin of subsociality is likely in this case. The presented data indicates that within Diaea two origins of subsociality are possible. Our results help to understand the evolution of sociality in thomisids and support the hypothesis that permanent sociality in spiders has evolved multiple times relatively recently from subsocial ancestors.

Keywords: Thomisid phylogeny, social spider, social evolution, *Diaea,* Araneae

Introduction

The evolution of sociality is puzzling considering that group living entails costs such as competition for resources and a high risk of accumulating pathogens or parasites (Hughes et al., 2002; Wilson et al., 2003). Generally, sociality may evolve when benefits outweigh the costs (Alexander, 1974). In many vertebrates, kin selection (Hamilton, 1964) and ecological constraints are thought to facilitate independent origins of sociality (Davis et al., 2011; Émlen, 1991; Faulkes et al., 1997). The same likely holds true for social spiders that form groups that always consist of family members (Avilés, 1997; Lubin and Bilde, 2007).

Sociality is rare amongst spiders, which are typically aggressive towards conspecifics, including kin. Less than 25 of the over 44,500 described spider species are known to be permanently social or 'quasisocial' (Agnarsson et al., 2006; Avilés, 1997; Lubin and Bilde, 2007; Platnick, 2014) and about 70 are temporarily social or 'subsocial' (Yip and Rayor, 2013). Though being rare, social behavior has been documented across various spider families (Agnarsson et al., 2006; Bilde and Lubin, 2011; Lubin and Bilde, 2007; Yip and Rayor, 2013). Quasisocial (hence 'social') spiders likely evolved from subsocial ancestors via gradual prolongation of communal activities of siblings, and elimination of the dispersal phase (Agnarsson et al., 2006; Bilde et al., 2005; Johannesen et al., 2007; Lubin and Bilde, 2007) and subsocial behavior has evolved at least 18 times independently (Agnarsson et al., 2006; Bilde and Lubin, 2011; Yip and Rayor, 2013). Short dispersal distances in subsocial species and the lack of premating dispersal in permanently social species result in highly inbred mating systems (Bilde et al., 2005; Lubin and Bilde, 2007; Lubin et al., 2009; Ruch et al., 2009). Inbreeding, in turn, may lead to loss of genetic variability in social spiders restricting diversification (Avilés, 1997, Agnarsson et al., 2013a). Extant social lineages tend to be relatively young ranging from a few hundred thousand years to about two million years (my) (Agnarsson et al., 2013a; Johannesen et al., 2007). In contrast, subsocial species are outbred and the limited available evidence suggests they may persist over much longer periods (Agnarsson et al., 2013a). Understanding the patterns of origin and persistence of subsocial and social lineages in a phylogenetic context is thus important.

There is molecular phylogenetic evidence for multiple origins of sociality within two spider families, the cobweb spiders (Theridiidae) and the velvet spiders (Eresidae). In the Theridiidae, where nine independent origins of sociality in three genera are documented (Agnarsson et al., 2006; Avilés, 1997; Avilés and Bukowski, 2006), social lineages predominantly contain only a single species, indicating a lack of diversification and high extinction rates (Agnarsson et al., 2006). In the eresid genus *Stegodyphus*, three independent origins of sociality have resulted in exclusively single-species social lineages as well, although with somewhat higher intraspecific mtDNA variability than observed in social theridiids (Johannesen et al., 2007). The single origin of subsociality in the middle Miocene (16 mya) recently hypothesized for group-living huntsman spiders (*Delena*, Sparassidae) (Agnarsson and Rayor, 2013) further supports the idea that a subsocial lifestyle can be maintained

over relatively long evolutionary time spans.

Group-living huntsman spiders are special among subsocial and social spiders in that Sparassidae lack a communal capture web (Agnarsson and Rayor, 2013; Avilés, 1997) and the same holds true for crab spiders (Thomisidae). More than 2,100 thomisid species in 174 genera are described worldwide (Platnick, 2014). Four of them, all Australian, are group-living, with similar biology but varying social complexity. They construct nests from leaves which serve as foraging areas (Evans, 1998a; Main, 1988) and protective retreats (Evans, 1998a; Unglaub et al., 2013) Subsocial crab spiders hunt by ambushing prey and females feed their offspring (Evans, 1998b; Ruch et al., 2014b). Spiderlings cooperate in nest construction, hunting and feeding for several months (Evans, 2000; Ruch et al., 2014a).

Thomisid taxonomy is poorly understood and many genera need revision (Benjamin et al., 2008; Garb and Gillespie, 2006; Szymkowiak, 2007). According to the current classification three of the group-living thomisids belong to the genus *Diaea* (Evans, 1995). The only permanently social thomisid is *D. socialis* Main, 1988 (Rowell and Main, 1992). Only little is known about the biology *D. inornata*, but like *D. socialis* it has a female-based sex-ratio and like *D. ergandros* (but different from *D. socialis*) it has an annual life-cycle (Evans, 1995). The subsocial lifestyle of the fourth species, *Xysticus bimaculatus* Koch, 1867, was only recently discovered (Ruch et al., 2014c) and its lifestyle seems very similar to *D. ergandros*. Offspring disperse relatively late and the presence of an adult living female seems beneficial for spiderling survival (Ruch et al., 2014c). The discovery of a subsocial species outside the genus *Diaea* indicates that sociality may have evolved more than once in Thomisidae. Alternatively, taxonomic and classificatory uncertainties might disguise a common origin of sociality in Thomisidae (e.g. Agnarsson & Rayor (2013) for Sparassidae). Only few molecular phylogenetic studies have been performed on crab spiders and none of them included group-living thomisids (Benjamin et al., 2008; Garb and Gillespie, 2006, 2009).

Here, we explore the phylogenetic relationship of Australian thomisids including the four group-living species. We test whether the permanently social *D. socialis* has evolved from subsocial *Diaea* and estimate the age of sociality in thomisids. Further, we aim to test the hypothesis that subsociality evolved multiple times independently in this clade.

Material and Methods

Data collection and phylogenetics

We collected living thomisids in 2012 and 2013 in NSW, QLD, TAS and WA, Australia (Figure 1). Whole spiders were preserved in 90% Ethanol. In addition, specimens which have been collected in 2008 in NSW, QLD and WA and preserved in 70% Ethanol were used for DNA extraction. In total, we extracted DNA from 93 crab spiders belonging to 26 species (Table 1). Specimens were identified based on original taxonomic descriptions and comparisons with type material. Species were categorized as having a non-social, subsocial, or social behavior according to previous behavioral classifications (Avilés and Harwood, 2012; Lubin and Bilde, 2007). Because social or subsocial behavior may

have been overlooked in certain species (see Ruch et al., 2014c), we also took into account whether spiders occurred solitarily or in groups at the collections sites. Three undescribed species that require separate mentioning are *Diaea* sp. 2, *Diaea* sp. 3, and *Diaea* ID35, because these cluster amongst the social/subsocial *Diaea* species and their behavioral classification is critical to the inference of the origins of social behavior in thomisids. *Diaea* sp. 2 and *Diaea* sp. 3 were collected in heath land as single juvenile individuals using a sweep net. Due to their occurrence as singletons and the absence of communal nests in the area we consider a non-social lifestyle very likely for these otherwise unknown species. *Diaea* ID35 was collected as adult female and produced an egg sac in the laboratory. The spiderlings of this species started cannibalizing each other a few days after hatching. This observation is a clear indication for a non-social behavior.

DNA was extracted from one or two legs of each specimen or whole carapace of small spiders using a modified Proteinase K-extraction protocol. We amplified partial fragments for two mitochondrial genes (16S rRNA (16S) and cytochrome c oxidase subunit I (COI)) and two nuclear genes (Histone H3 (H3) and the Internal Transcribed Spacer 2 (ITS2)). For the loci COI, H3, and ITS2 we used primers and protocols as described in (Agnarsson, 2010, 2012; Agnarsson et al., 2013b; Agnarsson et al., 2007). For 16S we used the forward primer 16SA/12261 CGCCTGTTTACCAAAAACAT (Hedin, 1997) and the reverse primer SPID-ND1/13398 TCRTAAGAAATTATTTGAGC at an annealing temperature of 48°C (Simon et al., 1994). Amplified fragments were sequenced in both directions by the University of Arizona Genetic Core (Genbank accession numbers will be added) and then assembled and proofread using the Chromaseq module (Maddison and Maddison, 2011a) in Mesquite (Maddison and Maddison, 2011b) employing Phred (Green and Ewing, 2002) and Phrap (Green, 2009).

We augmented the taxon sampling with 121 species from GenBank (Table 2) (Benson et al., 2007). Short 16S fragments (shorter than 500 bases) were not included.

We aligned sequences using MAFFT (Katoh et al., 2005) through the EMBL-EBI online portal with 100 tree rebuilding replications and 100 max iterations. Protein coding gene sequences were translated and confirmed to contain no stop codons. For all analyses, gaps and ambiguous bases were treated as missing data. The gene matrices were concatenated in Mesquite (Maddison and Maddison, 2011b). We created several different matrices to test for the effects of missing data, including 'all data', '2 genes' (including only taxa for which sequences from at least 2/4 genes were available), and '4 genes' (including only taxa for which sequences from all 4 genes were available).

We partitioned the data by gene, and partitions were exported from Mesquite for model choice. The appropriate models for each gene were chosen using jModeltest v0.1.1 employing the Akaike information criterion (Posada, 2008). For each partition we employed the corresponding model of evolution for analyses: GTR + Γ + I for COI and 16S and GTR + Γ for ITS2 and H3. We analysed the data matrices using Bayesian inference and Maximum likelihood of individual gene trees as well as concatenated matrices. We ran the MC3 (Metropolis coupled

Figure 1: a) *Diaea ergandros* female feeding with offspring on a captured prey item on a natural nest constructed from *Eucalyptus* leaves. b) Juvenile *D. ergandros* in 'ambush position'. c) A natural nest of the subsocial spider *Xysticus bimaculatus*. d) An adult female *D. ergandros* (spider in the middle) on a natural nest with relatively large offspring. e) An adult male (dark brown) and female (greenish) *D. socialis*. f) Subadult *D. socialis* females taken out of their natural nest. g) An adult *X. bimaculatus* female (left) and male (right). h) An adult *X. bimaculatus* female in a natural nest constructed from several *Acacia* leaves. Scale bars = 1 cm.

Markov chain Monte Carlo) in MrBayes V3.1.2 (Huelsenbeck and Ronquist, 2001) for 30.000.000 generations, sampling every 1000 generations. Chain stationarity and appropriate burnin was verified using Tracer 1.5 (Drummond and Rambaut, 2007), and the first 20% of the

trees were discarded as burnin. Maximum likelihood analyses of the concatenated matrices were performed in RAxML with the same data partitioning and using GTR + Γ. RAxML dictates using the GTR substitution model and a Γ model of rate heterogeneity was assumed. ML searches were repeated 100 times and the tree maximizing likelihood of the data was preferred. Due to weak support for many deeper nodes we evaluated the effect of selectively removing taxa with long branches (*Cebrenninius rugosus* in the 2-gene dataset and *Zygometis* sp. in the 4-gene dataset), which improved the support values in the Bayesian analysis but not in the ML analysis. We present all analyses including the long branches.

Individual-gene analyses under maximum likelihood were performed using RAxML (Stamatakis, 2006) as implemented in raxmlGUI v. 1.3 (Silvestro and Michalak, 2011). The number of independent ML searches was set to 10 and 1000 thorough bootstrap replicates were calculated.

The bayesian tree based on the 4-gene concatenated alignment was imported to Mesquite and ancestral state reconstructions were performed using three categorical behavioral character states: solitary living (non-social), subsocial, and social. Ancestral states were reconstructed using a maximum parsimony approach as well as the maximum likelihood method. Marginal probability reconstruction was performed using standard settings in Mesquite with model Mk1 (est.) and an estimated rate of 0.77960431 as well as -log likelihood of 17.46485515. Likelihoods are reported as proportional likelihoods; threshold when decisions made was set to 2.0 (standard setting).

We estimated node ages using a relaxed clock based on spider-specific substitution rates estimates for mitochondrial genes across several spider groups (Bidegaray-Batista and Arnedo, 2011; Kuntner et al., 2013) and using the occurrence of Thomisidae in Baltic amber (about 40 mya) as a minimum age calibration point. The mitochondrial substitution rate parameter (ucld.mean) was assigned a normal prior with mean=0.0112 and SD=0.001, and the age of Thomisidae (split between Thomisidae and Anyphaenidae in this study) was set as a lognormally distributed prior with a mean of 42 and log(stdev) of 0.3, with 95% of the prior distribution spanning 25-70 mya. For the nuclear genes substitution mean starting rates were set at an order of magnitude slower than the reported mitochondrial rate (Kuntner et al. 2013) and assigned uniform flat priors.

Results

Phylogenetic relationships of Australian thomisids

Within Thomisidae, several clades were relatively well supported and results were generally congruent among methods in the concatenated datasets. The ‘4 gene’ dataset only included Australian thomisids and was statistically best supported (Figure 2).

In all concatenated datasets (Bayesian inference and Maximum likelihood for ‘2 gene’, ‘4 gene’ data) and the COI and 16S single marker analyses, the Australian *Diaea* were distributed across two distinct clades and were not monophyletic. The subsocial/social *Diaea* formed a well-supported clade also including

three undescribed solitary thomisids. This clade was distinct from most of the solitary species *D. evanida* and *D. variabilis* as well as *D.* aff. *variabilis*, which together comprised a second monophyletic *Diaea* clade.

The subsocial *Xysticus bimaculatus* was found to form a sister-group relationship with an undescribed thomisid in both concatenated datasets. Its sister clade was *Cymbacha similis* in the '2 gene' dataset (Figure 3) and the *Cymbacha* clade including *Cymbacha similis* in the '4 gene' dataset (Figure 2). However, *Cymbacha* was only monophyletic in the '4 gene' dataset, while *Cymbacha similis* did not cluster with other *Cymbacha* in the '2 gene' dataset.

All of the above mentioned species belong to the *Thomisus* clade (*sensu* Benjamin et al. (2008)). *Sidymella* and *Stephanopis* were recovered in the '2 gene' dataset as *Stephanopis* clade sensu Benjamin et al. (2008), however neither genus was monophyletic (Figure 3).

Multiple origins and age of subsociality in Thomisidae

All social/subsocial *Diaea* were closely related in one well-supported *Diaea* clade (Figure 2, 3). The permanently social *D. socialis* and the subsocial *D. inornata* formed a robust sister-group relationship that had an estimated age of 10.4 my (Figure 4). Genetic structure in *D. socialis* was relatively flat (low intra-specific

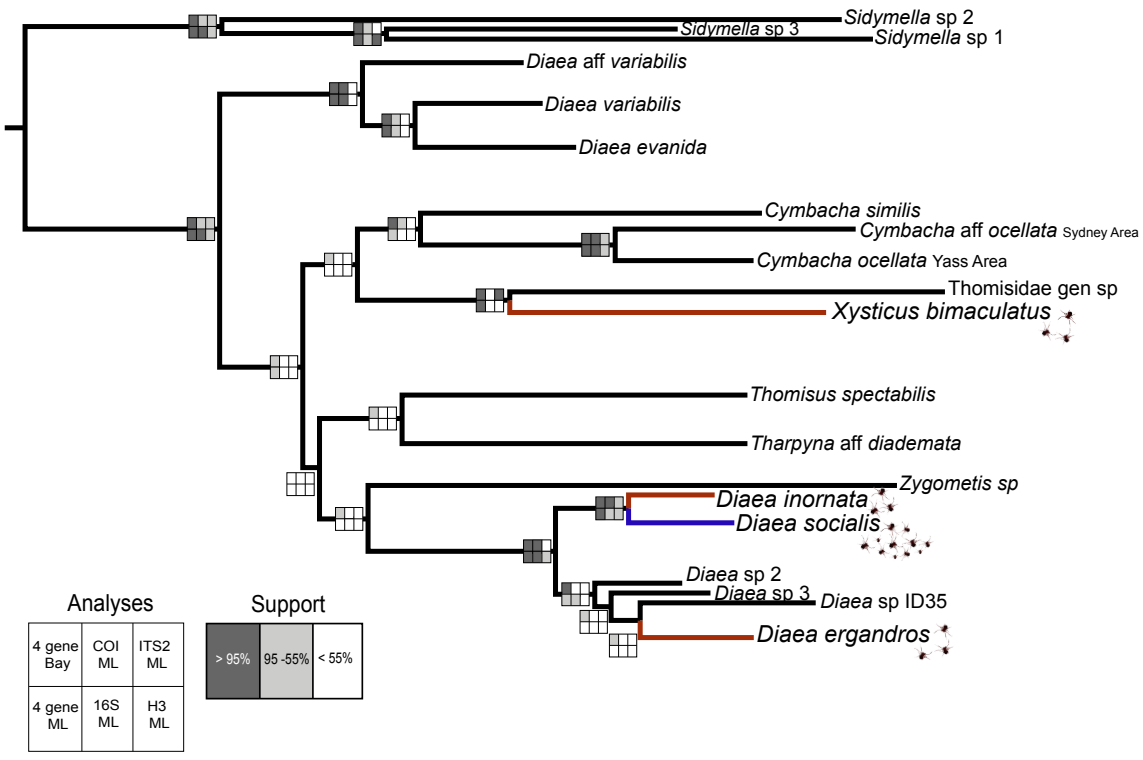


Figure 2: Summary tree based on results of the Bayesian (Bay) as well as the Maximum Likelihood (ML) analyses of the concatenated '4-gene' dataset and the results of individual genes (only representing Australian species). The boxes in front of the nodes show support for each analysis (either posterior probabilities or bootstrap values). '4 gene Bay' (upper left side of the box) represents posterior probability support of the concatenated '4-gene' dataset, '4 gene ML' (lower left side of the box) represents the ML analysis of the concatenated '4-gene' dataset, 'COI ML' (upper middle of the box) the ML analysis of the single gene COI, '16S ML'(lower middle of the box) the ML analysis of the single gene 16S, 'ITS2 ML' (upper right side of the box) the ML analysis of the single gene ITS2 and 'H3 ML' (lower right side of the box) the ML analysis of the single gene H3. Dark grey boxes show node-support greater than 95%, light grey boxes support ranging between 55 and 95% and white boxes support less than 55%. Subsocial lineages are shown in red, the social lineage in blue.

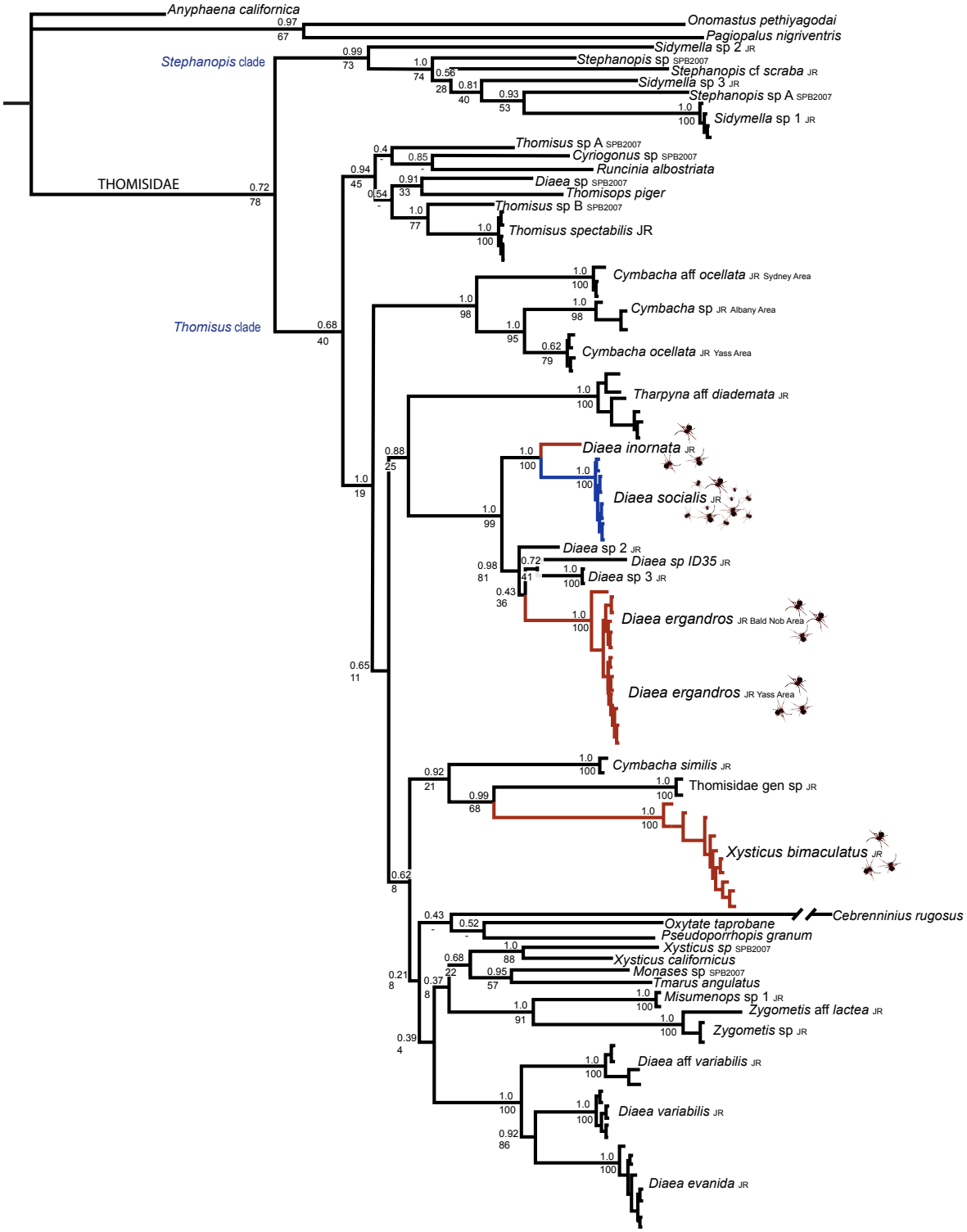
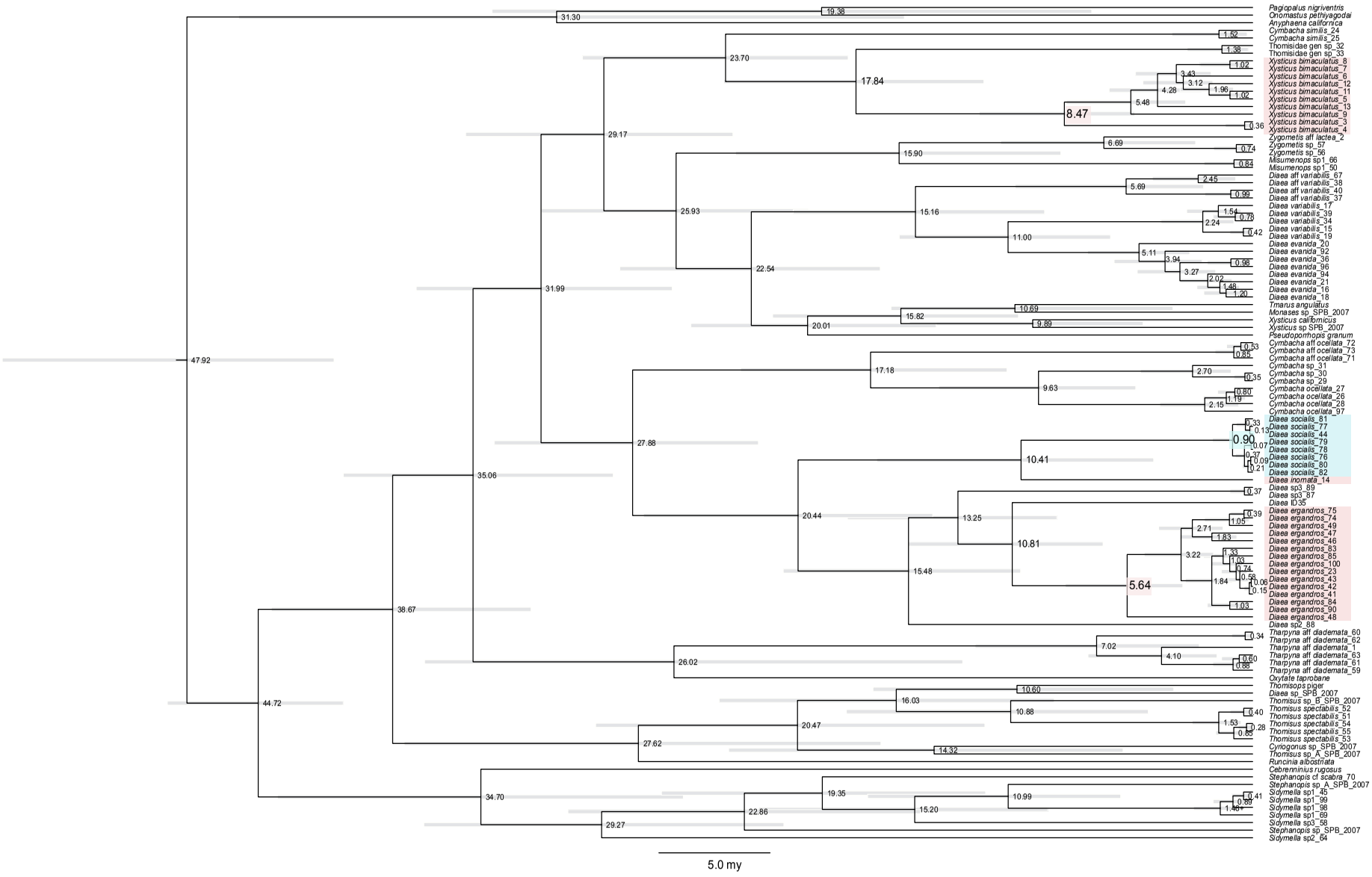


Figure 3: Summary tree based on results of the Bayesian as well as the Maximum likelihood analyses of the concatenated '2-gene' dataset. The dataset represents both, Australian species (marked as JR) and species obtained from GenBank with at least two of the four genes available. The numbers at the nodes represent posterior probabilities (upper number) and bootstrap support values (lower number). Relationships of the subsocial (red) and social (blue) taxa roughly mirror those suggested by the '4-gene' analyses. All subsocial/social taxa belong to the Thomisus clade sensu Benjamin et al. 2008.

Figure 4 (opposite site): Fossil-calibrated tree estimating node ages. Subsocial species are highlighted in red, the permanently social *Diaea socialis* is highlighted in blue.


Pagiopalus nigriventris
Onomastus pethiyagodai
Anyphaena californica
Cymbacha similis_24
Cymbacha similis_25
Thomisidae gen sp_32
Thomisidae gen sp_33
Xysticus bimaculatus_8
Xysticus bimaculatus_7
Xysticus bimaculatus_6
Xysticus bimaculatus_12
Xysticus bimaculatus_11
Xysticus bimaculatus_5
Xysticus bimaculatus_13
Xysticus bimaculatus_9
Xysticus bimaculatus_3
Xysticus bimaculatus_4
Zygometis aff lactea_2
Zygometis sp_57
Zygometis sp_56
Misumenops sp1_66
Misumenops sp1_50
Diaea aff variabilis_67
Diaea aff variabilis_38
Diaea aff variabilis_40
Diaea aff variabilis_37
Diaea variabilis_17
Diaea variabilis_39
Diaea variabilis_34
Diaea variabilis_15
Diaea variabilis_19
Diaea evanida_20
Diaea evanida_92
Diaea evanida_36
Diaea evanida_96
Diaea evanida_94
Diaea evanida_21
Diaea evanida_16
Diaea evanida_18
Tmarus angulatus
Monases sp_SPB_2007
Xysticus californicus
Xysticus sp SPB_2007
Pseudoporrhopis granum
Cymbacha aff ocellata_72
Cymbacha aff ocellata_73
Cymbacha aff ocellata_71
Cymbacha sp_31
Cymbacha sp_30
Cymbacha sp_29
Cymbacha ocellata_27
Cymbacha ocellata_26
Cymbacha ocellata_28
Cymbacha ocellata_97
Diaea socialis_81
Diaea socialis_77
Diaea socialis_44
Diaea socialis_79
Diaea socialis_78
Diaea socialis_76
Diaea socialis_80
Diaea socialis_82
Diaea inornata_14
Diaea sp3_89
Diaea sp3_87
Diaea ID35
Diaea ergandros_75
Diaea ergandros_74
Diaea ergandros_49
Diaea ergandros_47
Diaea ergandros_46
Diaea ergandros_83
Diaea ergandros_85
Diaea ergandros_100
Diaea ergandros_23
Diaea ergandros_43
Diaea ergandros_42
Diaea ergandros_41
Diaea ergandros_84
Diaea ergandros_90
Diaea ergandros_48
Diaea sp2_88
Tharpyna aff diademata_60
Tharpyna aff diademata_62
Tharpyna aff diademata_1
Tharpyna aff diademata_63
Tharpyna aff diademata_61
Tharpyna aff diademata_59
Oxytate taprobane
Thomisops piger
Diaea sp_SPB_2007
Thomisus sp_B_SPB_2007
Thomisus spectabilis_52
Thomisus spectabilis_51
Thomisus spectabilis_54
Thomisus spectabilis_55
Thomisus spectabilis_53
Cyriogonus sp_SPB_2007
Thomisus sp_A_SPB_2007
Runcinia albostriata
Cebrennninus rugosus
Stephanopis cf scabra_70
Stephanopis sp_A_SPB_2007
Sidymella sp1_45
Sidymella sp1_99
Sidymella sp1_98
Sidymella sp1_69
Sidymella sp3_58
Stephanopis sp_SPB_2007
Sidymella sp2_64
5.0 my

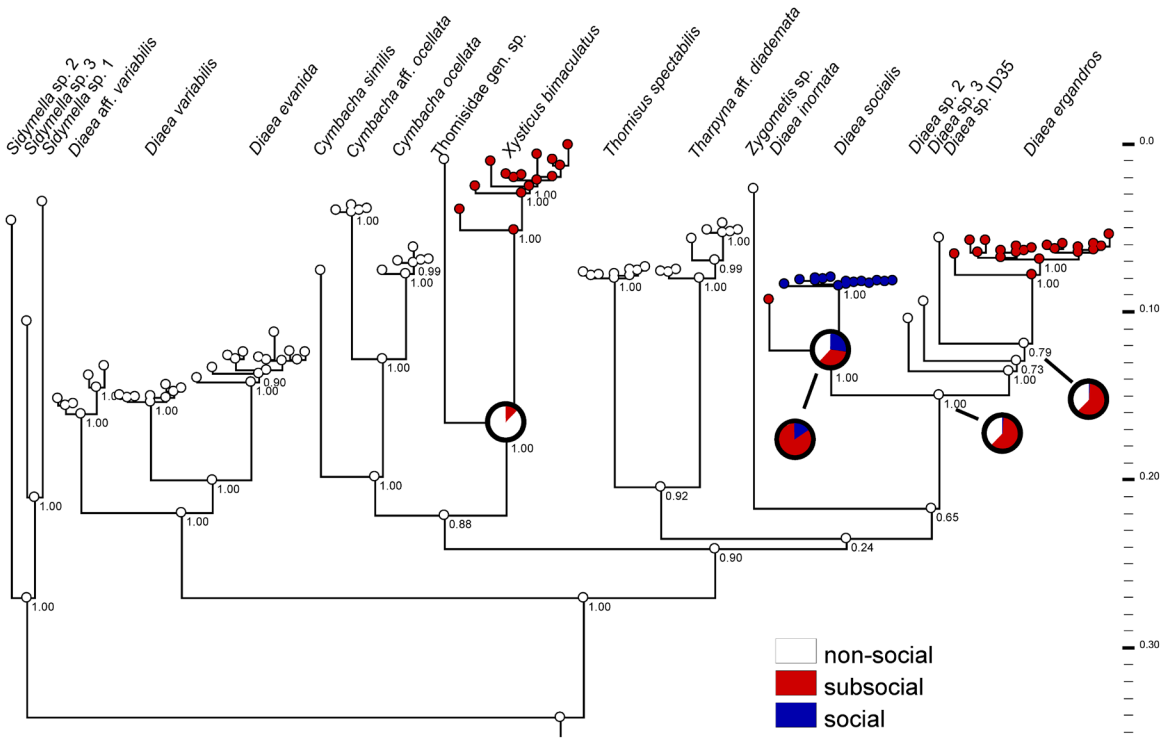


Figure 5: The phylogeny of subsocial/social Thomisidae reconstructed based on Bayesian inference and a four-loci concatenated dataset. Maximum-likelihood ancestral state reconstruction was used to map the distribution of the three behavioral character states "non-social" (white), "subsocial" (red), and "social" (blue) on nodes representing ancestral species. Pie charts indicate likelihood of the respective character state present in the respective ancestral species. Enlarged pie charts indicate nodes that have >0 likelihood for at least two character states; those charts shown next to the nodes represent results of a more conservative estimation where uncertainty about the behavioural state of *Diaea* sp. 2 and *Diaea* sp. 3 was taken into account. The graph shows two to three independent origins of subsociality in Thomisidae: While leaving the two above mentioned species unscored leaves the question regarding two potential origins of group living in *Diaea* open, the subsocial *Xysticus bimaculatus* is not closely related to any group-living *Diaea* and an independent evolution of its subsocial behavior is well supported in both approaches. The scale shows the proportion of nucleotide change.

variability) compared to the other species. At roughly 0.9 my, observed diversity within *D. socialis* was younger than that within the subsocial *D. ergandros* (~ 5.6 my) and *X. bimaculatus* (~ 8.5 my, Figure 4). As only a single specimen was collected for *D. inornata*, no conclusions about intra-specific divergence can be made.

The subsocial *D. ergandros* was comprised of two well-supported clades that correspond to the geographical distribution (app. 1000 km distance between collection sites, Figure 3). It clustered with three undescribed solitary *Diaea* species, and the common ancestor of *D. ergandros* and its sister *Diaea* ID35 was estimated to be 10.8 my old (Figure 4).

Due to the position of the three solitary *Diaea* spp. amongst the subsocial and social species, the ancestral state reconstructions suggested that sociality may have two independent origins within *Diaea* (Figure 5). Scoring these species as solitary resulted in a likelihood of 98% that the last common ancestor of this *Diaea* clade was non-social (non-social in the MP approach, see appendix Figure 6) and thus two independent origins of social behavior in *Diaea* were favoured. This subsocial behavior originated between 10.8 my and 5.6 my in *D. ergandros* and within the last 20.4 my in *D. inornata* or its ancestor. However, since slight uncertainty remains and theoretically the singly collected

juveniles were dispersing members of subsocial or social groups, we conservatively reanalysed the data leaving these species unscored (Figure 5). In this case, solitary lifestyle of the last common ancestor had a likelihood of only 39.9% whereas a subsocial lifestyle would be more likely with 57.9% (equivocal in MP analysis) and thus social behavior may have evolved much earlier, more than 20.4 my in the last common ancestor of the subsocial/social *Diaea* clade (Figure 5). Although we consider the behavioral classification of *Diaea* sp. 2 and *Diaea* sp. 3 as non-social to be solid, we have to point out that considering this slight uncertainty the possibility for only a single origin of social behavior in *Diaea* is given.

Relatively similar to the age of the subsocial behavior observed in *Diaea* species (at least 10-11 my), subsocial behavior in *X. bimaculatus* has evolved after its origin 17.8 my ago. *X. bimaculatus* was not closely related to any of the *Diaea* clades but clustered with an undescribed thomisid. Both the ML and MP ancestral state reconstructions strongly supported an independent origin of subsociality in this species from similar behavior in *Diaea*. The likelihood of non-social lifestyle in the ancestor of *X. bimaculatus* was 93.4% (unequivocal in MP reconstruction). The last common ancestor of the social *Diaea* and *X. bimaculatus* was clearly reconstructed as a solitary species. Accordingly, social behavior in this lineage likely existed for at least 8.5 my.

Discussion

Multiple independent origins of complex social behavior have been reported across various groups of arthropods including insects (Crespi et al., 1998; Gibbs et al., 2012; McLeish et al., 2007) as well as crustaceans (Duffy et al., 2000), but also spiders (Agnarsson et al., 2006; Johannesen et al., 2007). We explored the phylogenetic relationships of group-living Australian thomisids and asked whether sociality has evolved multiple times within this family. Our study lacks the taxon sampling and resources to robustly resolve deeper level phylogenetics of thomisids, with weak support characterizing many deeper nodes as in the study of Benjamin et al. (2008) based on a similar set of genes. Nevertheless, our results unambiguously answer our main question: the results strongly support at least two independent origins of sociality, and independently so in all analyses. The close relationship of the social/subsocial *Diaea* indicates a potential common origin of sociality in this clade given that the trees shown rather represent the evolution of the genes than that of the species. However, based on the collection data we have to assume a solitary lifestyle for three undescribed *Diaea* species, which cluster amidst the subsocial/social *Diaea* species. The ancestral state reconstructions therefore favour two origins of sociality within *Diaea* and an additional origin in *Xysticus bimaculatus*.

In some animals, social behavior is successful in the long-term evolutionary perspective as suggested by the existence of species-rich social clades, for instance in Australian thrips (Thysanoptera) (Crespi et al., 1998) and several eusocial Hymenoptera, such as bees (Brady et al., 2006; Gibbs et al., 2012). In spiders, however, permanently-social lineages typically consist of single species and generally show “flat” genetic structure indicating costs of inbreeding outweigh

benefits in the long term (Agnarsson et al., 2006; Agnarsson et al., 2013a). We dated the origin of *D. socialis* more recently than the subsocial thomisids, agreeing with other observations that social species are relatively young compared to subsocial species (Agnarsson et al., 2013a). Our data describe a possible age-range, rather than a definite age. Given the higher ages of the subsocial clades, our data support the hypothesis that subsociality is evolutionarily stable in comparison to sociality, as found for other spiders (Agnarsson et al., 2006; Agnarsson and Rayor, 2013).

Like other permanently social spiders, *D. socialis* is inbred as females mate within their natal nest and nests have a female-biased sex ratio (Main, 1988; Rowell and Main, 1992). Spiderlings of two subsocial species (*D. ergandros, X. bimaculatus*) only disperse shortly before mating, indicating that they may be transitory species between subsocial and social. In *D. ergandros*, spiders show a highly structured subdivision of populations and spiders are locally inbred (Evans and Goodisman, 2002). This further supports the idea that *D. ergandros* might be at a transitory stage in between subsociality and permanent sociality. However, other than in permanently social spiders, local inbreeding does not result in a female-biased sex-ratio in *D. ergandros* (Evans, 1995) and our data show a genetic distinction between the two main *D.* ergandros sampling locations, indicating persistence and relative isolation of the two corresponding populations long enough to allow for diversification. In *D. socialis,* with its rather limited distribution (Evans, 1997) and low age, we failed to detect such a diversification between sampling areas. Although we have limited evidence, this finding is consistent with the hypothesis that permanently social spider lineages fail to diversify due to high extinction rates (Agnarsson et al., 2013a).

Our results support the *Thomisus* clade as proposed by Benjamin et al. (2008). However, we found the genera *Cymbacha*, *Diaea*, *Tharpyna* and *Xysticus* reciprocally paraphyletic. Missing data and long branches seem to affect the analyses and suggest that a denser taxon sampling and additional loci are required to solve the deeper level phylogeny of Thomisidae. These findings moreover confirm previous suggestions that the Australian Thomisidae are in need of taxonomic revision (Benjamin et al., 2008; Lethinen, 2002; Szymkowiak, 2007; Szymkowiak and Dymek, 2012).

Although our results might provide a valuable starting point, this task is ultimately beyond the scope of this article. During the process of species identification we encountered various problems: a huge proportion of species are likely to be undescribed yet (marked as "aff." when similar to any particular described species; or "sp."); Australian thomisids are often difficult to identify because of insufficient quality of the antique descriptions (usually from the late 19th century) (Lethinen, 2002; Szymkowiak, 2007). Poor taxonomic descriptions resulted in misclassifications in the genus *Misumenops* as well (Garb and Gillespie, 2006). In a molecular-phylogenetic study, *M. rapaensis* from the Austral Islands was found to be more closely related with *Diaea* and not with other *Misumenops* (Garb and Gillespie, 2006). The polyphyletic status of the genus *Diaea* worldwide, with two endemic Australian clades further emphasizes the need of a systematic revision of Thomisidae.

Conclusions

We provide the first molecular phylogeny of group-living Australian Thomisidae. Subsociality has evolved at least two and potentially three times independently in thomisids. Resolving crab spider phylogeny is a challenging task and in this study we were only able to resolve a limited number of nodes at the generic level. Our study nevertheless offers progress in our understanding of Australian thomisid classification, and hence the origins of group-living, including novel molecular data for previously unstudied species. The likely polyphyly of *Diaea* and the implied multiple origins of sociality highlight an urgent need for further taxonomic and phylogenetic work on this family.

Note:

The following name changes have been proposed during the typesetting of the manuscript (Szymkowiak, 2014): *Diaea ergandros, D. inornata,* and *D. socialis* have become *Australomisidia ergandros, A. inornata,* and *A. socialis*. *Diaea evanida* and *D. variabilis* are now *Lehtinelagia evanida* and *L. variabilis.* The newly erected genera correspond to the two distinct Australian *Diaea* presented in the molecular phylogeny.

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Table 1: DNA was extracted from 26 species from New South Wales (NSW), Queensland (QL), Western Australia (WA) and Tasmania.

Species	Origin	Sex	Found in/ on	COI	16S	H3	ITS2
Cymbacha aff. *ocellata* 73	Sydney, NSW	female	leaf	KP056140	KP055878	KP056048	KP055960
Cymbacha aff. *ocellata* 71	Sydney, NSW	female	leaf	KP056138	KP055876	KP056046	KP055958
Cymbacha aff. *ocellata* 72	Sydney, NSW	female	leaf	KP056139	KP055877	KP056047	KP055959
Cymbacha ocellata 26	Murrumbateman, NSW	female	leaf	KP056095	KP055839	KP056003	KP055923
Cymbacha ocellata 27	Murrumbateman, NSW	female	leaf	KP056096	KP055840	KP056004	KP055924
Cymbacha ocellata 28	Murrumbateman, NSW	female	leaf	KP056097	KP055841	KP056005	KP055925
Cymbacha ocellata 97	Boorowa Park, NSW	female	leaf		KP055898	KP056069	KP055978
Cymbacha similis 24	Yass, NSW	female	leaf	KP056093	KP055837	KP056001	
Cymbacha similis 25	Yass, NSW	female	leaf	KP056094	KP055838	KP056002	KP055922
Cymbacha sp 29	Albany, WA	female	leaf	KP056098		KP056006	KP055926
Cymbacha sp 30	Albany, WA	female	leaf	KP056099		KP056007	KP055927
Cymbacha sp 31	Albany, WA	female	leaf	KP056100		KP056008	KP055928
Diaea aff. *variabilis* 38	Brisbane, QL	female	flower	KP056107	KP055848	KP056015	KP055934
Diaea aff. *variabilis* 40	Maclean, NSW	female	flower	KP056109	KP055850	KP056017	KP055936
Diaea aff. *variabilis* 37	Brisbane, QL	female	flower	KP056106	KP055847	KP056014	KP055933
Diaea aff. *variabilis* 67	Mona Vale Road, NSW	female	flower	KP056135	KP055874	KP056043	KP055955
Diaea ergandros 100	Yass, NSW	female	leaf-nest	KP056163	KP055901	KP056072	KP055979
Diaea ergandros 23	Yass, NSW	NA	leaf-nest	KP056092	KP055836	KP056000	KP055921
Diaea ergandros 41	Yass, NSW	NA	leaf-nest	KP056110	KP055851	KP056018	
Diaea ergandros 42	Yass, NSW	NA	leaf-nest	KP056111	KP055852	KP056019	
Diaea ergandros 43	Yass, NSW	female	leaf-nest	KP056112	KP055853	KP056020	
Diaea ergandros 46	Bald Nob, NSW	male	leaf-nest	KP056115	KP055856	KP056023	KP055938
Diaea ergandros 47	Bald Nob, NSW	female	leaf-nest	KP056116	KP055857	KP056024	KP055939
Diaea ergandros 48	Bald Nob, NSW	female	leaf-nest	KP056117	KP055858	KP056025	KP055940
Diaea ergandros 49	Bald Nob, NSW	male	leaf-nest	KP056118	KP055859	KP056026	KP055941
Diaea ergandros 74	Bald Nob, NSW	NA	leaf-nest	KP056141	KP055879	KP056049	KP055961
Diaea ergandros 75	Bald Nob, NSW	NA	leaf-nest	KP056142	KP055880	KP056050	KP055962
Diaea ergandros 83	Yass, NWS	NA	leaf-nest	KP056150	KP055888	KP056058	KP055970
Diaea ergandros 84	Yass, NWS	NA	leaf-nest	KP056151	KP055889	KP056059	KP055971
Diaea ergandros 85	Yass, NWS	NA	leaf-nest	KP056152	KP055890	KP056060	
Diaea ergandros 90	Yass, NWS	NA	leaf-nest	KP056156	KP055894	KP056064	KP055974
Diaea evanida 16	Brisbane, QL	female	flower	KP056086	KP055830	KP055994	KP055915
Diaea evanida 18	Brooms Head, NSW	female	flower	KP056088	KP055832	KP055996	KP055917
Diaea evanida 20	Sydney, NSW	female	flower	KP056090	KP055834	KP055998	KP055919
Diaea evanida 21	Brooms Head, NSW	male	flower	KP056091	KP055835	KP055999	KP055920
Diaea evanida 36	Sydney, NSW	female	flower	KP056105	KP055846	KP056013	KP055932
Diaea evanida 92	Sydney, NSW	male	flower	KP056158	KP055895	KP056066	KP055975
Diaea evanida 94	Sydney, NSW	male	flower	KP056159	KP055896	KP056067	KP055976
Diaea evanida 96	Sydney, NSW	female	flower	KP056160	KP055897	KP056068	KP055977
Diaea inornata 14	Tasmania	female	leaf-nest	KP056084	KP055828	KP055992	KP055913
Diaea socialis 44	Albany, WA	female	leaf-nest	KP056113	KP055854	KP056021	KP055937
Diaea socialis 76	Albany, WA	male	leaf-nest	KP056143	KP055881	KP056051	KP055963
Diaea socialis 77	Albany, WA	male	leaf-nest	KP056144	KP055882	KP056052	KP055964
Diaea socialis 78	Albany, WA	female	leaf-nest	KP056145	KP055883	KP056053	KP055965
Diaea socialis 79	Albany, WA	female	leaf-nest	KP056146	KP055884	KP056054	KP055966
Diaea socialis 80	Mt. Barker, WA	male	leaf-nest	KP056147	KP055885	KP056055	KP055967
Diaea socialis 81	Mt. Barker, WA	female	leaf-nest	KP056148	KP055886	KP056056	KP055968
Diaea socialis 82	Albany, WA	male	leaf-nest	KP056149	KP055887	KP056057	KP055969
Diaea sp. *ID*35	Brisbane, QL	female	flower	KP056104	KP055845	KP056012	KP055931
Diaea sp2 88	Thredbo, NSW	female	flower	KP056154	KP055892	KP056062	KP055973

Species	Origin	Sex	Found in/ on	COI	16S	H3	ITS2
Diaea sp3 87	Thredbo, NSW	female	flower	KP056153	KP055891	KP056061	KP055972
Diaea sp3 89	Thredbo, NSW	female	flower	KP056155	KP055893	KP056063	
Diaea variabilis 15	Brisbane, QL	female	flower	KP056085	KP055829	KP055993	KP055914
Diaea variabilis 17	Brisbane, QL	female	flower	KP056087	KP055831	KP055995	KP055916
Diaea variabilis 19	Brisbane, QL	female	flower	KP056089	KP055833	KP055997	KP055918
Diaea variabilis 34	Brisbane, QL	female	flower	KP056103	KP055844	KP056011	KP055930
Diaea variabilis 39	Brisbane, QL	female	flower	KP056108	KP055849	KP056016	KP055935
Misumenops sp1 5*0*	Brisbane, QL	female	flower	KP056119		KP056027	
Misumenops sp1 66	Brisbane, QL	female	flower	KP056134		KP056042	
Misumenops sp2 91	Sydney, NSW	female	flower	KP056157		KP056065	
Sidymella sp1 45	Sydney, NSW	female	leaf	KP056114	KP055855	KP056022	
Sidymella sp1 69	Sydney, NSW	female	flower	KP056136	KP055875	KP056044	KP055956
Sidymella sp1 98	Sydney, NSW	female	flower	KP056161	KP055899	KP056070	
Sidymella sp1 99	Sydney, NSW	female	flower	KP056162	KP055900	KP056071	
Sidymella sp2 64	Sydney, NSW	female	NA	KP056133	KP055873	KP056041	KP055954
Sidymella sp3 58	Albany, WA	female	NA	KP056127	KP055867	KP056035	KP055948
Stephanopis cf .scabra 70	Hervey Bay, QL	female	bark	KP056137		KP056045	KP055957
Tharpyna aff. *diademata* 1	Tarramurra, NSW	male	bark	KP056073	KP055819	KP055980	
Tharpyna aff. *diademata* 59	Tarramurra, NSW	male	bark	KP056128	KP055868	KP056036	KP055949
Tharpyna aff. *diademata* 6*0*	Tarramurra, NSW	NA	bark	KP056129	KP055869	KP056037	KP055950
Tharpyna aff. *diademata* 61	Tarramurra, NSW	female	bark	KP056130	KP055870	KP056038	KP055951
Tharpyna aff. *diademata* 62	Tarramurra, NSW	female	bark	KP056131	KP055871	KP056039	KP055952
Tharpyna aff. *diademata* 63	Tarramurra, NSW	male	bark	KP056132	KP055872	KP056040	KP055953
Thomisidae gen sp 32	Brooms Head, NSW	female	flower	KP056101	KP055842	KP056009	
Thomisidae gen sp 33	Brooms Head, NSW	male	flower	KP056102	KP055843	KP056010	KP055929
Thomisus spectabilis 51	Brisbane, QL	female	flower	KP056120	KP055860	KP056028	KP055942
Thomisus spectabilis 52	Brisbane, QL	female	flower	KP056121	KP055861	KP056029	KP055943
Thomisus spectabilis 53	Brisbane, QL	female	flower	KP056122	KP055862	KP056030	KP055944
Thomisus spectabilis 54	Brisbane, QL	female	flower	KP056123	KP055863	KP056031	KP055945
Thomisus spectabilis 55	Brisbane, QL	male	flower	KP056124	KP055864	KP056032	KP055946
Xysticus bimaculatus 11	Brisbane, QL	male	leaf-nest	KP056081		KP055989	KP055910
Xysticus bimaculatus 12	Brisbane, QL	male	flower	KP056082	KP055826	KP055990	KP055911
Xysticus bimaculatus 13	Brisbane, QL	female	leaf-nest	KP056083	KP055827	KP055991	KP055912
Xysticus bimaculatus 3	Brisbane, QL	female	leaf-nest	KP056074		KP055982	KP055903
Xysticus bimaculatus 4	Brisbane, QL	female	leaf-nest	KP056075		KP055983	KP055904
Xysticus bimaculatus 5	Brisbane, QL	female	leaf-nest	KP056076	KP055821	KP055984	KP055905
Xysticus bimaculatus 6	Brisbane, QL	male	leaf-nest	KP056077	KP055822	KP055985	KP055906
Xysticus bimaculatus 7	Brisbane, QL	female	leaf-nest	KP056078	KP055823	KP055986	KP055907
Xysticus bimaculatus 8	Brisbane, QL	female	leaf-nest	KP056079	KP055824	KP055987	KP055908
Xysticus bimaculatus 9	Brisbane, QL	male	nest	KP056080	KP055825	KP055988	KP055909
Zygometis aff. *lactea* 2	Airlie Beach, QL	female	flower		KP055820	KP055981	KP055902
Zygometis sp 56	Airlie Beach, QL	male	NA	KP056125	KP055865	KP056033	KP055947
Zygometis sp 57	Airlie Beach, QL	female	flower	KP056126	KP055866	KP056034	

Table 2: Genbank accession numbers for the species added from GenBank

Species	COI	H3	citation
Anyphaena californica	DQ628605	DQ628633	Spagna & Gillespie 2008
Pagiopalus nigriventris	EU168155	EU157106	Benjamin et al. 2008
Onomastus pethiyagodai	EU168160	EU157109	Benjamin et al. 2008
Amyciaea forticeps		EU157135	Benjamin et al. 2008
Aphantochilus sp. SPB-2007		EU157140	Benjamin et al. 2008
Bobaropactus sp. SPB-2007	EU168187		Benjamin et al. 2008
Boliscus sp. SPB-2007		EU157128	Benjamin et al. 2008
Borboropactus cinerascens		EU157126	Benjamin et al. 2008
Camaricus sp. SPB-2007		EU157125	Benjamin et al. 2008
Cebrenninius rugosus	EU168175	EU157134	Benjamin et al. 2008
Coriarachne utahensis	GU682878		iBOL
Coriarachne versicolor		EU157136	Benjamin et al. 2008
Cyriogonus sp. SPB-2007	EU168168	EU157118	Benjamin et al. 2008
Diaea praetexta	DQ174402		Garb & Gillespie 2006
Diaea sp. JEG-089	DQ174398		Garb & Gillespie 2006
Diaea sp. JEG-692	DQ174399		Garb & Gillespie 2006
Diaea sp. JEG-696	DQ174430		Garb & Gillespie 2006
Diaea sp. JEG-697	DQ174429		Garb & Gillespie 2006
Diaea sp. JEG-699	DQ174428		Garb & Gillespie 2006
Diaea sp. SPB-2007	EU168169	EU157119	Benjamin et al. 2008
Diaea subdola	EU168174	EU157124	Benjamin et al. 2008
Ebelingia kumadai	JN817241		Jang & Hwang
Ebrechtella tricuspidata	JN817240		Jang & Hwang
Epidius parvati	EU168163	EU157114	Benjamin et al. 2008
Haplotmarus sp.	EU168173	EU157123	Benjamin et al. 2008
Lysiteles coronatus	JN817245		Jang & Hwang
Lysiteles sp. SPB-2007	EU168184		Thomisidae
Mecaphesa asperata	HQ979279		iBOL
Mecaphesa naevigera	DQ174387		Garb & Gillespie 2006
Mecaphesa sierrensis	HQ979227		iBOL
Mecaphesa sp. East Maui	FJ590816		Garb & Gillespie 2006
Mecaphesa sp. West Maui	FJ590788		Garb & Gillespie 2006
Misumena vatia	JN817244		Jang & Hwang
Misumenoides formosipes	DQ174396		Garb & Gillespie 2006
Misumenops anguliventris	DQ174376		Garb & Gillespie 2006
Misumenops aridus	DQ174385		Garb & Gillespie 2006
Misumenops cavatus	DQ174377		Garb & Gillespie 2006
Misumenops celer	DQ174393		Garb & Gillespie 2006
Misumenops dalmasi	DQ174370		Garb & Gillespie 2006
Misumenops devius	DQ174395		Garb & Gillespie 2006
Misumenops discretus	DQ174375		Garb & Gillespie 2006
Misumenops editus	DQ174378		Garb & Gillespie 2006
Misumenops facundus	DQ174381		Garb & Gillespie 2006
Misumenops hiatus	DQ174386		Garb & Gillespie 2006
Misumenops imbricatus	DQ174380		Garb & Gillespie 2006
Misumenops importunus	DQ174392		Garb & Gillespie 2006
Misumenops insulanus	DQ174384		Garb & Gillespie 2006
Misumenops junctus	DQ174388		Garb & Gillespie 2006
Misumenops kanakanus	DQ174390		Garb & Gillespie 2006
Misumenops melloleitaio	DQ174374		Garb & Gillespie 2006
Misumenops nepenthicola	EF419094	EF419123	Su et al. 2007
Misumenops nigrofrenatus	DQ174383		Garb & Gillespie 2006

Species	COI	H3	citation
Misumenops pallidus	DQ174397		Garb & Gillespie 2006
Misumenops perkinsi	DQ174379		Garb & Gillespie 2006
Misumenops rapaensis	DQ174427		Garb & Gillespie 2006
Misumenops rothi	DQ174391		Garb & Gillespie 2006
Misumenops rufithorax	DQ174389		Garb & Gillespie 2006
Misumenops sp. JEG-461	DQ174371		Garb & Gillespie 2006
Misumenops sp. JEG-701	DQ174372		Garb & Gillespie 2006
Misumenops temihana	FJ590849		Garb & Gillespie 2009
Monases sp. SPB-2007	EU168172	EU157122	Benjamin et al. 2008
Oxytate striatipes	JN817243		Jang & Hwang
Oxytate taprobane	EU168161	EU157112	Benjamin et al. 2008
Ozyptila arctica	GU683805		iBOL
Ozyptila gertschi	GU682487		iBOL
Ozyptila praticola	HQ924470		iBOL
Phrynarachne katoi	JN817247		Jang & Hwang
Pseudoporrhopis granum	EU168170	EU157120	Benjamin et al. 2008
Runcinia acuminata	EU168166		Benjamin et al. 2008
Runcinia albostriata B		EU157130	Benjamin et al. 2008
Runcinia albostriata	EU168178	EU157116	Benjamin et al. 2008
Stephanopis sp. A SPB-2007	EU168167	EU157139	Benjamin et al. 2008
Stephanopis sp. B SPB-2007		EU157137	Benjamin et al. 2008
Stephanopis sp. C SPB-2007		EU157138	Benjamin et al. 2008
Stephanopis sp. SPB-2007	EU168185	EU157117	Benjamin et al. 2008
Stephanopis sp. SPB-2007	EU168185	EU157117	Benjamin et al. 2008
Synema globosum	JN817246		Jang & Hwang
Talaus sp. SPB-2007		EU157127	Benjamin et al. 2008
Thomisidae sp. SPB-2007		EU157133	Benjamin et al. 2008
Thomisops piger	EU168171	EU157121	Benjamin et al. 2008
Thomisus granulifrons	EU168162		Benjamin et al. 2008
Thomisus sp. A SPB-2007	EU168164	EU157115	Garb & Gillespie 2006
Thomisus sp. B SPB-2007	EU168176	EU157129	Benjamin et al. 2008
Tibellus maritimus	KC502847		Smith & Adamowicz
Tibellus oblongus	JN817239		Jang & Hwang
Tmarus angulatus	HQ924573		iBOL
Tmarus angulatus B SPB-2007		EU157111	Benjamin et al. 2008
Tmarus piger	JN817248		Jang & Hwang
Tmarus rimosus	JN817249		Jang & Hwang
Xysticus audax	JQ412462		Briscoe et al. 2013
Xysticus bicuspis	HQ924597		iBOL
Xysticus californicus	EU168181	EU157131	Benjamin et al. 2008
Xysticus canadensis	DQ127516		Barrett & Hebert
Xysticus concretus	JN817252		Jang & Hwang
Xysticus cristatus	FR775767		Virant-Doberlet et al. 2011
Xysticus deichmanni	GU684397		iBOL
Xysticus durus	GU684350		iBOL
Xysticus elegans	GU683407		iBOL
Xysticus ellipticus	GU683928		iBOL
Xysticus emertoni	GU682874		iBOL
Xysticus ephippiatus	JN817250		Jang & Hwang
Xysticus ferox	HQ979287		iBOL
Xysticus fraternus	DQ174400		Garb & Gillespie 2006
Xysticus funestus	GU682875		iBOL
Xysticus insulicola	JN817251		Jang & Hwang
Xysticus labradorensis	GU683754		iBOL

Species	COI	H3	citation
Xysticus luctans	GU682560		iBOL
Xysticus luctuosus	GU683927		iBOL
Xysticus nigromaculatus	GU683895		iBOL
Xysticus obscurus	KC502859		Smith & Adamowicz
Xysticus punctatus	GU682529		iBOL
Xysticus sicus	JN817253		Jang & Hwang
Xysticus sp. 1 WOCS-2009	FJ899833		Davey & Symondson
Xysticus sp. 2 WOCS-2009	FJ899834		Davey & Symondson
Xysticus sp. 3-GAB	HQ924402		iBOL
Xysticus sp. Akaiwa	AB564738		Sonoda et al.
Xysticus sp. MCH-2003	AY297423		Maddison & Hedin
Xysticus sp. S316		DQ665704	Maddison & Needham 2006
Xysticus sp. SPB-2007	EU168182	EU157132	Benjamin et al. 2008
Xysticus triangulosus	KC502861		Smith & Adamowicz
Xysticus triguttatus	GU683802		iBOL

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Virant-Doberlet,M., King,R.A., Polajnar,J. and Symondson,W.O. (2011) Molecular diagnostics reveal spiders that exploit prey vibrational signals used in sexual communication *Molecular Ecology* 20 (10), 2204-2216

Appendix

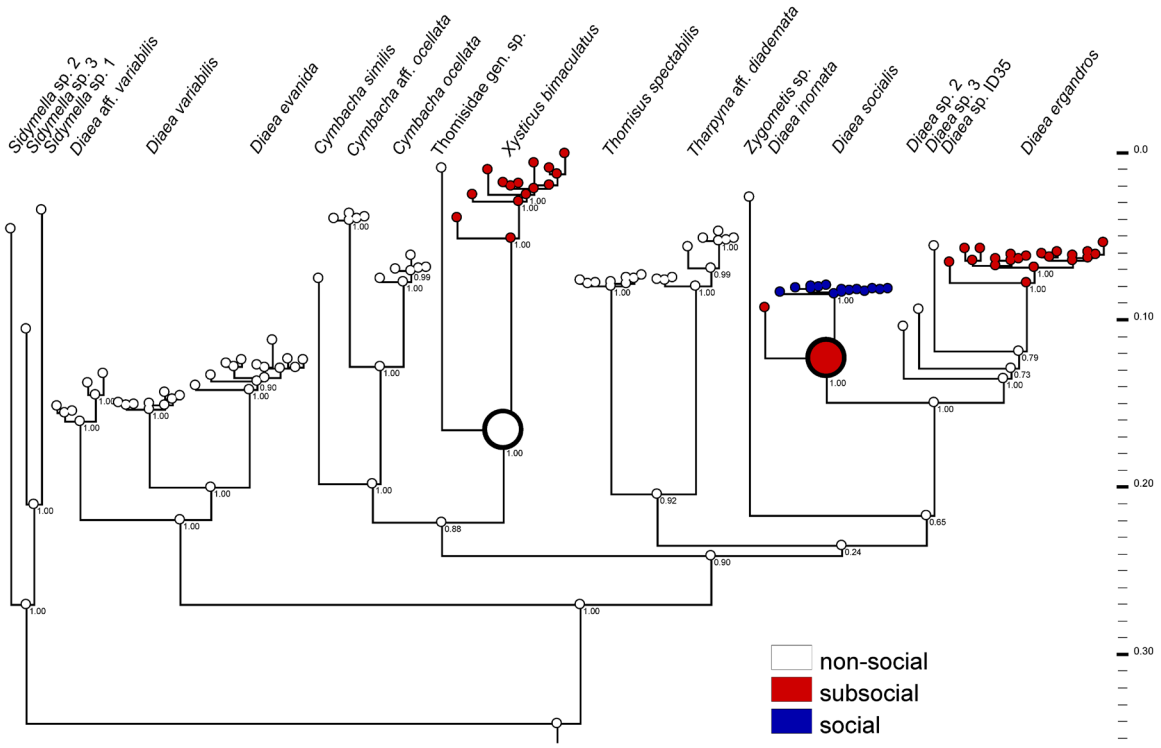

Figure 6: Parsimony ancestral states reconstruction based on the '4-gene' Bayesian analyses showing the independent origins of subsociality in Thomisidae.

Chapter 4

Mating behaviour and natural mating rates in a subsocial spider

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Unpublished manuscript

Abstract

Genetic relatedness is an important factor for the evolution of cooperation and can be secured by low mating rates (monogamy). Relatedness is further increased when females mate with a brother so it has been suggested that inbreeding may have substantial kin-selected benefits and lead to higher cooperation. Social spiders evolved from subsocial ancestors by losing their dispersal phase and are characterised by a highly inbred mating system. The subsocial crab spider *Diaea ergandros* lives in family groups and spiders cooperate in nest construction and foraging until they mature and disperse. The species seems to be transitory between subsocial and permanently social as nestmate relatedness is high and populations are locally inbred. Hence, we expect that females mate with a single male, perhaps a brother, thus increasing genetic relatedness and thereby cooperation of their offspring. Contrary to our predictions, *D. ergandros* females did not actively seek or avoid inbreeding. Although female mating rates were low in natural nests, our data do not support the existence of a monogamous mating system in this species. This suggests that mating decisions do not only depend on securing high offspring cooperation, and that multiple mating in *D. ergandros* may exist to counteract negative effects of inbreeding.

Keywords: *Diaea ergandros*, inbreeding, social spider, microsatellites, paternity

Introduction

Genetic relatedness is an important condition for cooperation to evolve and the family is considered to be the root of sociality in many taxa (Hamilton, 1964; Maynard Smith, 1964). Relatedness between sibling group members can for example be increased by low female mating rates. By mating with a single male, all of a female's offspring share on average half of their genes with their siblings, which may increase cooperation between these highly related individuals and thus the inclusive fitness of parents and offspring (Hamilton, 1964).

There is ample evidence that the transition from a solitary lifestyle to sociality is facilitated by low mating rates (monogamy hypothesis) (Boomsma, 2009; Boomsma et al., 2011; Cornwallis et al., 2010; Hughes et al., 2008). In eusocial insects and many cooperatively breeding birds, monogamy is a key to understanding the evolution of sociality (Boomsma, 2009; Cornwallis et al., 2010; Warrington et al., 2013). It has been suggested that cooperation may be further increased by inbreeding due to substantial kin-selected benefits (Kokko and Ots, 2006). Indeed, some social taxa are characterised by intra-colony mating and inbreeding, such as naked mole rats (Jarvis et al., 1994) or social spiders (Lubin and Bilde, 2007). However, inbreeding usually leads to an accumulation of deleterious recessive alleles and often negatively affects fitness (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009; Keller and Waller, 2002), yet may also purge deleterious alleles (Bilde et al., 2005; Pusey and Wolf, 1996), and may be favoured when the costs of inbreeding depression are lower than the costs of inbreeding avoidance (Waser et al., 1986).

Solitary and social spiders differ in their preference for mating partners. Solitary spiders preferentially outbreed and avoid mating with siblings. This is especially true when they have already mated once with a sibling, for example in the spider genus *Argiope* (Zimmer, personal communication). When they have mated with a sibling, they reduce the negative effects of inbreeding by multiple mating and cryptic female choice (Welke and Schneider, 2009). In contrast, social spiders have switched from outbreeding to inbreeding mating systems (Agnarsson et al., 2013; Avilés, 1997; Bilde et al., 2005; Lubin and Bilde, 2007; Riechert and Roeloffs, 1993). Highly inbred social spiders can have high fixation index (F_{ST}) values (Avilés and Purcell, 2012; Lubin and Bilde, 2007), for example F_{ST} values of up to 0.96 in the social spider *Anelosimus eximius* (Agnarsson et al., 2010), suggesting no exchange between colonies since F_{ST} values increase (maximum = 1.0) when subpopulations become fixed for different alleles. Solitary species, for example *Argiope bruennichi,* have low F_{ST} values ranging between 0.02 and 0.06 (Zimmer et al., 2014).

Social spiders evolved from subsocial (periodically social) species through the elimination of a dispersal phase (the so-called 'subsocial route') (Agnarsson et al., 2006; Avilés, 1997; Lubin and Bilde, 2007; Wickler and Seibt, 1993). Several experimental studies of subsocial spiders have shown that higher relatedness facilitates cooperation (Ruch et al., 2009b; Ruch et al., 2014a, b; Schneider and Bilde, 2008), hence the monogamy hypothesis may apply to these spiders as well. Provided that current subsocial species resemble the ancestors

of social spiders we may expect that females mate only once and produce cooperative full-sibs. In addition, they may not resist inbreeding or may even seek incestuous matings, further increasing relatedness in their brood. To date, natural mating rates of many subsocial spider species are unknown (Lubin and Bilde, 2007).

The subsocial crab spider *Diaea ergandros* appears to be at a particularly advanced transitory stage between subsocial and social because individuals only disperse as adults presumably after mating in their natal nest (Avilés, 1997; Evans, 1998a). Hence, we expect individuals of this species to ensure high genetic relatedness within the nest. Evans and Goodisman (2002) studied the genetic structure of *D. ergandros* populations using allozymes and found that nestmate relatedness was relatively high (r=0.44), and indeed *D. ergandros* young show high levels of cooperative behaviour in nest building, hunting and foraging (Evans, 1998a, 1999; Ruch et al., 2014a). Females and males mature in the same nest, suggesting that they interbreed but direct evidence is lacking. Such a mechanism would resemble mechanisms in permanently social spiders where mated females found new inbred colonies (Lubin and Bilde, 2007; Schneider et al., 2001).

However, a curious peculiarity of *D. ergandros* is that unrelated individuals are known to enter groups and stay (Evans, 1998b; Evans and Goodisman, 2002). There are estimates that up to 45 % of nests may contain strangers (Evans, 1998a) and if they also mate within this group, there would be less inbreeding, resulting in less cooperation and potentially outbreeding depression (Lynch, 1991; Pusey and Wolf, 1996). Experiments have shown that *D. ergandros* spiders are able to recognize kin (Evans, 1998b, 1999). Whether kin-recognition is used to avoid or promote incestuous mating is unknown as detailed descriptions of the mating behaviour and potential differences between sibling or non-sibling matings are lacking. Considering that *D. ergandros* is a transitory species between subsocial and permanently social we predict that females should be more reluctant to mate with a stranger than with a brother.

Although the majority of offspring are sired by a single mother and father in *D. ergandros*, some natural nests were reported to contain individuals that could have been produced by multiply mating (polyandrous) females (Evans and Goodisman, 2002). Multiple mating reduces offspring relatedness and would further reduce offspring cooperation. In Evans and Goodisman's (2002) study marker variability was low, which may have led to an underestimation of female mating rates. We developed microsatellite markers to reinvestigate the natural mating rates of the species and to test whether these are consistent with predictions from the monogamy hypothesis. For the latter we collected females with egg sacs or newly hatched offspring and analysed the paternity rates with two polymorphic microsatellite markers.

Methods

Male courtship and mating behaviour

The spiders were collected at eleven locations from their natural habitat around Yass (34°55'20.50"S, 149°6'15.53"E) and Boorowa (34°25'53.31"S, 148°43'49.47"E), NSW (Australia) and transferred to Macquarie University, Sydney

between April and June 2011 (Austral autumn). Nests were dissected in the laboratory; afterwards we provided nesting material (*Eucalyptus* leaves) and food (*Bactocera tryoni* and *Drosophila* sp.), and raised the spiderlings in their family groups in plastic containers (500 ml). Before maturation, in August (the Austral Spring), all spiders were separated and kept individually in plastic containers (100 ml) to avoid mating prior to the actual observations. They were kept under semi-natural light conditions and the temperature ranged between 20° - 28° C. The spiders were fed with *Bactocera tryoni* and *Drosophila* sp. twice a week and were provided with water every second day. Upon maturation, between September and February, they were weighed to the nearest 0.1 mg by using an electronic balance (Mettler Toledo New Classic MS) and measured with callipers. Males matured slightly earlier than females, with first adult males being found in late August and first adult females in early September. However, most males and females reached maturity simultaneously around November and December (Austral Summer) in the laboratory.

We qualified and quantified the mating behaviour of male and female first copulations (N = 36 pairs). For the mating trials, we transferred the female with the help of a paintbrush into a small, transparent plastic vial (5 cm diameter). After one minute, the male was transferred into the vial as well. We noted the time until male and female had first contact (touching the legs), the time until the male jumped onto the females' opisthosoma, time of courtship and total copulation duration. We documented any reluctant or aggressive behaviour by females, such as i) moving away from the male, ii) spreading the forelegs when the male approaches or iii) bucking when the male is on top of the females' opisthosoma. Moreover, we noted whether the male or the female terminated the copulation. We observed male and female behaviour for another five minutes after the copulation was terminated.

Precopulatory mate choice

Spiders were collected within their natural nests from six different locations around Yass in 2010 and sent to the laboratory at the University of Hamburg, Germany. Nests were dissected and spiders were housed individually in 250 ml plastic containers until maturation around October to December. They were kept under semi-natural light conditions (12 h light, 12 h dark) and the temperature ranged between 23° - 25° C. The spiders were fed with *Calliphora* sp. and *Drosophila* sp. twice a week and were provided with water every second day.

We randomly assigned inbred mating couples, where a virgin female was mated with a virgin male from the same nest (N = 10 pairs). In the outbreeding treatment, virgin females were mated once to an unrelated virgin male from a foreign nest collected at a different location (N = 22 pairs). Females and males were transferred into the mating arena (transparent plastic vials) as described above. We noted whether females showed any form of aggressive or reluctant behaviour when being confronted with either a related or unrelated male. Aggressive or reluctant behaviour included i) moving away from the male, ii) spreading the forelegs when the male approaches or iii) biting the male. We also recorded the copulation duration of all trials.

Natural mating rates in the field

Microsatellite marker isolation and primer design

We extracted genomic DNA from legs of spiders using the 5 PRIME ArchivePure DNA Kit (5 PRIME, Hamburg, Germany) according to the manufacturer's protocol in 2012. Pooled DNA of one male and female specimen was then sequenced on one lane of a 454 sequencer. Library preparation and sequencing were conducted according to the manufacturer's protocols (454 Life Sciences, Branford, USA). The resulting 454 reads were filtered for tandem repeats using Tandem Repeats Finder (Benson, 1999) and resulted in 834 potential repeats. Primers were then designed for 50 microsatellite loci using the Primer3 plus software (Untergasser et al., 2007). PCRs were run using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and at an annealing temperature of 58°C. Twelve of the 50 microsatellite loci could be successfully amplified.

Fragment-length analysis was carried out on an Applied Biosystems 3730 DNA Analyzer and microsatellite alleles were called using the software GeneMapper 4.0 (Applied Biosystems). We detected an unexpectedly high rate of problematic microsatellite loci for the species. Most of the tested loci were either completely monomorphic or showed high noise during genotyping (e.g. multiple allele calls) and

Table 1: Summary of primers for *Diaea ergandros* (ten monomorphic and two polymorphic).

Primer name	Forward Primer 3'-5' Reverse Primer 3'-5'	Product Size (bp)	Repeat type	Number of alleles	Colour tag
Derg07	TTGCTAATGGGGGCACAC	225-276	ATT	9	FAM
	TTGGACAACACATATTTCAGGA				
Derg09	GGCAAATTTTGGTCATCACG	141	CAA	1	FAM
	GATGAAAGCAAGGATAGTTTCTCA				
Derg11	TCCTGGACCACTCTTTCGTT	326	AATA	1	FAM
	TTGAAAGGCTTATGTTGCACT				
Derg13	TTGAATGCAAAATGTAGCCAAT	351-373	TAA	6	FAM
	TTGCCTTTAACCTCGATTGC				
Derg22	TCATCAACAACAACAACAACCA	247	GAA	1	FAM
	GTCGTCCTCGTCCTCGTAGA				
Derg37	TTTTCTGTTCGCGCAATGC	272	AAT	1	FAM
	AGGGACATTCAAATGCCTGTTG				
Derg39	TGGACAGACGAGGCACTAAG	296	AAAG	1	FAM
	ACAGTCGACTCTCGTTAATTCG				
Derg42	CCCCAGTAACTGACACATTAAGG	252	AAT	1	HEX
	ACACTGGTTCACTTTGTGTCAG				
Derg45	GGTGCAACGTTGTAGAAGCG	246	ATC	1	FAM
	ACCGACTCTAGAACACGCAAC				
Derg47	GGCAACGTTGTAGAAGCGAC	245	ATC	1	HEX
	ACCGACTCTAGAACACGCAAC				
Derg49	ACACAATAACTCAAACTGGAAACG	138	AAAT	1	HEX
	AGGTGCTCCATAGATCTGCC				
Derg53	ACTTGGACAAAACTTTCAAAAGCC	193	AT	1	FAM
	CCAGACGTGAAGCAATGCAG				

could not be properly genotyped. Thus, out of 12 tested loci, only two could be used for further analyses (Table 1). We genotyped 37 individuals (only subadult males and females collected in 2012 or adult females collected in 2013) from four collection sites (at least 10 km distance between sites). Expected (He) and observed heterozygosity (Ho) as well as inbreeding coefficient (F_{IS}) values were calculated using Microsatellite Analyser (MSA) 4.05 (Dieringer and Schlötterer, 2003). Pairwise F_{ST} values were calculated using Arlequin version 3.5.1.3. F_{ST} is the fixation index and high F_{ST} values (maximum 1.0) suggest no exchange between colonies when subpopulations become fixed for different alleles. F_{IS} is the inbreeding coefficient and high values imply high levels of inbreeding, meaning an excess of homozygotes.

Using genepop on the web (Raymond and Rousset, 1995; Rousset, 2008), we tested the loci for Hardy-Weinberg equilibrium (Table 2).

Paternity tests

We collected spider nests from six locations in southern New South Wales (Yass, 34°55'20.50"S, 149°6'15.53"E, each site at least 10 km apart from another) and one location in northern New South Wales (Bald Nob, 29°37'44.26"S, 151°58'37.00"E) in January and February 2013, at which time mothers with newly hatched spiderlings are most common. We extracted DNA from 23 adult females (i.e. putative mothers) and 11-15 spiderlings (i.e. putative offspring) from each of 23 nests (total spider $N = 334$), and amplified the DNA with the primers of the two polymorphic loci as described above. Paternity was analysed with the software GERUD 2.0 (Jones, 2005) for 21 families, which is a relatively conservative method of estimating paternity in spiders (Tuni et al., 2012).

Data Analyses

Statistical analyses were carried out with R version 2.15.3 (R Development Core Team 2013) and JMP 9.0 (SAS Institute Inc., USA). Continuous data were tested for normal distribution (Shapiro-Wilk-Test) and for equal variances. All statistical tests are 2-tailed ($\alpha = 0.05$). Descriptive statistics are given as mean ± standard error (SE).

Results

Male courtship and mating behaviour

After being placed into the vial, most males started searching for females. First contact (= males touched the female with his forelegs) occurred after 5:33 ± 1:07 minutes (range 0:01 - 29:20 min). After the first contact, the male started to court. The following sequence of behaviours was observed most commonly. First, the male jumped onto the opisthosoma of the female and tapped with his forelegs on the female (Figure 1). Second, the male started to lay down silk threads over the prosoma of the female (Figure 1), which is similar to some *Xysticus* spiders (Foelix, 2011). Third, the male moved over and under the female's opisthosoma to access her genital opening. Fourth, copulation occurred; the male inserted both palps consecutively to deposit sperm. During courtship, 16 females were passive (= not moving at all), while the other 20 kept moving for 0:51 ± 0:19 min in the

Table 2: Expected and observed heterozygosity (He, Ho) and Hardy-Weinberg equilibrium (HWE) and Fis for both polymorphic loci. Spiders were collected at three collection sites north and one collection site south (Kiers Rd) of the town Yass.

Primer		Collection Site			
		Alpaca Farm	Kiers Road	Normanhurst	T6 (Lachlan VW)
Derg07	Number of individuals	12	5	12	8
	Number of alleles	6	4	6	7
	Ho	0.75	0.2	0.75	0.5
	He	0.81	0.73	0.78	0.8
	P-value HWE	0.08	0.009*	0.24	0.013*
	Fis (Pop)	0.05	0.72	0.01	0.36
Derg13					
	Allele Number	6	4	6	4
	Ho	0.91	0.4	0.75	0.25
	He	0.8	0.71	0.82	0.74
	P-value HWE	0.24	0.15	0.32	0.009*
	Fis (Pop)	-0.17	0.42	0.07	0.66

vial while the male was courting. Six females showed a resistant behaviour during courtship, such as bucking and trying to get rid of the male while the male was courting. None of the females showed aggressive behaviour, such as spreading the legs or biting.

The courtship duration ranged between 1:06 and 43:40 minutes (6:30 ± 1:30 min). Courtship duration was positively correlated with the duration until females stopped moving (pairwise correlation: $r^2 = 0.71$, $P < 0.0001$). For the copulation, the male moved either to the left or right side and inserted the palp ($N = 12$ left, $N = 24$ right). After a short courtship sequence, he copulated into the remaining side. The total copulation duration ranged between 4:00 and 66:58 minutes (24:22 ± 2:30 min). In 30 cases, the male terminated the copulation and moved away, the female started removing the silk after 2:22 ± 0:34 minutes. Six females actively terminated the copulation, however only one of them showed a resistant behaviour prior to copulation, while the others were passive prior to copulation.

Precopulatory mate choice

We did not observe any aggressive female behaviour during the mating trials with either related or unrelated males. Three females showed resistant mating behaviour as described above during courtship, two in the outbred and one in the inbred treatment. The copulation duration did not differ between inbred (21:00 ± 2:00 min) and outbred (18:20 ± 1:50 min) mating couples ($t = -1.08$, $P = 0.29$, $N = 32$). Only few of the females successfully produced egg sacs and none of the egg sacs hatched so that we could not compare possible differences in offspring quantity, quality and offspring cooperation due to the mating treatment.

Microsatellite markers

We found deviations from Hardy-Weinberg equilibrium (HWE) in two populations in at least one locus (Table 2). In all cases we recorded a heterozygote deficit. Pairwise F_{ST} values were generally low and did not significantly differ

Table 3: Pair-wise genetic statistics (Fst, bottom left) and *P*-values (top right) for four collection sites of *Diaea ergandros*. Populations were not significantly differentiated (indicated by *P*-values >0.05).

	Alpaca Farm	Kiers Road	Normanhurst	T6 (Lachlan VW)
Alpaca Farm		*P* = 0.3	*P* = 0.06	*P* = 0.11
Kiers Road	0.023		*P* = 0.12	*P* = 0.44
Normanhurst	0.025	0.065		*P* = 0.59
T6 (Lachlan VW)	0.042	0.05	0.008	

between the collection sites, meaning that there is some exchange between these populations.

Natural mating rates in the field

We collected 23 females with their newly hatched offspring to determine the natural mating rates. In one nest, we did not find an adult female, but instead a subadult female that was not related to the offspring and we excluded this nest from the paternity analysis. Three nest contained spiderlings that were larger than the rest and were considered to be 'intruders'. These individuals were excluded from the paternity analysis. Ten of the 21 analysed broods were sired by a single father, while 11 broods required a minimum of two fathers to explain the offspring's genotype (Table 4).

Table 4: Summary of the GERUD 2.0 results for paternity estimates obtained from the analyses of two polymorphic microsatellite markers for broods from seven different collection sites. For each family (nest), the minimum number of fathers and the number of father solutions (testing pairwise combinations of potential paternal genotypes if the number of fathers is > 1) for the analysed progeny are shown.

Nest ID (# offspring)	Min # fathers	Father solutions	Collection site	Total # spiderlings/nest
DE292 (14)	2	1	Alpaca Farm	21
DE294 (14)	1	1	Alpaca Farm	43
DE326 (14)	1	1	Alpaca Farm	15
DE328 (14)	1	1	Alpaca Farm	22
DE329 (14)	2	2	Alpaca Farm	36
DE331 (14)	2	14	Alpaca Farm	25
DE332 (14)	2	16	Alpaca Farm	18
DM24 (14)	1	1	Bald Nob	20
DM33 (10 + 3 intruder)	1	1	Bald Nob	25
DE316 (11)	2	8	Kiers Road	11
DE317 (14)	2	10	Kiers Road	25
DE318 (14)	1	1	Kiers Road	47
DE319 (14)	1	1	Kiers Road	22
DE322 (15)	2	10	Kiers Road	17
DE296 (14)	2	8	Normanhurst	14
DE298 (13 + 1 intruder)	1	1	Normanhurst	33
DE301 (14)	1	1	Normanhurst	37
DE304 (10 + 3 intruder)	2	6	T6	32
DE308 (14)	2	9	T6	22
DE315 (14)	1	1	Yass River Road	35
DE313 (14)	2	18	Yass Valley Way	29

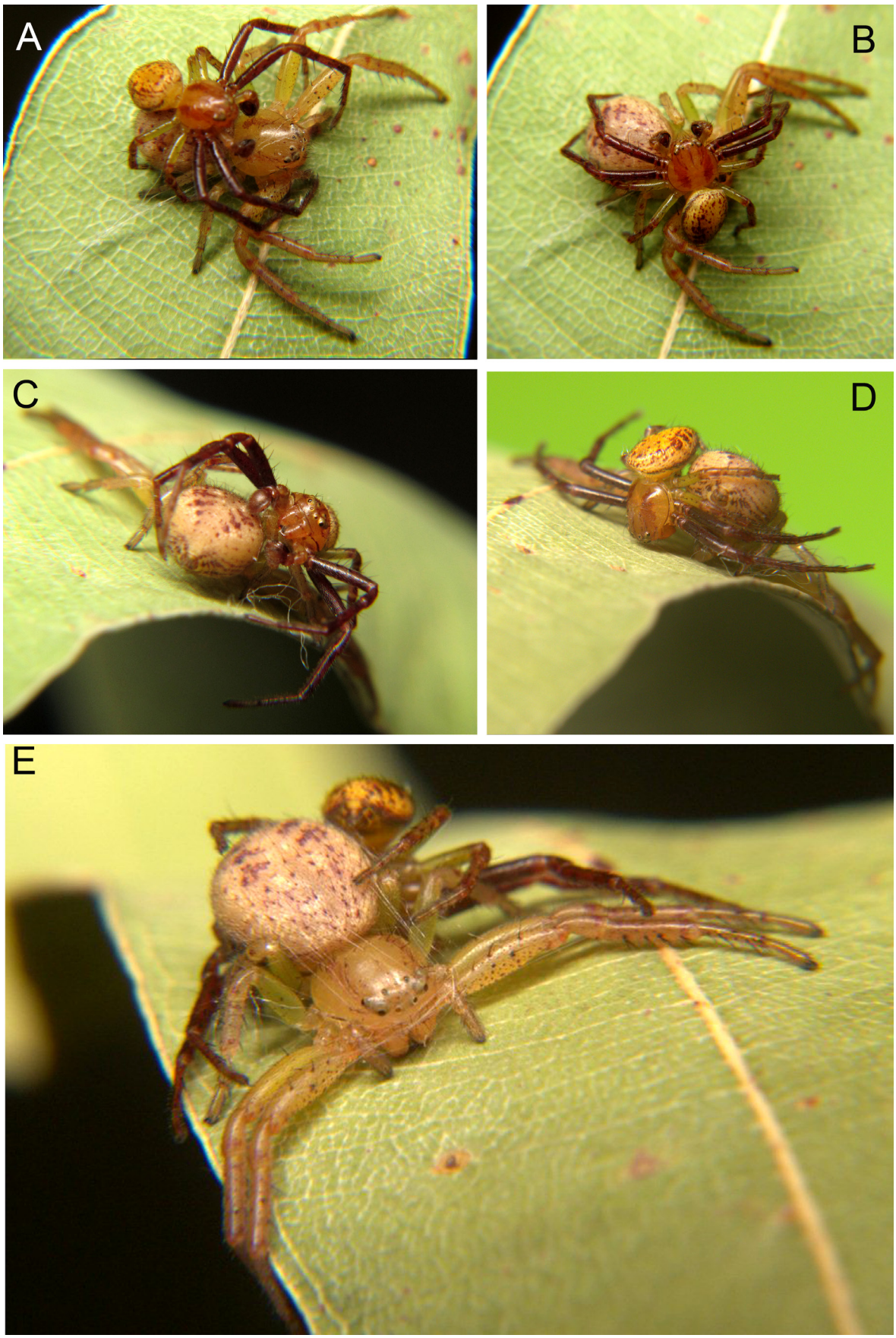

Figure 1: Courtship and mating position in *Diaea ergandros.* A-C: male courtship during which the male covers the female prosoma with silk. D: male assuming the mating position. E: female covered with silk during mating. Photos: Adam Wilkins

Discussion

Natural mating rates and mate choice

Our data do not support the hypothesis that *Diaea ergandros* females actively secure high relatedness among their offspring. Firstly, most females accepted males without an obvious process of selection. Second, the mating trials did not provide evidence for female choice of related over unrelated males. Third, the genetic analyses found monogamy only half the time. Nevertheless, the natural mating rates in *D. ergandros* were relatively low, with all non-monogamous families sired mostly by just one additional father. These low female mating rates are partly consistent with Evans and Goodisman's (2002) findings, although the power of detecting polyandrous females was higher in our study. Multiple mating but rather low mating rates under natural conditions are common in subsocial spiders of the genus *Stegodyphus*, which results in mixed paternities (Maklakov et al., 2005; Ruch et al., 2009a; Schneider, 1997; Tuni and Berger-Tal, 2012; Tuni et al., 2012).

The finding that half of the studied *D. ergandros* broods were sired by more than one father raises the question why females mate multiply. In *D. ergandros*, offspring cooperation has fitness consequences. For example, nest construction activity is higher and thus mortality lower in juvenile sibling groups compared to groups consisting of unrelated spiders (Evans, 1999). A possible explanation may be that multiple mating is not a result of female choice. Both, female and male *D. ergandros* are capable of multiple mating (females may accept up to four males, personal observation, Evans & Ruch) and females do not become more resistant with increasing mating trials in the laboratory (personal observation, Ruch). In *Stegodyphus lineatus*, polyandry does not seem to be a result of female choice either (Tuni et al., 2012). However, these subsocial spiders behave differently from *D. ergandros*; female *S. lineatus* become very aggressive after a first copulation and multiple mating seems to be a result of male manipulation rather than female choice (Maklakov and Lubin, 2004; Tuni et al., 2012). Multiple mating is costly for female *S. lineatus* since males may kill offspring of a first clutch (Schneider and Lubin, 1996, 1997), which may explain the high levels of aggression when re-mating and the low mating rates. Moreover, male mate search is costly in *Stegodyphus* and the operational sex ratio is biased towards females, which further explains low mating rates (Berger-Tal and Lubin, 2011).

An alternative explanation for the acceptance of multiple mating in *D. ergandros* may be that the costs of rejecting an additional mating partner are higher than the costs of reduced offspring cooperation. It is possible that both full-sibs and half-sibs show similar levels of cooperation, in which case multiple mating would not be costly in terms of reduced cooperation. However, differences between full-sibs and half-sibs remain to be studied.

It is also conceivable that the benefits of increased cooperation in highly related groups are offset by the costs of low genetic diversity, such as lowered immunity to pathogens, and so this trade-off may differ between locations and years. In bumble bees (*Bombus* spp.) females are usually monandrous (Schmid-Hempel and Schmid-Hempel, 2000) but polyandrous females produce workers that are less prevalent

to infections with a parasite (Baer and Schmid-Hempel, 2001). Similarly, heterozygous termites have greater immunity to pathogens (Calleri et al., 2006). Although multiple mating is beneficial in terms of reduced infection risk in bumble bees, reproductive success was highest in monandrous female and those that were mated with four males, but low when females mated with two males (Baer and Schmid-Hempel, 2001). It has been suggested that polyandry is costly in terms of cooperation and colony life but that these costs are compensated for when the mating frequency further increases (Baer and Schmid-Hempel, 2001). In *Diaea ergandros*, cooperation (facilitated through monandry) may in some years positively affect fitness by allowing the group to utilise a larger prey spectrum and better survive food shortage while in other years highly related and potentially inbred groups are more susceptible to decease and to be wiped out by a contagious disease a lot quicker than groups with a higher genetic diversity. Long-term monitoring of survival of groups in relation to their genetic composition is required to investigate these possibilities.

The results of the mating trials indicate that female *D. ergandros* neither prevent nor prefer mating with close kin. Similarly, females of the subsocial spiders *Stegodyphus lineatus* and *S. tentoriicola* do not avoid mating with relatives (Bilde et al., 2005; Ruch et al., 2009a). Although *D. ergandros* seems to be a transitory species between subsocial and permanently social and a previous study indicated relatively high levels of inbreeding (Evans and Goodisman, 2002), inbreeding may still be costly in *D. ergandros*. Especially if deleterious recessive alleles are still present in the population, females may benefit from mating with an unrelated mating partner, which may also explain the acceptance of immigrating spiderlings (this study & (Evans and Goodisman, 2002)). We could not estimate the costs of inbred mating because none of the egg sacs hatched in captivity, but future studies may focus on potential fitness costs of inbreeding in subsocial crab spiders.

Low variability of microsatellite markers

We only found two polymorphic microsatellite loci in *Diaea ergandros*. Similarly, allozyme loci were moderately polymorphic in this species (Evans and Goodisman, 2002). Microsatellite markers are usually abundant in the genome, highly polymorphic and easily genotyped (Agrawal et al., 2001; Krehenwinkel and Tautz, 2013; Liu and Cordes, 2004; Rowe et al., 1997), thus the high frequency of problematic loci was somewhat unexpected. Structural genomic changes in the species, e.g. polyploidy, might be a possible reason for the problems we faced during genotyping and the clarification of this issue will require additional research. Nevertheless, the two polymorphic markers showed a relatively high variability and allowed detecting multiple paternities in *D. ergandros.* Likewise, paternity patterns in two species of praying mantis were successfully estimated using two polymorphic loci (Umbers et al., 2011) and four loci resolved paternity patterns in the subsocial spider *S. lineatus* (Tuni et al., 2012).

Conclusion

Results from this study suggest that *D. ergandros* does not actively seek or avoid inbreeding but that the conditions of mating within the nest sometimes promote inbreeding and high relatedness within groups (Evans and Goodisman, 2002). On the contrary, half of the analysed broods were sired by at least two different fathers, which may result in high genetic diversity but theoretically reduces offspring cooperation. This suggests that the maintenance of high cooperation is not the only selection factor behind mating decisions and that inbreeding may still negatively affect *D. ergandros*. Future studies should focus on costs and benefits of genetically diverse broods.

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Chapter 5

Offspring dynamics affect food provisioning, growth and mortality in a brood-caring spider

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Abstract

In brood-caring species, family members are faced with a conflict over resource distribution. While parents are selected to adapt the amount of care according to their offspring's needs, offspring might be selected to demand more care than optimal for parents. Recent studies on birds have shown that the social network structure of offspring affects the amount of care and thus the fitness of families. Such a network structure of repeated interactions is likely influenced by within-brood relatedness. We experimentally manipulated the group composition in a brood-caring spider to test how the presence of unrelated spiderlings affects the dynamics between female and brood as well as within broods. Broods consisting of siblings grew better and had a lower mortality compared with mixed broods, no matter whether the caring female was a genetic or foster mother. Interestingly, we found that foster mothers lost weight when caring for sibling broods, while females caring for mixed broods gained weight. This indicates that females may be willing to share more prey when the brood contains exclusively siblings even if the entire brood is unrelated to the female. Resource distribution may thus be negotiated by offspring dynamics that could have a signalling function to females.

Key words: Parent-offspring conflict, sociality, cooperation, social network structure

Introduction

In species with parental care, parents and offspring are faced with conflicts over resource distribution (Parker and Macnair, 1979; Trivers, 1974). Offspring may be under selection to demand a higher amount of care than parents might be willing to provide, especially if parents need to retain resources for future broods. Since parental care is limited, offspring compete with each other over sharing this resource (Mock and Parker, 1997; Roulin and Dreiss, 2012).

Two main modes of resource allocation have been suggested to be relevant in this context: in scramble competition models it is assumed that parents are rather passive and that competition between offspring determines how resources are distributed (Macnair and Parker, 1979). Honest signalling models assume that parents actively allocate resources depending on the need of the offspring (Andrews and Smiseth, 2013; Godfray, 1991, 1995a).

Recent studies suggest that the resolution of parent-offspring conflicts involves repeated interactions among all group members (Parker et al., 2002; Royle et al., 2012). Royle et al. (2002) point out that determining control mechanisms is complex and that offspring- and female control represent the two ends of a 'power continuum'. Hence, the resolution of the conflict strongly depends on the context, including a network of interactions (Royle et al., 2002).

While most studies dealing with conflicts over parental care show that offspring compete with each other over resources, siblings may also cooperate to increase the overall amount of parental care (Roulin and Dreiss, 2012). Cooperation will be favoured when inclusive fitness gains exceed the direct costs of cooperating (Hamilton, 1964; Mock and Parker, 1997). Generally, interacting individuals in family conflicts are (at least partly) related so that any conflict will potentially entail indirect fitness costs.

However, relatedness among broods may vary when females mate multiply (polyandry), mobile young immigrate into an existing family group (Evans and Goodisman, 2002) or due to brood parasitism (Muller et al., 1990). Offspring should be less competitive towards closely related nestmates (Hamilton, 1964), but should become less likely to share as relatedness decreases (Godfray, 1995b; Royle et al., 1999).

Offspring migration into a foreign group can be found in the subsocial crab spider *Diaea ergandros*. Spiderlings that lost their mother can migrate into foreign nests (Evans, 1998b; Evans and Goodisman, 2002) and thus females may face a brood that contains a mixture of own and unrelated offspring. A molecular study (using allozyme markers) on the genetic structure of 28 sampled *D. ergandros* nests that contained a putative mother showed that in 75% of the nests offspring were likely produced by the present mother and a single father. In 21.4% of the nests spiderlings could not be assigned to the mother's genotype, indicating that foreign spiderlings immigrate into nests. In one case (3.6%) paternity was shared between at least two fathers (Evans and Goodisman, 2002).

In these spiders, females hunt and share prey with the offspring (Evans, 1995, 1998b) and some females are consumed by their offspring (matriphagy, Evans et al., 1995), while others stay alive until the spiderlings mature (Ruch, personal observation). Evans (1998b) showed

that females recognize own offspring. Cues that allow discrimination between kin and non-kin have not been identified in crab spiders, but in other subsocial spiders cuticular hydrocarbons are possible kin recognition cues (Grinsted et al., 2011).

As spiders digest externally (Foelix, 2011), female *D. ergandros* cannot individually allocate food to specific spiderlings, but they may allow spiderlings to feed with them and leave more food for the brood. In a situation where broods consist of a mixture of own and foreign offspring, females may selectively allow own offspring to join her feeding.

Alternatively, females may share food independently of their relatedness to the brood. In this case, food distribution may depend on competition between offspring, while the female is mostly passive (Royle et al., 2002). Accordingly, we predict that the dynamics between female and the brood and also among the brood vary depending on the relatedness between them.

If females are largely in control of food allocation and base allocation on the presence of kin cues, female care (food provisioning) should gradually decrease with increasing proportions of foster offspring in her brood. Reducing investment is beneficial for the mother if this increases the probability of producing a second brood. We observed that female *D. ergandros* are able to produce a second clutch in cases where the first one failed (Ruch, personal observation). In other semelparous subsocial spiders females may also produce a second brood when the first brood is removed (Schneider and Lubin, 1997). Reduced maternal investment is likely to result in higher spiderling mortality and lower spiderling growth when the proportion of foster offspring is high (Figure 1 A). Assuming that females mostly control the rate of food provisioning, we predict that females caring for own offspring leave more food to the brood and thus lose weight while those caring for foster offspring retain more food and gain weight. We further predict that only those females caring for own offspring tolerate matriphagy.

If competition between offspring is the predominant influence on food distribution (Roulin and Dreiss, 2012), spiderlings may adjust cooperation in sharing food to the average degree of relatedness within the brood (Hamilton, 1964). If offspring mostly control food distribution and siblings cooperate more than non-siblings, we predict that broods consisting of siblings grow better and have a lower mortality compared with broods of mixed offspring (Figure 1 B).

To test these predictions about group dynamics between females and offspring and the effects of immigrating spiderlings, we manipulated brood composition and decreased relatedness between females and their respective offspring groups. We then monitored female hunting behaviour, mass development and mortality as well as offspring growth and mortality.

Methods

Study Species

Diaea ergandros Evans, 1995 (Thomisidae) is a semelparous, subsocial spider that inhabits the foliage of *Eucalyptus* trees in closed-canopy forests (Evans, 1997) as well as trees along roadsides in Southeastern Australia. Broods usually originate as the offspring of a single

female that migrated from her natal colony after mating. Females construct nests from *Eucalyptus* leaves and produce a single egg sac (Evans, 1995). After the spiderlings have hatched, the female continues to expand the nest and catches prey to feed her young. Offspring usually stay in their natal nest and communally continue to extend the nest with new leaves. Nests serve as foraging areas and spiders hunt without a capture web by ambushing prey (Evans, 1998a).

Experimental setup

We collected *D. ergandros* nests from their natural habitat near the town Yass in New South Wales (Australia, 34°55'20.50"S, 149°6'15.53"E) in February 2012. We cut whole nests off trees with either gardening cutters or expandable branch cutters in heights ranging from fifty cm to approximately ten metres. Nests were dissected in the laboratory and spiderlings were counted and weighed to the nearest 0.1 mg by using an electronic balance (Mettler Toledo New Classic MS). Adult living females were weighed and their prosoma width was measured with digital callipers.

We only collected nests with very young spiderlings, thereby decreasing the likelihood that foreign spiderlings have already migrated into the group. Asymmetries in relatedness in the broods due to multiply mating females cannot be excluded, however the rate of polyandrous females was very low in a previous study (Evans and Goodisman, 2002).

For the experiment, we randomly assigned females into four treatments with varying degrees of relatedness to their allocated spiderlings: 0%, 33%, 66%, or 100% of the brood were

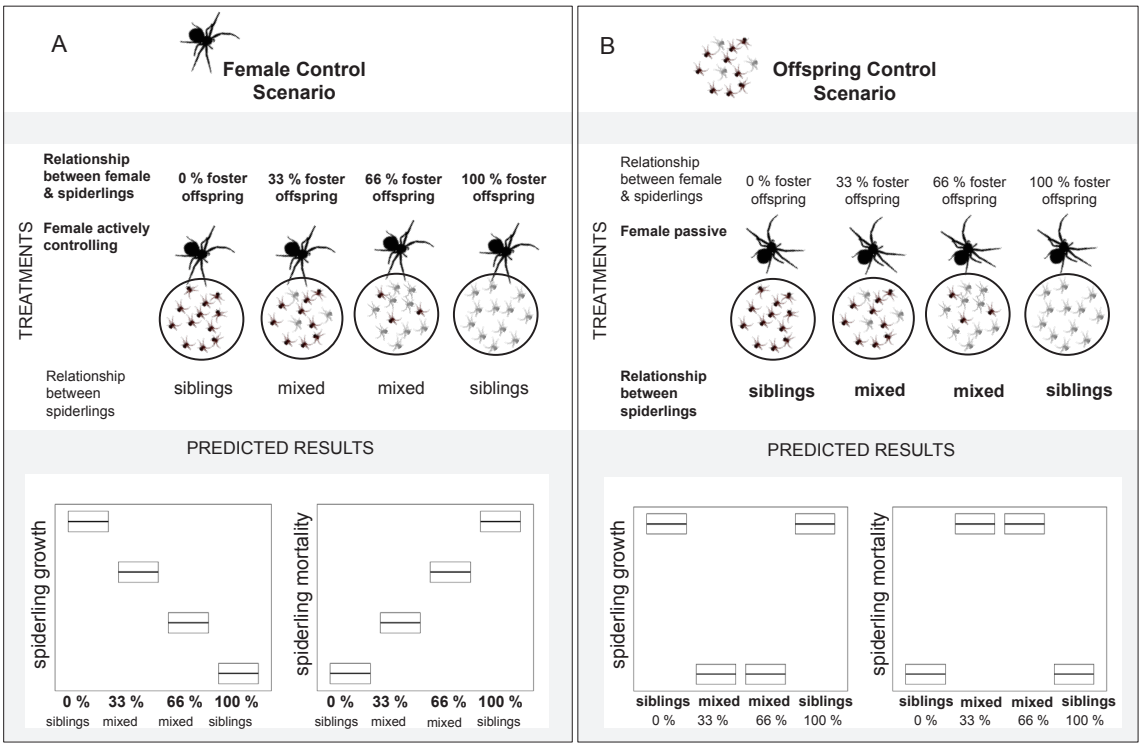

Figure 1: Predicted outcomes for the two different scenarios for offspring growth and mortality. A) Assuming that females are largely in control of resource allocation, we predict offspring growth to gradually decrease with increasing numbers of foster offspring and mortality to increase. B) Assuming competition between offspring as major control over food distribution, we predict that the sibling broods have a higher growth and lower mortality than mixed broods.

Table 1: Initial female mass, number of given spiderlings and mean spiderling mass before the experiment was started.

Treatment	Female mass [mg]	No. of added spiderlings	Spiderling mass (average [mg])	Spiderling mass (median [mg])	$N_{replicates}$
0% foster	28.6 ± 2.2	24.3 ± 1.6	2.38 ± 0.17	2.53	10
33% foster	27.2 ± 2.1	24.2 ± 1.8	2.43 ± 0.19	2.6	10
66% foster	24.7 ± 1.4	25.5 ± 1.4	2.05 ± 0.19	2	10
100% foster	26.7 ± 1.9	25.0 ± 1.2	2.20 ± 0.20	1.98	10
Female	27.6 ± 2.0	NA	NA	NA	12

foster offspring, taken from a different female (=collected at least 5 km apart from each other to minimize maternal relatedness); the remaining percentage were the female's own offspring. Since eggs mostly fail to hatch in the laboratory (Ruch, personal observation) we were unable to set up a cross-foster familiarity control, where offspring are assigned immediately after they hatch. This means that those females caring for own offspring may be more familiar with the brood compared with those females caring for foster offspring and that siblings are more familiar with each other compared with broods consisting of mixed offspring, which may affect group dynamics. The absence of a familiarity control does not allow conclusions about the mechanism of kin recognition, which however was not an aim of the study. When spiderlings migrate into foreign nests under natural conditions, it is assumed they are neither familiar nor related to the group.

From the offspring perspective, the group composition was the same in the 0 % and 100% foster treatment (all siblings) as well as in the 33% and 66% foster treatment (mixed broods from two different females). All females and spiderlings experienced the same procedure of being separated and weighed before the groups were formed.

Since female body mass varied, the number of spiderlings per female was based on female mass (sum mass spiderlings = female mass * 2) and ranged between 16 and 35 spiderlings. Our rational was that we wanted to standardise female body reserves in relation to offspring number. The body mass of the female was considered a resource in itself, since it has been found that females were consumed by their offspring (Evans et al., 1995). There was a negative correlation between the number of spiderlings per female and the initial spiderling mass (meaning the mass spiderlings had prior to the experiment, Pearson: $r = -0.59$, $P < 0.0001$): the larger the spiderlings the fewer were assigned to a female. The average number of allocated spiderlings per treatment as well as their mean initial body mass did not differ between treatments ($ANOVA_{No\ spiderlings}$: $r^2 = 0.01$, $F_{3,36} = 0.16$, $P = 0.9$; $ANOVA_{body\ mass}$: $r^2 = 0.05$, $F_{3,36} = 0.68$, $P = 0.6$, Table 1).

In an additional control, females were kept without offspring to monitor their mass development and whether they would produce a second clutch. Spider groups were kept in 750 ml plastic containers that were covered with gauze to allow airflow. Groups were checked and sprayed with water every two days. We recorded all dead spiderlings and whether the respective female was alive. The experiment was terminated after nine weeks.

Female hunting behaviour

All groups were fed with one *Calliphora* sp. (Diptera, approx. 50 mg) once a week. These flies were too big to be caught by the spiderlings. Thus, females had to catch the flies and could either feed themselves or share the prey with the spiderlings. We recorded whether females had caught the fly 24 hours after introduction to the containers.

Female mass development and matriphagy

Females were weighed every fortnight and we calculated their mass development by subtracting the initial mass from the final mass. To control for the overall effect of the presence of spiderlings on female mass development and mortality, we included a control of females without spiderlings. These were fed at the same frequency as the experimental females. In case a female died, we weighed the remains and calculated the loss of body mass from the previous weighing event.

Offspring mass development and mortality

Ten randomly chosen spiderlings of each group were individually weighed before the experiment and again after four weeks. We aimed to test whether female care affects offspring mass development and mortality depending on the relatedness to the female and between the offspring. Therefore, we analysed offspring mass development and mortality in the first four weeks when all females were still alive.

Statistical Analyses

Data analyses were performed using JMP 9.0.2 (*SAS Institute Inc., Cary, NC, USA*) and R version 2.15.3 (R Core Team, 2013). Descriptive statistics are given as mean ± standard error (SE). Continuous data were tested for normality using the Shapiro-Wilk W-test. Data that were not normally distributed were analysed using non-parametric tests.

The generalised linear model (GLM) and the generalised linear mixed model (GLMM) were performed in R using the *lme4* package. We simplified maximal models by stepwise elimination of the least significant variable and comparing the models with ANOVAs. We used the minimal adequate model (indicated by the lowest AIC) to identify determinants of the response variable. Post-hoc tests (Tukey contrasts) were performed with the *multcomp* package. The generalised estimating equation (GEE) was performed in R using the *geepack* package. We simplified the maximal model by stepwise elimination of the least significant variable and comparing the models with Wald statistics.

Box-and-whisker plots were plotted in R and the upper and lower whiskers show the range, the box shows median and interquartiles. Individual dots indicate outliers.

The percentage of successful prey capture by females (N = 148 observations) was analysed using a generalised linear mixed model (GLMM) with binomial error distribution. The maximal model to investigate whether a fly was caught (yes/no) included the following explanatory variables: treatment (percentage of foster offspring), female start mass and the week of the

feeding observation. Female ID was included as a random factor to control for repeated measurements.

Female mass development was analysed with a non-parametric Kruskal-Wallis test. We analysed female mortality using a Cox proportional hazard model ($N = 52$).

Offspring mass development was calculated by subtracting the initial group mass from the final group mass. We fitted a GLM with normal error structure and included treatment (percentage of foster offspring) and the average spiderling initial mass as well as their interaction. Differences between the four treatments were analysed using Tukey contrast. Offspring mortality was analysed using a GEE with binomial error structure and exchangeable association structure. Mortality (y/n) was the response variable and treatment and spiderling start weight as well as their interaction the explanatory variables ($N = 989$ spiderlings). Female ID was specified as grouping variable to control for measurements within the same group. Treatment was re-ordered to analyse differences between the treatments by comparing the coefficients of each treatment with the reference level.

Data are available in DRYAD: http://doi.org/10.5061/dryad.53942.

Results

Female hunting behaviour

We aimed to test whether the female's prey capture behaviour varies according to her relatedness with the brood. However, treatment ($P = 0.3$) did not significantly affect prey capture (average% of flies caught per treatment: *0% foster*: 50.0 ± 15.07, *33% foster*: 41.7 ± 18.0, *66% foster*: 35.0 ± 15.5, *100% foster*: 40.9 ± 21.2). Female initial mass was not significant either ($P = 0.4$) and both factors were eliminated from the final model, which showed that the percentage of successfully caught flies varied significantly between the feeding observations (*FO*), but without a discernable pattern of overall increase or decrease ($\chi^2_3 = 14.09$, $P = 0.00017$, $N_{females} = 40$, $N_{observations} = 148$, average% of flies caught *FO 1*: 45.7 ± 4.1, *FO 2*: 88.2 ± 6.4, *FO 3*: 8.9 ± 6.4, *FO 4*: 24.9 ± 1.7).

We observed females of all treatments sharing prey with offspring. As the spiderlings were not individually marked we could not differentiate whether females interacted differently with the offspring in the mixed broods.

Female mass development and matriphagy

Overall, female mass development was not significantly different between the five

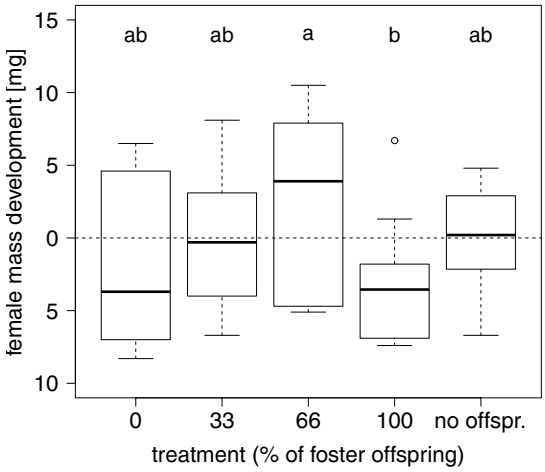

Figure 2: Female mass development depending on their relatedness to the offspring (0%, 33%, 66%, 100% foster) and for females without brood. Females caring for sibling broods (0% foster and 100% foster) lost weight and there was a difference with females of the 100% foster treatment losing significantly more mass than females caring for 66% foster offspring (a and b express the statistical difference, Wilcoxon multiple comparisons $P < 0.05$).

Table 2: Wald statistics (W) obtained from the GEE showing the differences in mortality between the four treatments.

Treatments	Estimate	SE	W	*P*
33% foster / 0% foster	0.86	0.52	2.76	0.096
66% foster / 0% foster	1.25	0.42	8.53	**0.004**
66% foster / 33% foster	0.39	0.51	0.57	0.45
100% foster / 0% foster	0.44	0.42	1.12	0.3
33% foster / 100% foster	0.42	0.51	0.69	0.4
66% foster / 100% foster	0.81	0.44	3.4	0.065

treatments over the duration of the experiment (Kruskal-Wallis test: $\chi^2_4 = 7.01$, $P = 0.13$). The median values initially increase with decreasing relatedness to the mother (Figure 2) but then drop in the 100% foster treatment where we expected the greatest maternal weight gain. A Wilcoxon multiple comparison revealed that females of the 66% foster treatment gained significantly more mass than females of the 100% foster treatment ($Z = 2.0$, $P = 0.045$, Figure 2). Females caring for own offspring (0% foster) lost mass, while females caring for 33% foster offspring and females without offspring neither gained or lost mass, however all treatment comparisons except for the above mentioned one were not significantly different (Wilcoxon each pair, all $P > 0.05$).

Twenty-five females died over the course of the experiment but female mortality was not significantly different between the five treatments (Cox proportional hazard model: $\chi^2_4 = 5.93$, $P = 0.2$). We never observed matriphagy and the mass loss between the last weighing event before the female had died and the day she died was not significantly different between the treatments (ANOVA: $r^2 = 0.07$, $F_{4,21} = 0.41$, $P = 0.8$). A single female (in the 0% foster treatment) lost more than 80% of body mass, but it is unclear whether the spiderlings may have fed on her. We regularly observed spiderlings sitting on the body of alive females (across all treatments), but we never observed them feeding on a female body. Across all treatments, the average mass loss of females was 28.6 ± 4.3% and suggests that matriphagy was not relevant in this experiment. None of the females produced a second clutch.

Offspring mass development and mortality

Initial spiderling body mass had an influence on final mass (meaning that spiderlings with a higher initial body mass had a higher final body mass, Pearson: $r = 0.86$, $P < 0.0001$) and was therefore included as a covariate (there was no difference between treatments prior to the experiment, see Table 1). Corrected for initial body mass

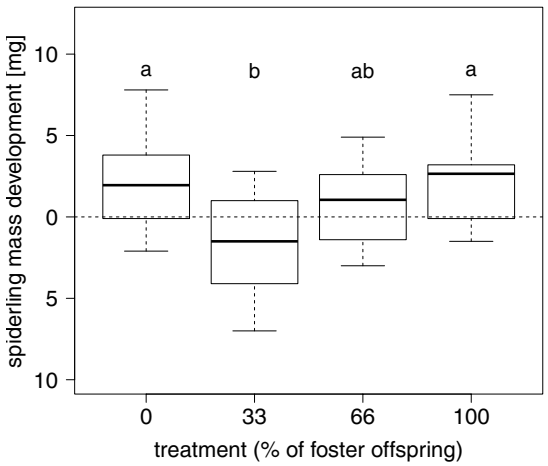

Figure 3: Offspring mass development depending on their relatedness to the caring female (0%, 33%, 66%, 100% foster). Spiderlings of the sibling broods (0% foster & 100% foster) gained significantly more mass than those belonging to the 33% foster treatment, while the 66% foster treatment was not significantly different from the other treatments (a and b express the statistical difference, Tukey contrasts $P < 0.05$).

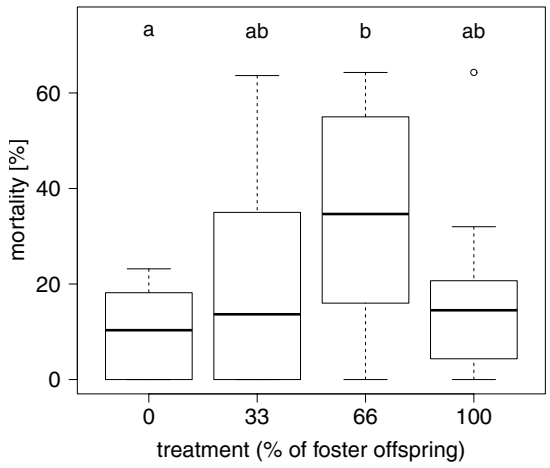

Figure 4: Differences in offspring mortality between the four treatments (0%, 33%, 66%, 100% foster; a and b express the statistical difference, Wald test $P < 0.05$).

(GLM: $F_{1,35} = 4.49$, $P = 0.04$), spiderling mass development was significantly different between the four treatments (GLM: $F_{3,35} = 3.89$, $P = 0.017$, Figure 3), with spiderlings of the sibling treatments (0% foster & 100% foster) gaining more weight than spiderlings of mixed broods.

Offspring mortality was predicted by treatment (GEE: $\chi^2_3 = 8.98$, $P = 0.03$) and negatively affected by spiderling start mass ($\chi^2_1 = 7.12$, $P = 0.008$). Spiderlings belonging to the 0% foster treatment had a significantly lower mortality than spiderlings belonging to the 66% foster treatment (Table 2). There was a trend that spiderlings of the 100% foster treatment had a lower mortality compared with the 66% foster treatment, albeit not significant (Table 2, Figure 4).

Discussion

We experimentally manipulated the group composition of *Diaea ergandros* broods to test how the presence of unrelated spiderlings affects dynamics between female and brood as well as within broods. We found that broods consisting of siblings grew better compared with mixed broods, independent of their relatedness to the caring female. Our results are consistent with a scenario where resource distribution is more strongly influenced by interactions between offspring than by female interests.

Contrary to predictions of the maternal control hypothesis, females lost mass when caring for broods consisting of siblings only (0% and 100% foster treatment). This suggests that females shared more food with siblings than with mixed broods. Indeed females caring for 66% foster offspring (mixed broods) gained mass and spiderlings in this treatment had the highest mortality. These results indicate that i) females do vary the amount of prey they share with offspring groups, however the pattern of food sharing does not consistently decrease with decreasing relatedness to the brood and ii) offspring dynamics may have a signalling function that affects the food provisioning behaviour even of foster mothers.

A previous study of the same species concluded that females provide more care for own offspring, as females caught more prey for own offspring than for foster offspring and own offspring grew better than foster offspring (Evans, 1998b). These different findings may be due to different experimental procedures. In our experiment, we provided a single large fly per female and all females were equally likely to capture prey regardless of the relatedness to their brood. Evans (1998b) on the other hand offered two slightly smaller flies which may have resulted in a different hunting and food-sharing pattern. The contrasting results indicate the presence of a flexible hunting behaviour depending on the available prey type.

Moreover, we did not find matriphagy, suggesting that these spiders may be plastic in both, their hunting behaviour and whether matriphagy occurs or not. In fact, these two may be linked: in situations where exclusively large prey items are available and offspring are not able to overwhelm them, the presence of a hunting mother may be more beneficial than consuming her. In situations where small prey items are dominating, spiderlings may be able to hunt on their own and would have an additional nutritional benefit by consuming the mother (Salomon et al., 2005). Plasticity in matriphagy has been demonstrated in another subsocial spider. In *Stegodyphus lineatus*, matriphagy occurred significantly later or not at all when females were caring for an experimentally reduced number of offspring (Salomon et al., 2005). Plasticity in brood-caring behaviour is also common in birds and several factors, including prey availability (Chiaradia and Nisbet, 2006) and parents' personalities (Westneat et al., 2011) may affect the amount of care.

When feeding on a communal prey item, spiderlings in mixed broods may behave differently compared with siblings, since spiderlings of the mixed treatments are only related to a part of the group. In addition, genetic variation within the brood might lead to phenotypic variation in foraging efficiency, which may result in some individuals foraging better than others (Beauchamp et al., 1997). Direct competitive interactions between spiderlings may be more frequent in mixed broods, for example by excluding unrelated and/or unfamiliar group members from foraging, while siblings may cooperate and thus gain inclusive fitness when the direct costs of sharing are lower than the benefits (Hamilton, 1964; Mock and Parker, 1997).

An important next step to test this mechanism is to individually mark spiderlings and observe their foraging behaviour as well as interactions between female and offspring more closely. In barn swallows, unrelated nestlings were competing more intensely, and it was suggested that kin selection may be the mechanism to resolve this conflict (Boncoraglio et al., 2009). Unrelated spiderlings might also be more reluctant than highly related broods to contribute their digestive enzymes to a common prey item which ultimately reduces feeding efficiency and growth rates. This was found in the subsocial spider *Stegodyphus lineatus*, where related spiders extracted more mass out of a common prey and grew better than unrelated spiders (Schneider and Bilde, 2008).

Similarly, differences in extracting prey may explain the overall reduced growth within mixed broods in our experiment. However, even though we assorted the groups at a very early stage in their life, we cannot distinguish whether effects of relatedness or familiarity cause the differences in our study. *Diaea ergandros* individuals are able to recognize kin (Evans, 1999), thus kin discrimination could potentially cause these differences. In other subsocial spiders, sibling-specific cuticular hydrocarbons are possible kin recognition cues (Grinsted et al., 2011) and these might exist with a similar function in *D. ergandros* as well.

In our experiment, sibling broods that were fed by an unrelated foster mother did not differ in growth from those sibling broods with genetic mothers. This result suggest that offspring dynamics as described above may be a signal

that prompts even unrelated foster mothers to leave more of the liquefied prey for the brood. Offspring dynamics may for example affect the conflict over food provisioning in birds (Royle et al., 2012). In great tits (*Parus major*), females and males provide food differently depending on the social network structure of offspring. Females provide more food to small and medium sized offspring groups which show a stronger social network structure than large groups, while the amount of male care is negatively correlated with a strong network structure and thus males provide more food when caring for large groups (Royle et al., 2012).

Mixed broods in our experiment may have sent a weaker signal due to a lower network structure and thus females caring for mixed broods may have ingested more food themselves. Contrary to our prediction, however, this did not result in the production of a second clutch. Even though we observed that females can produce a second clutch shortly after the first one failed, it seems that females are unable to produce another clutch after the first one has hatched. Evans et al. (1995) described that the ovaries of *D. ergandros* degrade, producing trophic eggs after oviposition and there seems to be no plasticity, even in cases where all offspring are removed.

The idea that food-provisioning behaviour of females may be more dependent on offspring dynamics than on female-offspring discrimination is supported by the finding that all females were equally likely to hunt and share the prey item. Such a lack of discrimination has also been shown in birds, where parents of a semi-colonial swallow species do not discriminate between calls of genetically related or foster nestlings (Leonard et al., 1997) and burying beetles (*Nicrophorus vespilloides*), where females care for unrelated larvae (Muller et al., 1990). An explanation may be that the costs of alloparental care could be relatively low compared to the cost of a rejection error (Keller, 1997). Costs of alloparental care may further be outbalanced when an increased group size has positive effects (Kokko et al., 2001; Unglaub et al., 2013), such as enhanced defence against predators.

In conclusion, we showed that immigrating spiderlings have a negative effect on spider group dynamics. This effect might be imposed by the non-relatedness and/or unfamiliarity of immigrant spiderlings. The challenge for future research is to identify the mechanism that causes the differences and also to investigate the interactions of immigrating individuals with the family group more closely.

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Chapter 6

Families hunt more successfully: effect of group composition on hunting and communal feeding

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Abstract

Group activities that require an initial investment are liable to be exploited. This situation can, for example, be found in group-hunting lions, but also in subsocial and social spiders, in which several individuals capture single large prey items. Individuals could save investment by contributing less to the hunt but also during feeding by saving their external digestive enzymes. Such dynamics have been partly explored in subsocial and social web-building spiders, but are likely to differ when groups hunt in the absence of a web. Subsocial crab spiders hunt without webs and forage communally. Their nests usually comprise related individuals, although groups accept immigrating spiderlings from foreign nests, which may affect competition among group members. We aimed to test whether hunting and communal feeding differ depending on spiderling group composition and formed experimental treatments consisting of either (1) family members, (2) family members including two foreigners or (3) assorted spiderlings. Group hunting was more frequently found among family members and was positively correlated with spiderling mass increase. Family groups fed in consistently larger numbers and grew better compared to assorted groups but also compared to family groups with only two foreigners. The latter finding suggests that even a few immigrants may negatively affect communal activities.

Keywords: *Diaea ergandros*, foraging, group hunting, inclusive fitness, sociality, Thomisidae

Introduction

Group hunting can be found in vertebrates, for example in carnivorous mammals (Bailey et al., 2013), birds (Bednarz, 1988; Bowman, 2003) and fish (Strubin et al., 2011) but also in invertebrates such as ants and spiders (Duncan and Crewe, 1994; Kim et al., 2005; Witte et al., 2010) with varying complexity among species. Individual investment in the group hunt and the benefits, namely the sharing of prey, may be distributed unevenly among group members. This can be the result of a hierarchical social organization such as in group-hunting wolves, *Canis lupus* (Fritts and Mech, 1981) or cheating group members, for example in lion packs, *Panthera leo* (Packer and Ruttan, 1988). Cheaters are those individuals in a group that benefit from the cooperation of others by investing less than their fair share in cooperative actions (West et al., 2007). The potential for cheating can cause group hunting to become unstable. Therefore, this behaviour should persist only if hunting success is significantly improved by hunting in a group compared with hunting singly, for example when targeting large prey (Packer and Ruttan, 1988). The benefits of group hunting are similar in vertebrates and invertebrates: groups are able to overwhelm large prey (Avilés, 1997; Binford and Rypstra, 1992; Lubin and Bilde, 2007; Yip et al., 2008).

Social spider species live their entire life in communal nests and build much larger capture webs than solitary species. Similarly, subsocial spiders live in communal nests as juveniles; however, unlike social spiders they disperse prior to maturity (Yip and Rayor, 2013). The communal construction of a large capture web is a major characteristic of most of the subsocial and social species that have been described (Avilés, 1997; Lubin and Bilde, 2007). By hunting large prey items, subsocial and social spiders communally create a common good that is consumed as a group (Schneider and Bilde, 2008): all the feeding spiders inject their digestive enzymes into the prey and suck out the liquefied mass (Foelix, 2011). The outcome for individual group members will depend on how the prey is distributed among the members of the hunting group and whether it is shared with others that did not engage in the hunt (Ward and Enders, 1985; Whitehouse and Lubin, 1999).

Group hunting and food sharing are often found among related individuals in carnivorous mammals (Bailey et al., 2013), which is also true for spiders (Lubin and Bilde, 2007). Food sharing can also be found among unrelated individuals when the immediate benefits of sharing exceed the costs (Stevens and Gilby, 2004). Benefits from group feeding may be asymmetric and conflicts are predicted for all subsocial and social spider species. These conflicts might be mitigated by inclusive fitness benefits when sharing with relatives (Hamilton, 1964). For example, there is evidence that relatedness reduces competition between nestmates in two subsocial *Stegodyphus* species (Ruch et al., 2009; Schneider and Bilde, 2008).

In systems in which family groups accept immigrating individuals the situation might be different. In the subsocial huntsman spider, *Delena cancerides*, almost 50% of the colonies contain immigrant spiders (Yip et al., 2012). A similar situation can be found in *Diaea ergandros*, a subsocial crab spider, where up to 44 % of the nests contain unrelated spiderlings

(Evans, 1998b). Although they lack a capture web, these spiders may hunt and feed in groups (Ruch, personal observation).

Subsocial crab spiders usually live in family nests, but they recognize and accept unrelated migrating juveniles (Evans, 1998b, 1999). Juveniles (spiderlings) seem to migrate only when the conditions in their original nest are unsuitable, for example when the caring female is no longer present (Evans, 1998a). When *D. ergandros* spiderlings immigrate into a nest, they are most likely to be neither related (Evans and Goodisman, 2002) nor familiar with the other group members, which possibly affects group dynamics. For example, offspring dynamics seem to affect the amount of female care: females share more prey with broods consisting of siblings than with broods consisting of a mixture of their own and foster offspring (Ruch et al., 2014).

Immigrating spiderlings could, however, benefit other group members because larger groups seem to be less vulnerable to predation (Evans, 1998a; Unglaub et al., 2013). Another benefit of accepting immigrants, at least shortly before maturation, might be the increased potential for outbreeding (Evans, 1999). Nevertheless, conflicts over group activities such as communal hunting and feeding are likely to be pronounced in groups with high levels of immigration because here unrelated spiders compete for resources.

To investigate group hunting and foraging dynamics in groups with and without immigrant spiderlings, we conducted an experiment under laboratory conditions over 2 years. In the first year, we formed two treatments with groups consisting of family members or unrelated (assorted) spiderlings. In the second year, we had the same two treatments and added a third treatment which consisted of predominantly family members, but included two immigrants. We predicted that spiderlings living in family groups would be more likely to invest in communal activities than family groups including foreigners and that assorted groups would invest the least in group activities.

Methods

Study species

Diaea ergandros Evans, 1995 is a semelparous, subsocial crab spider (Thomisidae) that inhabits the foliage of *Eucalyptus* forests in southeastern Australia (Evans, 1997). Subsocial crab spiders are special among social spiders since they do not build capture webs, which is considered a major characteristic explaining the maintenance of social groups (Evans, 1995; Evans and Main, 1993).

Females disperse after mating and build a brood chamber from several *Eucalyptus* leaves. They hunt by ambushing prey, provide food to their offspring and some females are consumed by their offspring (matriphagy; Evans et al., 1995). Spiderlings overwinter together and cooperate in nest construction and feeding (Evans, 1995).

Spiders are able to recognize kin (Evans, 1999). Communal feeding is known to be present in social and subsocial crab spiders; yet group hunting has not been reported (Evans and Main, 1993). Relatedness of nestmates is high in this species (r = 0.44; Evans and Goodisman, 2002), although immigrant spiders from other nests can be found (Evans, 1998b), thereby diluting group relatedness. Mixed groups with several instars

within one nest were found to be less related than homogeneous groups that consisted mainly of spiderlings of the same instar (Evans, 1999).

General protocol

The first trials of the experiment were conducted in 2011. The same protocol was applied again in 2013. The spiders for the laboratory experiment in both years (2011 and 2013) were collected in March with their nests from *Eucalyptus* trees around Yass (34°55'20.50"S, 149°6'15.53"E) and Boorowa (34°25'53.31"S, 148°43'49.47"E). Depending on the height of the tree, we cut whole nests with either gardening cutters or expandable branch cutters at heights ranging from 50 cm to approximately 10 m. In the laboratory, nests were dissected and spiderlings were counted. Broods were generally very young which reduces the likelihood of finding immigrant spiderlings within nests (Evans, 1999).

Similar-sized spiderlings were selected from their natal nests (N_{2011} = 24, N_{2013} = 17) and separated into two main treatments: groups that consisted of either seven family members (all from the same nest, N_{2011} = 10, N_{2013} = 11) or seven assorted spiderlings (all from different nests, collected at least 1 km from each other, N_{2011} =

Table 1: Differences between the treatments (assorted/family/family + 2 foreign)

	Assorted	Family	Family + 2 foreign	Test	*P*
2011 mass before	2.93 ± 0.10	2.89 ± 0.15		Assorted/family: Wilcoxon: Z = - 0.40 N = 18	0.69
2011 mass after	4.01 ± 0.21	4.39 ± 0.22		Assorted/family: see results mass increase, Table 2	
2013 mass before	5.47 ± 0.43	5.32 ± 0.57	5.65 ± 0.69	Assorted/family: Wilcoxon: Z = - 0.45 N = 22	0.65
				Family/family + 2: paired t: t_{10} = 1.39 N = 22	0.2
2013 mass after	6.04 ± 0.49	7.36 ± 0.73	7.03 ± 0.96	Assorted/family: see results mass increase, Table 2 Family/family + 2: see results mass increase	
2011 CV start	0.23 ± 0.011	0.23 ± 0.018		Assorted/family: t test: t_{17} = - 0.38, N = 18	0.71
2011 CV end	0.27 ± 0.021	0.18 ± 0.014		Assorted/family: t test: t_{17} = - 3.18, N = 18	**0.007**
2013 CV start	0.18 ± 0.017	0.18 ± 0.022	0.19 ± 0.02	Assorted/family: t test: t_{20} = - 0.07, N = 21	0.95
				Family/family + 2: paired t: t_{10} = 1.05, N = 22	0.3
2013 CV end	0.21 ± 0.009	0.20 ± 0.010	0.21 ± 0.022	Assorted/family: t test: t_{20} = - 0.68, N = 21	0.5
				Family/family + 2: paired t: t_{10} = 0.16, N = 22	0.87

Differences in mean group mass before and after the experiment are shown as well as the coefficient of variation (CV) of spiderlings before and after the experiment (= 'start', 'end'). Descriptive data are shown as mean ± SE for 2 years separately (2011 and 2013). Bold *P* values show significant differences.

10, N_{2013} = 11). Although a group size of seven is lower than the average group size in natural nests, we chose it to ensure that all spiders could feed on the same prey item at the same time. Of 222 nests opened, 13% contained fewer than 10 spiders (Ruch, personal observation); thus a group size of seven is not unrealistic. Moreover, not all spiderlings feed simultaneously in natural nests (Ruch, personal observation).

In 2011, we observed that only very few spiderlings moulted over the course of the experiment. The same was true in 2013 and so we individually marked spiderlings with nontoxic watercolour (Plaka Farbe, Figure 1). The small colour dots were applied to the opisthosoma of each spiderling with slender blades of grass. In 2013, we used the collected broods for an additional treatment consisting of five individually marked family members (each from the same family as in the sibling treatment) and added two marked foreign spiderlings from different nests ('family + 2 foreign'). We added this treatment to simulate a more realistic group composition with mostly siblings and few immigrants. These groups did not differ in their initial spiderling mass from the corresponding family groups (Table 1). Because of the paired design, this additional treatment was not included in the main analyses comparing family and assorted groups, but was analysed separately and each group was compared with its corresponding family group (except for individual mass increase).

Figure 1: Five individually marked spiderlings feeding on a common prey item in 2013. The coloured dots allowed us to track each individual.

Spiderlings were individually weighed to the nearest 0.1 mg on an electronic balance (Mettler Toledo New Classic MS) and placed into petri dishes (10 cm diameter) with a leaf-shaped paper towel as shelter. Even though spiderlings were collected at the same time of year, spiderling weights differed significantly between 2011 and 2013 (Wilcoxon: Z = - 5.1, N = 40, $P < 0.0001$; 2011: 2.90 ± 0.08 mg; 2013: 5.4 ± 0.35 mg), but not between the experimental groups (Table 1). We thus analysed both years separately.

In both years, spiderlings were checked daily during a 2-week habituation phase before the actual experiment was started and we did not find obvious differences in spiderling distribution in the petri dish between the two treatments (e.g. the spiderlings of family groups did not tend to cluster more than the assorted spiderlings).

During this time the spiderlings were fed once with a fly (*Bactrocera tryoni* and *Musca domestica* in 2011; *Musca domestica* in 2013) and spiderlings of both treatments were observed to feed communally on the prey item. In 2011, two of the groups escaped after a few days and were replaced. These two groups were excluded from all analyses that included the initial spiderling mass. In cases in which spiderlings moulted in 2013, we weighed and re-marked them.

We compared spiderling group mass between the start and the end of the experiment (11 weeks after the spiderlings were placed into the petri dishes) and calculated the relative mass increase of the two main treatments (family and assorted; relative mass increase, Table 2) and the additional 'family + 2 foreign' treatment in 2013. The relative mass increase of spiderlings was calculated using the formula [Sum group mass/ individuals alive at the end of the experiment]/ [Sum group mass/individuals alive at the start of the experiment]. We also calculated the coefficient of variation in body mass (ratio of the standard deviation to the mean (CV_{bm})) for all treatments (family, assorted, family + 2 foreign) before and after the experiment (CV, Table 1). In 2013, we were able to calculate each individual's mass increase (Table 3).

Feeding experiment

After 2 weeks of habituation to the petri dishes, the feeding experiment was started. Trials were conducted blind with respect to treatment, meaning that the observer did not know which treatments were observed. A CO_2-anaesthetized *B. tryoni* (2011) or *M. domestica* (2011 and 2013) fly was weighed to the nearest 0.1 mg and placed into the petri dish. The flies started moving in the petri dishes after a few seconds and we recorded when and how many spider(s) attacked.

In 2011 we only recorded how many spiders bit into the prey item in the first 2 seconds, without having a clear definition for a group attack. Based on our experience in 2011, we defined a group attack in 2013 as joining the hunt (biting into the prey item) while the fly was still alive and moving. Flies usually stopped moving around 10 seconds after the attack, depending on which body part the spiderling(s) had attacked.

After a successful attack, we defined spiders as 'feeding' when the fly stopped moving. Spiderlings joining others feeding on the fly after it had stopped moving were categorized as having 'joined feeding', but not as having 'joined an attack'. All members of the group could theoretically join feeding after the actual prey capture event.

We determined the number of feeding spiderlings every 15 minutes for 2 hours (nine observations) and calculated the average number of communally feeding spiderlings within the 2 hour feeding period (number of communally feeding spiderlings, analyses of the main treatments family and assorted, Table 2). We conducted a pairwise comparison of the average number of communally feeding spiderlings between family groups and the corresponding siblings from the same family with two foreign spiderlings. In 2013, we also recorded which individual initiated attacks and the total feeding time of each individual.

If the prey was not attacked within 2 hours, the trial was terminated and the group was tested again the next day. If they did not attack on that day, they were tested once again a day later and if there was still no attack they then had to wait until the next week of feeding trials. We also compared the proportion of successful attacks in the two treatments (attack success, Table 2).

The flies that were not attacked during these 2 hours were weighed again to calculate the weight loss from desiccation (N_{2011} = 119, N_{2013} = 57 trials). Some flies escaped and could not be weighed again. The weight loss from desiccation of those flies that were not attacked

was 3.33 ± 0.29% in 2011 (N_{flies} = 119) and 4.37 ± 0.48% in 2013 (N_{flies} = 57).

We recorded 129 attacks in 256 trials (50.39%) in 2011 and 150 attacks in 207 trials (72.46%) in 2013 during the experimental phase of 7 weeks. In the unsuccessful trials, spiderlings either did not attempt to attack the prey item (N_{2011} = 96, N_{2013} = 51 trials) or a single individual attacked the prey item but lost it again (N_{2011} = 31, N_{2013} = 6 trials).

After a successful attack (N_{2011} = 98, N_{2013} = 144), the fly was removed after the 2 hour feeding period and weighed again. We calculated the percentage of extracted prey mass and compared it between the two treatments (% extracted prey mass, Table 2). Although we aimed to record one successful feeding trial per group once a week, intervals between successful feeding trials were variable owing to unsuccessful trials.

Data analyses

Data analyses were carried out with JMP 9.0 (*SAS Institute Inc., Cary, NC, U.S.A.*) and R version 2.15.3 (*The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org*). Continuous data were tested for normal distribution (Shapiro–Wilk test) as well as for equal variance and transformed to fulfil the model assumptions if necessary. Parametric tests were applied when the data fulfilled the criteria of normal distribution of the residuals; otherwise nonparametric tests were used. All statistical tests are two tailed (α = 0.05). Descriptive statistics are given as mean ± SE.

Generalized linear models (GLMs) were fitted in R. For the binomial GLMs, we used a quasibinomial error structure (owing to overdispersion, details in Table 2). Since we observed groups over several weeks, all analyses dealing with correlated data were analysed with models that allow correcting for these correlations (generalized least squared 'GLS').

We used the *nlme* package in R for fitting the GLSs. In the GLSs, we included group ID as a grouping variable to control for measurements of the same group and specified the correlation structure as (1) temporal to correct for repeated measurements over several weeks (number of communally feeding spiders, % extracted prey mass) or (2) exchangeable when there was no time dependency (individual mass increase).

We simplified maximal models (both GLM and GLS) by stepwise elimination of the least significant variable (always starting with the interaction term) and compared the models with ANOVAs to test whether the explanatory power of the model was reduced. The response variables and explanatory variables for each model are explained in Tables 2 and 3.

Results

Attack success

In 2011, family groups had an attack success rate of 49.9 ± 6.6% and assorted groups 42.5 ± 6.5% (N_{broods} = 20). The attack success rate was not significantly different between family and assorted groups (Table 2).

In 2013, family groups had a significantly higher attack success rate (80.0 ± 4.4%) than assorted groups (64.35 ± 4.9%, Table 2) and family groups had a significantly higher success rate than family groups with two foreigners (62.33 ± 5.5%; paired *t* test: t_{10} = 2.29, P = 0.04).

Table 2: Analyses of the hunting and foraging experiment in 2011 and 2013

Response	Analyses	Explanatory variables	Test	*df*	*P*
Attack success	GLM with quasibinomial distribution and logit link function N_{2011} = 18 N_{2013} = 22	Treatment (family/assorted)	2011: *F* = 0.96	1,17	0.34
			2013: *F* = 5.52	1,21	**0.03**
		Mean initial spiderling mass	2011: *F* = 0.75	1,16	0.4
			2013: *F* = 3.23	1,20	0.09
		Treatment*mean initial spiderling mass	2011: *F* = 1.21	1,15	0.23
			2013: *F* = 0.26	1,19	0.61
Number of communally feeding spiderlings	GLS with normal distribution, correlation structure specified as temporal (AR1) for group ID and time N_{2011} = 18 groups, 86 trials N_{2013} = 22 groups, 144 trials	Treatment (family/assorted)	2011: *L-ratio* = 6.46	3	**0.01**
			2013: *L-ratio* = 12.78	4	**< 0.0001**
		Mean initial spiderling mass	2011: *L-ratio* = 0.03	4	0.87
			2013: *L-ratio* = 4.79	4	**0.03**
		Treatment*mean initial spiderling mass	2011: *L-ratio* = 1.71	5	0.19
			2013: *L-ratio* = 0.06	5	0.81
% total extracted prey mass (angular transformation)	GLS with normal distribution, correlation structure specified as temporal (AR1) for group ID and time N_{2011} = 18 groups, 85* trials N_{2013} = 22 groups, 144 trials *2011: one outlier excluded owing to weighing error	Treatment (family/assorted)	2011: *L-ratio* = 6.12	4	**0.01**
			2013: *L-ratio* = 13.32	3	**< 0.0001**
		Mean initial spiderling mass	2011: *L-ratio* = 4.62	4	**0.03**
			2013: *L-ratio* = 1.0	4	0.31
		Treatment*mean initial spiderling mass	2011: *L-ratio* = 1.44	5	0.23
			2013: *L-ratio* = 0.37	5	0.54
% per capita extracted prey mass (angular transformation)	GLS with normal distribution, correlation structure specified as temporal (AR1) for group ID and time N_{2011} = 18 groups, 85* trials N_{2013} = 22 groups, 144 trials *2011: one outlier excluded owing to weighing error	Treatment (family/assorted)	2011: *L-ratio* = 0.23	4	0.63
			2013: *L-ratio* = 13.31	3	**< 0.0001**
		Mean initial spiderling mass	2011: *L-ratio* = 3.82	3	**0.05**
			2013: *L-ratio* = 1.03	4	0.3
		Treatment*mean initial spiderling mass	2011: *L-ratio* = 0.001	5	0.97
			2013: *L-ratio* = 0.37	5	0.54
Relative mass increase	GLM with normal distribution and identity link function N_{2011} = 18 N_{2013} = 22	Treatment (family/assorted)	2011: *F* = 1.28	1,16	0.27
			2013: *F* = 31.58	1,21	**< 0.0001**
		Average number of successful feeding trials	2011: *F* = 8.6	1,17	**0.01**
			2013: *F* = 0.67	1,20	0.42
		Treatment*average number of successful feeding trials	2011: *F* = 0.32	1,15	0.57
			2013: *F* = 0.47	1,19	0.5
Mortality%	GLM with quasibinomial distribution and logit link function N_{2011} = 18 N_{2013} = 22	Treatment (family/assorted)	2011: *F* = 0.14	1,15	0.71
			2013: *F* = 0.13	1,19	0.72
		Average number of successful feeding trials	2011: *F* = 6.15	1,17	**0.025**
			2013: *F* = 2.38	1.21	0.14
		Mean initial spiderling mass	2011: *F* = 0.15	1,16	0.71
			2013: *F* = 0.08	1,20	0.78
		Treatment*average number of successful feeding trials	2011: *F* = 0.006	1,14	0.94
			2013: *F* = 1.3	1,18	0.27

All models were analysed separately for the 2 years and test statistics are thus displayed separately. Test statistics, *df* and *P* values of non-significant variables stem from the step when a variable was dropped from the model; thus *df* vary depending on the number of remaining variables. Bold *P* values show significant variables that remained in the final model.

Group hunting and communal feeding

In 2011, we only recorded the number of spiders biting into the prey item within the first 2 s of the attack. In 18 of 98 (18.36%) successful attacks, two or more spiders bit into the prey item simultaneously. We observed this exclusively in family groups in nine of 10 groups. However, since we did not define a group attack prior to the start of the experiment, we did not analyse these trials in more detail.

In 2013, we defined a group attack as joining the hunt while the fly was still moving and observed 15 group attacks in family groups, 10 in family groups including two foreigners and three group attacks in assorted groups. Averaged for each group, we found that family groups were significantly more likely to attack as a group than assorted groups (group attacks$_{family}$: 1.36 ± 0.4; group attacks$_{assorted}$: 0.27 ± 0.14; Wilcoxon test: Z = 2.09, N = 22, P = 0.036), while the number of group attacks did not differ between family groups and family groups including two foreigners (paired sign test: M = -1.5, P = 0.45). In the family + 2 treatment, the group-attacking individuals were siblings in seven cases, in two cases a family member and an unrelated spiderling attacked together and in one case two foreigners attacked together. Group attacks were always successful, whereas attacks of single spiders could result in the spider losing the prey (N_{2011} = 31, N_{2013} = 6 trials).

After a prey item was captured, we observed communal feeding in all treatments, meaning that spiderlings that had not contributed to the hunt were able to feed on the fly. It was rare that all spiderlings of the group fed simultaneously but family groups fed in significantly larger numbers than the assorted groups in both years (Figure 2) and the number of communally feeding spiderlings was predicted by the average initial spiderling mass in 2013 (Table 2). Family groups moreover fed in larger numbers than the corresponding family groups including two foreigners (paired t test: t_{10} = 3.4, P = 0.007).

During the 2 hour feeding period, attacked flies lost on average 28.68 ± 1.90% of their body mass in 2011 and 35.29 ± 1.99% in 2013. The percentage of total extracted prey mass was significantly different between family and assorted groups in both years and was moreover explained by the initial spiderling mass in 2011

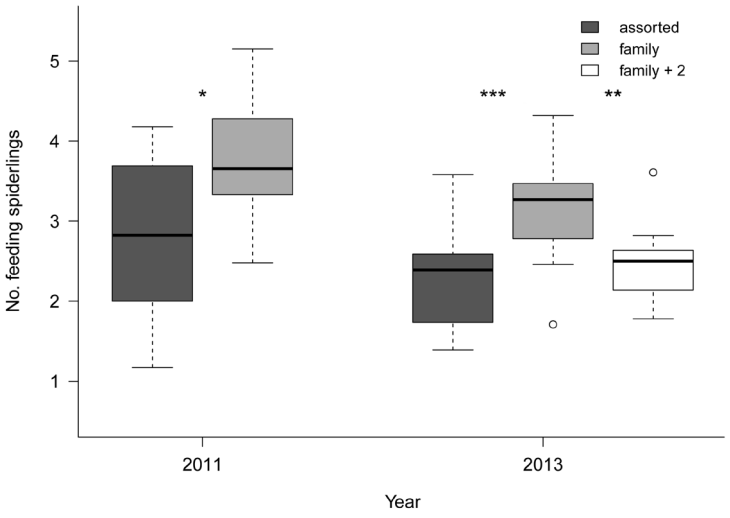

Figure 2: The average number of communally feeding spiderlings in family and assorted groups, shown separately for 2011 and 2013, as well as family groups including two foreign spiderlings in 2013 (family + 2). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are outliers.Dark grey boxes show assorted groups, light grey family groups and white boxes 'family + 2 foreign' groups. Asterisks indicate a statistically significant difference between the treatments. * P < 0.05; ** P < 0.01; *** P < 0.0001.

Table 3: Analyses of individual mass increase in 2013

Response	Analyses	Explanatory variables	Test	*df*	*P*
Individual mass increase	GLS with normal distribution, correlation structure specified as exchangeable (compound symmetry) for group ID *N* = 33 groups, 214 individuals	Treatment (family/assorted/family + 2)	*L-ratio* = 23.38	5	**< 0.0001**
		Number of each individual's attacks	*L-ratio* = 9.95	6	**0.002**
		Total feeding time	*L-ratio*= 7.45	6	**0.006**
		Total feeding time * number of each individual's attacks	*L-ratio* = 0.38	7	0.54

Test statistics, *df* and *P* values of non-significant variables stem from the step when a variable was dropped from the model, and df vary depending on the number of remaining variables. Bold *P* values show significant variables that remained in the final model.

(Table 2), with smaller spiderlings feeding in larger numbers Family groups extracted more prey mass than family groups with two foreign spiderlings (paired *t* test: $t_{10} = 4.09$, $P = 0.002$; Figure 3).

The per capita extracted prey mass (calculated as the total mass extracted/number of feeding spiders) was significantly different between family and assorted groups in 2013 (Table 2), but not between family groups and family groups including two foreigners (paired *t* test: $t_{10} = 0.26$, $P = 0.8$). In 2011, it was only predicted by the initial spiderling mass (Table 2), with larger spiderlings extracting more prey mass

Spiderling growth

We compared spiderling group mass when the spiderlings were placed into the petri dishes and after the experiment. We found that family groups had a higher relative mass increase compared with both, the corresponding family groups with two foreign spiderlings (paired *t* test: $t_{10} = 3.39$, $P = 0.007$; Figure 4) and the assorted groups (only significantly higher in 2013; Figure 4, Table 2). In 2011, the relative mass increase was significantly higher in groups with more successful feeding trials (Table 2).

Although the coefficient of variation in body

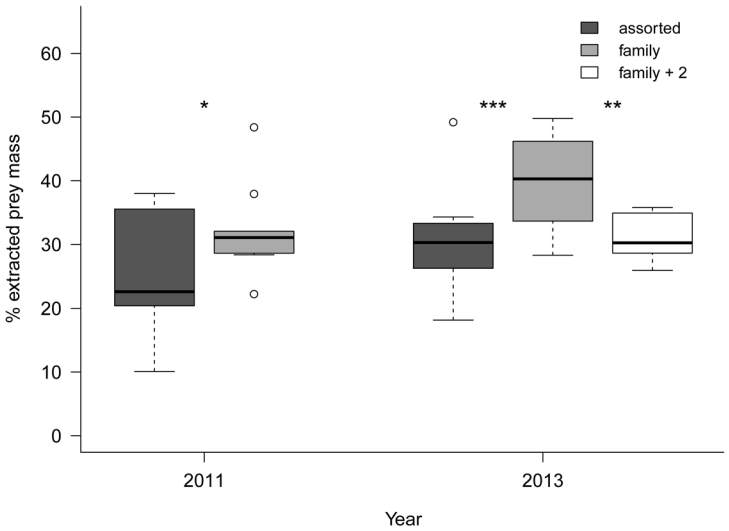

Figure 3: Percentage of prey mass that spiderlings extracted from a prey item in family and assorted groups, displayed separately for 2011 and 2013, as well as family groups including two foreign spiderlings in 2013 (family + 2). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are outliers. Asterisks indicate a statistically significant difference between the treatments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$. Dark grey boxes show assorted groups, light grey family groups and white boxes 'family + 2 foreign' groups.

mass (CV_{bm}) was similar for all treatments and both years at the beginning of the experiment (Table 1), we found a significantly lower CV_{bm} in family groups at the end of the experiment in 2011.

In 2013, when we followed each individual, the relative mass increase was higher when spiderlings initiated attacks and when they had a higher total feeding time (Table 3). In addition, the individual mass increase was significantly higher for spiderlings from family groups.

Mortality

Mortality did not differ between family and assorted groups, and treatment was dropped from the models of both years. The proportion of dead spiderlings was significantly higher in groups with less successful feeding trials in 2011 (Table 2). In 2013, mortality did not differ between family groups with two foreign spiderlings and the corresponding family groups (paired sign test: $M = 1.5$, $P = 0.38$).

Discussion

We tested whether group performance is affected by immigrating spiderlings in the non-webbuilding subsocial spider *D. ergandros*. As predicted, group hunting was more frequently found in family groups. Family groups moreover fed in larger numbers, extracted more prey mass and grew better compared with family groups including two foreigners and spiderlings in assorted groups.

Group hunting is predicted to be stable when it improves the individual hunting success (Packer and Ruttan, 1988). Even though group hunting occurred rarely in our laboratory experiment, spiderlings attacking as a group were always successful in overwhelming the prey, whereas singly attacking spiderlings faced the risk of losing the prey. Similar patterns were found in the group-hunting ant *Leptogenys diminuta*, in which individuals attack small prey items alone, but recruit other ants when attacking larger prey (Witte et al., 2010).

As in other taxa (Stevens and Gilby, 2004),

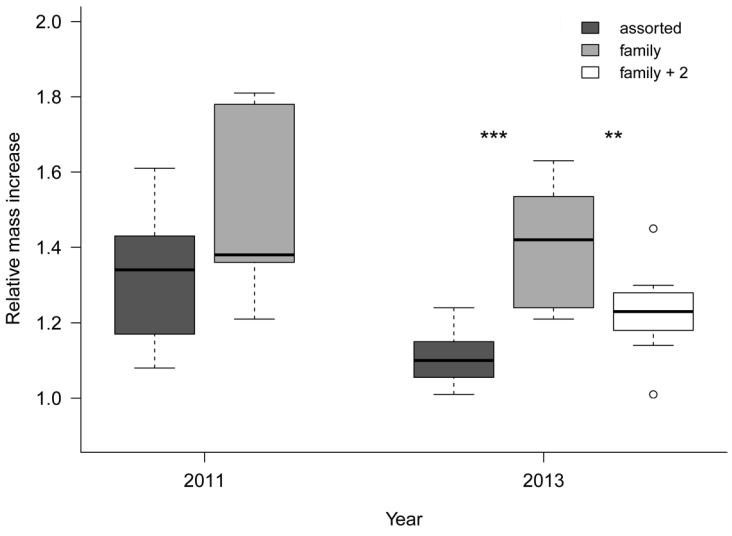

Figure 4: Relative mass increase of family and assorted groups in 2011 and 2013 as well as for family groups including two foreigners in 2013 (family + 2). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are outliers. Asterisks indicate a statistically significant difference between the treatments. ** $P < 0.01$; *** $P < 0.0001$. Dark grey boxes show assorted groups, light grey family groups and white boxes 'family + 2 foreign' groups.

group hunting in *D. ergandros* might increase the foraging success of all group members under natural conditions because of higher prey capture rates. By group hunting, the spiderlings might have access to resources that they could not exploit as solitary hunters, for example defended prey such as ants or wasps, which can be regularly found in *D. ergandros* nests (Evans, 1998a). The potential to hunt as a group allows increased flexibility towards large prey types (Rypstra, 1993) and could be interpreted as an adaptation to uncertain prey abundance and composition.

Assorted spiderlings in our experiment rarely attacked as a group; however, groups with only two foreigners and family groups did not differ in the frequency of group attacks. Thus, nests consisting of a high percentage of immigrant spiders might be unable to overwhelm well-defended and large prey when individuals rarely attack as a group.

In our experiment, initiating an attack was always beneficial for the attacking spiderlings. Those spiderlings that attacked the prey foraged for a longer time, which was positively correlated with growth. Similarly, attacking individuals of the web-building social spider *Stegodyphus dumicola* grew best (Whitehouse and Lubin, 1999). All these findings are based on experiments in the laboratory and exclude a potential predation risk for the attacking spiders themselves. Thus, initiating an attack may be costly in the presence of predators (Riechert and Hedrick, 1990; Unglaub et al., 2013).

Unlike web-building social spiders (Rypstra, 1993) and group-hunting ants (Witte et al., 2010), group-hunting crab spiders do not obviously recruit group members to join an attack, so their group hunting is a rather passive form of cooperation (Bailey et al., 2013). This differs from cooperatively hunting vertebrates with high cognitive abilities, in which individuals coordinate their hunting behaviour. Chimpanzees, *Pan troglodytes*, for example, adjust their own hunting behaviour depending on the action of another individual (Boesch and Boesch, 1989).

Besides the differences in group hunting between family and assorted groups, we found that family members fed in larger numbers and extracted a higher percentage of prey mass. Differences between family groups and assorted spiderlings were more pronounced in 2013 than in 2011, when the spiderlings were much smaller. This suggests that the hunting and foraging behaviour is affected by spiderling size. For example, spiderlings in 2011 had a much lower attack success rate and extracted a lower percentage of prey mass than spiderlings in 2013.

In spite of these differences, the direction of the treatment effect was the same in both years. Family groups shared the prey item among more individuals and these spiderlings also extracted more prey mass and grew better compared with assorted groups and families including two foreign spiderlings. The latter indicates that the presence of only a few immigrant spiderlings negatively affects group dynamics. The causes behind the effects are unknown and deserve further research.

Relative growth rates determine the reproductive success and thus fitness of colony members in the social spider *Anelosimus eximius* (Rypstra, 1993) , which may also apply in *D. ergandros*. In *Stegodyphus lineatus,* a web-building subsocial spider, sibling groups have an advantage in growth over nonsiblings as

well (Schneider and Bilde, 2008). In the latter species, the per capita extracted prey mass was lower when spiders were feeding communally in unrelated groups, perhaps because unrelated individuals contributed less digestive enzyme (Schneider and Bilde, 2008). In *D. ergandros*, we also found a higher per capita extracted prey mass in family groups than assorted spiderlings in 2013 and were able to link the lower individual mass increase to each individual's foraging activity. It is moreover possible that digestive enzymes are incompatible when unrelated spiderlings share prey (Schneider, 1996) which could explain the lower per capita extracted prey mass of assorted spiderlings in 2013.

Although some individuals hardly ever participated in hunting and feeding, which consequently resulted in lower growth, assorted spiderlings regularly shared prey. A possible explanation could be that sharing the prey is more beneficial than fighting over the prey item (Stevens and Gilby, 2004). We rarely observed fights or obvious monopolization of the prey and thus an interesting challenge would be to identify the mechanism that discourages individuals from participating in feeding.

The difference in hunting and feeding behaviour between our experimental groups might be the result of either low relatedness or a lack of familiarity in the assorted groups. Individuals that immigrate into natural nests of conspecifics are neither familiar nor closely related. Spider interactions can be affected by both, as reported in the non-webbuilding spider *Delena cancerides,* which accepts immigrating spiders (Yip et al., 2012). Relatedness and familiarity affect feeding performance in a range of taxa, including mammals (Valsecchi et al., 1996). In the subsocial spider *S. lineatus,* however, relatedness but not familiarity caused the above-mentioned differences in cooperative feeding behaviour (Schneider and Bilde, 2008).

A lack of familiarity is expected to cause behavioural differences in the early stages of our experiment but these differences would probably decline over time. For example, fish become familiar after around 12 days of cohabitation (Griffiths and Magurran, 1997). Since we found that group attacks took place at all stages of the experiment and spiderlings of all treatments were foraging communally from the beginning, we suggest that relatedness rather than familiarity might cause these differences in *D. ergandros*. As individuals are able to distinguish between related and unrelated spiders (Evans, 1999), it is likely that spiderlings invest more in group activities when living in family groups.

In conclusion, we found that group composition affected group-hunting performance and growth in *D. ergandros*. Spiders in the assorted groups invested less, which resulted in lower growth. However, accepting immigrants seems to have beneficial effects in subsocial crab spiders, such as better protection from predators in large groups (Unglaub et al., 2013). These benefits may outweigh the costs of reduced communal hunting and feeding and explain the acceptance of immigrant spiderlings.

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Chapter 7

Hunted hunters? Effect of group size on predation risk and growth in the Australian subsocial crab spider *Diaea ergandros*

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Abstract

A reduced predation risk is considered to be a major adaptive advantage of sociality. While most studies are concerned with non-predatory prey species, group-living predators are likely to face similar threats from higher-order predators. We studied the relationship between group size and predation risk in the subsocial crab spider *Diaea ergandros* by testing predictions from theoretical models including attack abatement as well as the formation of protective retreats. In a field survey, we found predatory clubionid spiders in 35% of the *D. ergandros* nests and as predicted, nest size did not correlate with predator presence. In a subsequent laboratory experiment we observed survival probability, nest construction activity and feeding behaviour including weight development between groups of different sizes as well as in the absence or presence of a predator. Large groups had an advantage in terms of survival and growth compared to smaller groups or single individuals. They also built significantly larger nests than smaller groups, supporting the idea of protective retreat formation being an adaptive benefit to group living. Even though clubionids did attack *D. ergandros*, they did not significantly affect overall mortality of *D. ergandros*. The feeding experiment showed that spiders fed on a larger proportion of flies in the presence of a predator. However, these groups gained significantly less weight compared to the control groups, indicating that the potential predators not only act as predators but also as food competitors, constituting a two-fold cost for *D. ergandros*.

Keywords: Sociality, spiders, group living, predation risk, intraguild predation, cooperation

Introduction

The evolution and maintenance of social behaviour are amongst the most puzzling themes in animal biology. Group living has been associated with numerous costs, such as competition for resources (Blumstein et al., 2002; Grand and Dill, 1999; Grove, 2012) and conflict over reproduction (Huchard and Cowlishaw, 2011; Macedo et al., 2004; Rypstra, 1993). It is generally assumed that substantial benefits must outweigh inherent costs of group living for sociality to evolve and to be maintained (Alexander, 1974).

A frequently proclaimed advantage of group living is a reduction of predation risk (Hamilton, 1971; Inman and Krebs, 1987; Sorato et al., 2012; Uetz and Hieber, 1994; Yip and Rayor, 2011). Two simple mechanisms that might account for this are the encounter effect and the dilution effect (Inman and Krebs, 1987; Turner and Pitcher, 1986; Uetz and Hieber, 1994). The encounter effect predicts that predators encounter group-living prey less frequently compared to singly-living prey that is scattered in a certain environment (Inman and Krebs, 1987). Moreover, the probability of detection by predators increases to a lesser degree than group size does, thus there is no linear effect that would predict that larger groups are more likely to be detected.

Once detection has occurred, the dilution effect predicts each individual´s risk of being captured as an inverse function of group size. Turner and Pitcher (1986) have suggested that the mechanisms of encounter avoidance and risk dilution simultaneously occur together as a single process which they termed attack abatement.

A recent model has shown that potential prey is less likely to be detected when aggregated, highlighting group living as a benefit of predator avoidance (Ioannou et al., 2011). On an individual level this implies a reduced relative risk for individuals in larger groups due to the dilution effect, as for example found in mobile systems like birds (Beauchamp, 2012; Sorato et al., 2012) and zooplankton (Jensen and Larsson, 2002).

The dilution effect can also be found in less mobile systems such as spiders: in the colonial spider *Metepeira incrassata*, the per capita risk of suffering from a kleptoparasite was reduced with increasing colony size (McCrate and Uetz, 2010). Although the vast majority of spiders are solitary, aggressive and potentially cannibalistic predators (Choe and Crespi, 1997; Whitehouse and Lubin, 2005), group living and sociality in spiders evolved many times independently (Lubin and Bilde, 2007). Spider colonies are relatively sedentary and thus particularly vulnerable to predation (Lubin, 1974; Rayor and Uetz, 1990; Rayor and Uetz, 1993). Hence, spiders provide an ideal model to examine costs of predation and potential benefits of group living.

The Australian subsocial crab spider *Diaea ergandros* Evans, 1995 belongs to one of the two genera that include social species (*Diaea* and *Delena* (Rowell and Aviles, 1995) which lack any form of a capture web, although capture webs are regarded as an important condition for the evolution of sociality in spiders (Avilés, 1997; Yip and Rayor, 2011). Hence, many benefits of group living for social spiders that mutually share a snare web may not apply in subsocial and social crab spiders (Yip and Rayor, 2011). Moreover, spiderlings stay together in

their natal communal nest from hatching until maturation (approximately eight months), much longer than other subsocial spiders. For example, in the subsocial spider *Stegodyphus lineatus* spiderlings usually disperse four weeks after hatching (Schneider, 1995).

During the period of group living, *D. ergandros* communally enlarge the nest by attaching more leaves and they also hunt in groups (Ruch, personal observation). Nest size increases with the number of group members and nests show a labyrinthine structure, which may protect against predators (Evans, 1998a). The most frequent predators seem to be other spiders (Clubionidae). Clubionids are vagrant spiders that infiltrate the nests of and prey on *D. ergandros* (Evans, 1998a). Thus, one of the benefits of group living may be increased protection from predation by clubionids. Furthermore, complex communal webs or nests may function as protective retreats, further reducing predation risk (Evans, 1998a; Henschel, 1998; Manicom et al., 2008; Seibt and Wickler, 1990). Evans (1998a) for example found that the lack of a protective retreat led to 100% mortality in *D. ergandros* when faced with *Clubiona robusta,* whereas those having a protective retreat had a mortality of 5%.

We aimed to investigate the role of and the relationship between group size and predation risk in *D. ergandros* in the field and in the laboratory. The central hypothesis is that predation risk is reduced by group living and that larger groups are more resistant to predators than smaller groups. Possible hypotheses that we investigated include attack abatement and the formation of protective retreats.

According to the idea of attack abatement, predator recognition is not a proportional function of group size. We predicted that infestation by clubionids and nest size only show a weak (if any) relationship, which we tested in a field survey. According to the dilution effect, we expected that individuals from large groups are less vulnerable to predation.

However, in addition to the dilution effect we predicted that living in large groups is beneficial due to more complex protective retreats. We tested the latter in the laboratory by quantifying how the presence of a predator affects survival of individuals living in groups of different sizes. In terms of the protective-retreat hypothesis we had two predictions. First, groups exposed to predators will increase nest construction activity and second, individuals are more likely to occupy the retreat in the presence of a predator. Finally, the presence of a predator can affect *D. ergandros* by altering their risk-taking behaviour, where we predict spiders to be less active, forage less and consequently gain less weight.

Methods

Study Species

Diaea ergandros Evans, 1995 (Thomisidae) is a non-territorial, periodically-social (subsocial) spider. This annual species inhabits the foliage of *Eucalyptus* trees in closed-canopy forests in South-Eastern Australia (Evans, 1997). In *D. ergandros*, groups originate as the offspring of a single female that migrated from her natal colony after mating. The female constructs a nest from several *Eucalyptus* leaves and lays a single egg sac containing 15-80 eggs therein (Evans, 1995).

After the eggs hatch, the mother continues to expand the brood chamber and catches

prey to feed her young. Finally, maternal care culminates in self-sacrifice (matriphagy). The mother develops trophic eggs that are never laid and she is eventually eaten by her young (Evans et al., 1995). After the mother´s death, the offspring stay in her nest and communally continue to append new leaves onto it. Leaves are tightly attached with silk threads and form a labyrinthine structure inside, which may protect against predators (Evans, 1998a). Furthermore, nests serve as foraging areas and spiders hunt without a capture web by ambushing prey (Evans, 1998a). Most commonly found prey items are Hymenoptera (ants and wasps) and Coleoptera (beetles) (Evans, 1998a). In the subsequent spring, the offspring mature and disperse after mating.

Clubiona robusta L. Koch, 1873 are mainly found under bark of *Eucalyptus* trees where they build silken retreats (Austin, 1984). Females can produce two broods per year without being restricted to a specific season. Thus, adults and juvenile spiders of all instars can be found throughout the year. These spiders are nocturnal and mainly prey on Hymenoptera and Coleoptera (Austin, 1984). *C. robusta* can be frequently found in and around *D. ergandros* nests and Evans (1998a) demonstrated that these spiders prey on *D. ergandros*.

Field Survey

A total of 88 nests were collected during April and June 2011 in Yass (34°55'20.50"S, 149°6'15.53"E) and Boorowa (34°25'53.31"S, 148°43'49.47"E) (NSW, Australia) and dissected in the laboratory at Macquarie University, Sydney. Adult females (if present) were weighed to the nearest 0.1 mg, using an electronic balance (Mettler Toledo New Classic MS) and the tibia-patella length of the first left leg was measured with digital callipers. Spiderlings were removed from the nests and counted. Ten individuals out of each nest were weighed in order to calculate the mean spiderling weight. Clubionids inside the nests were counted as well.

Group size may affect nest size, hence the following nest characteristics were recorded: Length (a) and width (b) of nests were measured using a digital calliper. Nest size was calculated by the formula for ellipsoid volume ($V = 4/3\pi \cdot a/2 \cdot b/2^2$). Possible nest entrances were identified and counted and we counted the number of brown (old) and green (fresh) leaves used for nest construction. After removal of spiders and debris, leaves were dried in an oven (70°C for 25.5 hours followed by 90°C for one hour) and weighed afterwards.

Experimental setup in the laboratory

Overall, 82 artificial groups of four different sizes (G1: one individual, G5: five individuals, G10: ten individuals and G25: 25 individuals) were established from 875 inhabitants of the collected nests. All groups consisted of siblings between 3rd and 5th instar (all referred to as spiderlings). Spiderlings were individually weighed before placing them into containers. Individual spiderling weight ranged from 4.04 mg to 18.4 mg (8.5 ± 0.11 mg, $N = 875$) before the experiment commenced. Each group was placed into an inverted plastic cup (capacity 500 ml) whose bottom was cropped and covered with mesh.

In every cup, we attached 22 deformable,

Table 1: Distribution of the 76 groups over four different group sizes and two treatments (predator present = experimental, predator absent = control)

Group size	*N* Exper. groups	*N* Control groups	Σ
single (G1)	9	8	17
small (G5)	10	10	20
medium (G10)	10	10	20
large (G25)	10	9	19
Σ	39	37	76

transparent plastic strips to the inside of the top surface in order to provide nesting material. From six of these groups some individuals escaped from the containers within the first few days. These groups were excluded from all statistical analyses except for the data on foraging, resulting in a total of 76 groups.

Since a number of individuals died over the course of the experiment, group sizes were now defined as ranges: *single*: one individual, *small*: two-five individuals, *medium*: six-ten individuals, *large*: eleven-25 individuals.

Spiders were sprayed with water daily and were fed with Queensland fruit flies, *Bactrocera tryoni* (Tephrididae) during a two-week habituation time.

After two weeks, we allocated half of the nests to the experimental treatment and half to the control treatment, making sure that the group sizes were balanced across both treatments (Table 1). We placed a single *Clubiona* spp. (65.19 ± 7.15 mg, $N = 58$) as predator into the containers of the experimental groups of each group size ($N = 39$, Table 1) over a period of four weeks. In cases where clubionids died (for unknown reasons) they were replaced. The remaining groups served as control groups ($N = 37$, Table 1) without the addition of a predator. We compared the survival probability, the nest construction activity as well as the foraging behaviour and weight gain between the different group sizes with and without a predator present. Weight gain was expressed as [(average spiderling weight end – average spiderling weight start)/(average spiderling weight start) * 100].

The average start weight did not differ between the three group sizes (Wilcoxon each-pair comparison, Table 2). All groups experienced a cohabitation phase of at least two weeks after the first weighing event and before the clubionids were placed into the containers of the experimental

Table 2. Weight development of spiderlings according to group size

	small	medium	large	Test	*P*
Mean body weights before experiment [mg]	7.3 ± 0.5	8.0 ± 0.62	9.2 ± 0.71	*Wilcoxon each-pair*	
	(*N* = 20)	(*N* = 19)	(*N* = 19)	small - large: *Z* = 1.9	0.06
				medium - large: *Z* = 1.2	0.22
				small - medium: *Z* = 0.7	0.46
Average weight gain/ day [%]	0.12 ± 0.1	0.07 ± 0.063	0.35 ± 0.078	*ANOVA*	
	(*N* = 13)	(*N* = 18)	(*N* = 19)	$F_{2,44} = 3.87$	**0.03**

Mean initial weight did not differ between group sizes. Large groups gained significantly more weight than medium groups (with and without a predator present) and also more than small groups (in the presence of a predator).

groups, leading to an average duration of 48 ± 1.3 days between the two weighing events. To control for the different durations of test periods, weight change was calculated and expressed as the relative (%) weight gain per day. Due to insufficient data points, *singles* had to be excluded from statistical analyses except for the analyses of nest construction activity and the survival analyses.

Survival probability: Effect of group size

We experimentally investigated the influence of group size and predation risk on survival by measuring mortality in *D. ergandros* groups over four weeks. Four weeks after clubionids were added to the experimental groups, the nests and those of corresponding control groups were dissected. Surviving *D. ergandros* were counted and weighed. The dilution effect predicts that mortality will decrease proportionally with group size. Here we test whether this statistical effect alone is present or whether there is an additional benefit of group size. We analysed the overall mortality using a Cox proportional hazard model and Kaplan-Meier survival estimations.

Protective retreats: Nest construction activity

To detect possible differences in nest construction activity due to group size or predation risk, the number of curled leaves (as a measure of nest construction activity) was recorded before clubionids were introduced to the experimental groups and two weeks thereafter. The number of curled leaves was determined as the difference between 22 available leaves and the number of uncurled leaves, which could be counted readily. The first measure was thought to reflect differences in nest construction activity due to group size whereas possible effects of or interactions with predation risk were investigated by analysing the difference of curled leaves between the second and the first count using Wilcoxon signed-rank tests.

Hunt or being hunted? Foraging in presence of a predator

After the introduction of a predator to half of the groups, a foraging experiment was started. Once a week for an overall period of four weeks (resulting in four trials), all groups were fed with Queensland fruit flies (*Bactrocera tryoni,* weight ~9 mg). We controlled for group size by adding one fly per every 2.5 living *D. ergandros*. To investigate the foraging behaviour of *D. ergandros* housed in differently sized groups in the presence and absence of predators, all groups were observed for a period of 60 minutes after the addition of flies. Within that period, we recorded whether and when clubionids attacked either *D. ergandros* or *B. tryoni.*

In order to investigate if group size and predation risk influence whether *D. ergandros* stay inside the nest (e.g. to seek a safe position) or rather tend to leave the nest (e.g. to catch prey), spiderlings outside the nest were counted. For the analyses, we excluded those groups where spiderlings had not yet built a nest.

The latency to first attack by *D. ergandros* on *B. tryoni* was recorded in order to determine effects of predation risk and group size. Since latency of first attack could not always be ascertained accurately due to the large number of observation groups, it was always expressed

according to the next fixed observation point (15, 30, 45 or 60 minutes).

For the analyses of the latency to attack we excluded all groups that did not attack within the 60-minutes observation period. Moreover, we recorded the number of flies being eaten by *D. ergandros*. Since there are alternative approaches to analyse data with repeated measurements we used generalised linear mixed models (GLMMs) and generalised estimating equations (GEE) (Zuur et al., 2009).

Statistical Analyses

Statistical data analyses were performed using JMP 9.0.2 (*SAS Institute Inc., Cary, NC, USA*) and R version 2.15.0 (*R Core Team, 2012*). JMP was used for all analyses except for the generalised linear mixed models (GLMMs) and the generalised estimating equations (GEE) (proportion of spiderlings outside the protective retreat, latency to attack and the proportion of eaten flies). Continuous data were tested for normal distribution using the Shapiro-Wilk W test.

Raw data from the field survey were *log*-transformed to achieve normal distribution allowing for the use of parametric tests (nest size, dry weight of leaves, number of leaves, number of entrances, mean weight of spiderlings inside the nest). If normal distribution could not be achieved, non-parametric tests were used. Descriptive statistics are given as mean ± standard error (SE) if not specified other.

The GLMMs were performed using the *lme4* package (under R version 2.15.1). Post-hoc tests for the GLMM with normal error distribution were conducted using the *multcomp* package (under R version 2.15.2). The GEEs were performed using the *geepack* package (under R version 2.15.2).

Table 3. GLMM analyses of the foraging experiment

Response variable	Analyses	Explanatory variables	χ^2_1 GLMM	*df* GLMM	*P* GLMM	N_{groups}
Proportion of spiderlings outside the nest	GLMM Error function: binomial (logit link) Random factor: group ID	Predator (present/absent)	3.7	8	0.054	48
		Group size (small, medium, large)	11.8	7	**0.003**	
		Predator * Group size	0.17	10	0.92	
		Feeding trial	23.74	7	**<0.0001**	
Latency to attack	GLMM Error function: normal (identity link) Random factor: group ID	Predator (present/absent)	0.52	6	0.47	57
		Group size (small, medium, large)	12.3	5	**0.002**	
		Predator * Group size	2.9	11	0.23	
		Feeding trial	1.8	9	0.61	
Proportion of flies being eaten	GLMM Error function: binomial (logit link) Random factor: group ID	Predator (present/absent)	9.3	3	**0.002**	62
		Group size (small, medium, large)	5.6	5	0.059	
		Predator * Group size	0.3	10	0.86	
		Feeding trial	4.2	8	0.24	

We tested the explanatory variables predator, group size as well as their interaction (*) and feeding trial for each response variable. '*df*' represents the degrees of freedom for the whole model and stem from the step when a variable was dropped from the model.

Data on the latency to attack were transformed (sqrt) to fulfil model assumptions; all other data fulfilled the model assumptions. All GLMMs and GEEs contained the same explanatory variables (Table 3, Appendix 1: Table 6).

For the GLMM, we included group ID as random factor to control for repeated measurements. We simplified maximal models by stepwise elimination of the least significant variable and comparing the models with ANOVAs. We always started eliminating with the interaction and compared the models until the most parsimonious model with the lowest AIC to explain the response variable was determined. The reported non-significant *P*-values and degrees of freedom stem from the step when a variable was dropped from the model.

For the GEEs group ID was specified as ID to control for repeated measurements and we specified the association structure as auto-regressive (AR1). We tested the significance of the variables with a Wald-test and eliminated those variables that were not significant (Appendix 1: Table 6). Since both approaches (GLMM and GEE) led to similar results, we only present those of the GLMM in the main text and moved the GEE results into an appendix.

Results

Field Survey

We examined 88 *Diaea ergandros* nests from the field and found clubionids (*Clubiona* spp.) in 35.23% (N = 31) of collected nests. Overall, we counted 92 clubionids of different instars. In two nests, there were more clubionids than *D. ergandros*: i) one female and one spiderling vs. 22 clubionids; ii) no *D. ergandros* vs. 13 clubionids. Both these outliers were excluded from the statistical analysis. In 17 % we found one or more clubionid nests (1.67 ± 0.98 nests, N = 15) near a *D. ergandros* nest (within 20 cm radius).

A living adult *D. ergandros* female (presumably the mother) was found in 62.5% of the nests (N = 55). The probability of clubionids present inside a nest was higher in the absence of an adult female (Likelihood-ratio test: χ^2_1 = 5.97, P = 0.01). As predicted, group size and nest size did not affect whether nests were infested by clubionid predators (Unpaired t-tests: t_{80} = - 1.28, P = 0.21 (group size); t_{83} = 0.31, P = 0.76 (nest size)). Furthermore, in infested nests group size and nest size did not correlate with the number of predators present (Spearman rank correlations: r_s = - 0.31, P = 0.13 (group size); r_s = 0.07, P = 0.71 (nest size)).

Nests with or without clubionids did not differ in any other measures (dry weight of leaves, number of leaves, percentage of green leaves, number of entrances, number of clubionid nests close by; all P > 0.28). Interestingly, those nests with clubionids present included heavier *D. ergandros* spiderlings than those without (Unpaired t-test: t_{77} = 2.16, P = 0.03).

Laboratory Experiment

Survival probability: Effect of group size

Overall, there was no difference in mortality whether predators were present or not. However, there was a significant effect of group size on mortality risk (Likelihood-ratio test: χ^2_3 = 55.41, P < 0.0001). Single individuals had the highest

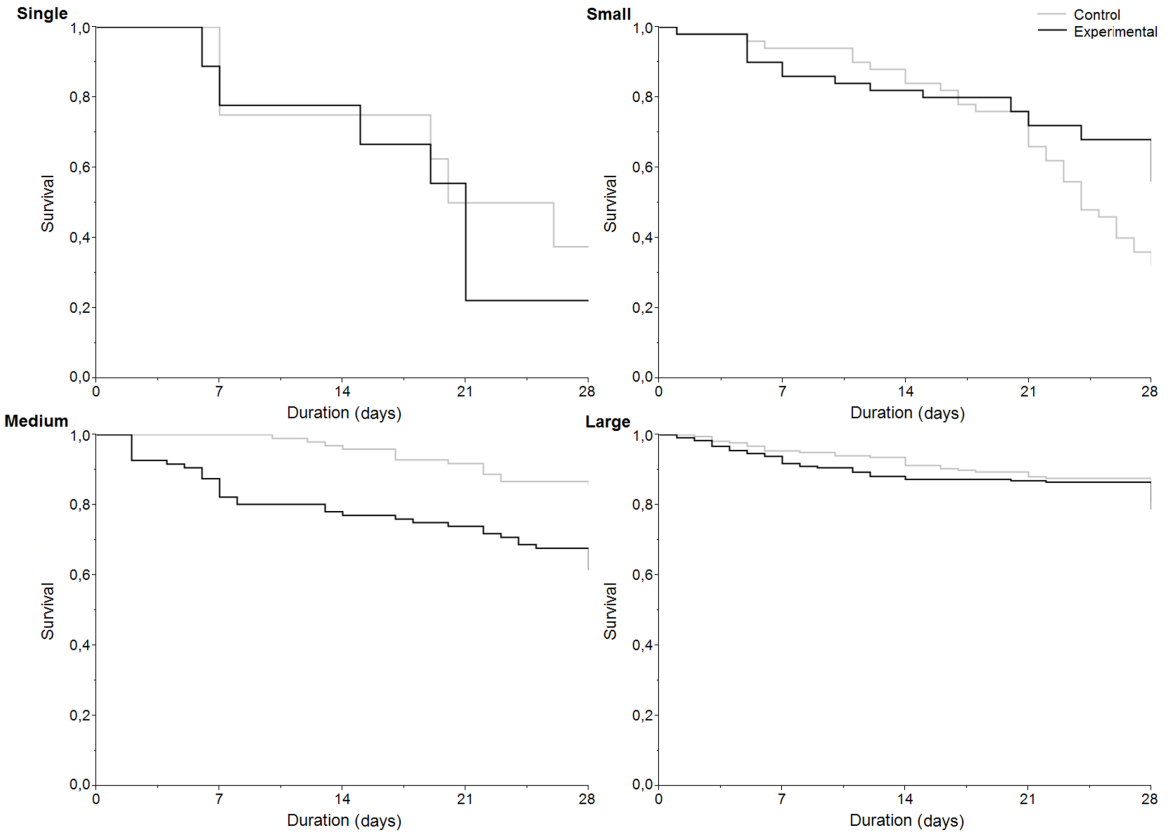

Figure 1. Kaplan-Meier survival estimations for single individuals, small, medium and large groups between experimental and control groups (predator present = experimental, predator absent = control). The presence of a predator had a significantly negative effect on survival in medium sized groups. Small groups had a lower survival probability in the control treatment. Survival was generally high in large groups and generally low in singles, independent of predator presence.

mortality risk and smaller groups had a higher mortality risk than larger groups. For example, the mortality risk for single individuals was 5.3 times higher than that for individuals of large groups (Table 4). While the main effect of predator presence was not significant (Likelihood-ratio test: $\chi^2_1 = 0.41$, $P = 0.52$), there was a significant interaction of group size and predation risk on survival (Likelihood-ratio test: $\chi^2_3 = 18.99$, $P = 0.0003$).

To investigate this interaction more closely, Kaplan-Meier survival estimations were used to display survival functions of different group sizes depending on predation risk graphically (Figure 1) and log-rank tests were conducted to test for statistical significance. In large groups ($\chi^2_1 = 0.49$, $P = 0.48$) and singles ($\chi^2_1 = 0.33$, $P = 0.57$) the presence of predators had no effect on spiderling mortality. While the mortality was overall very low in large groups, it was very high

Table 4. Hazard ratio (representing mortality risk), 95% confidence interval and *P*-value of a Cox proportional hazard model for group size and predation risk. Singles and small groups had a significantly higher mortality risk than medium and large groups. Mortality risk is not generally higher when a predator is present

Variables	Hazard ratio	95% confidence interval	*P*
single / small	1.58	0.80–2.87	0.18
single / medium	4.21	2.09–7.82	0.0002
single / large	5.26	2.69–9.29	<0.0001
small / medium	2.66	1.78–4.04	<0.0001
small / large	3.33	2.36–4.65	<0.0001
medium / large	1.25	0.85–1.79	0.22
E / C	1.45	0.46 - 4.90	0.53
E = experimental groups, C = control groups			

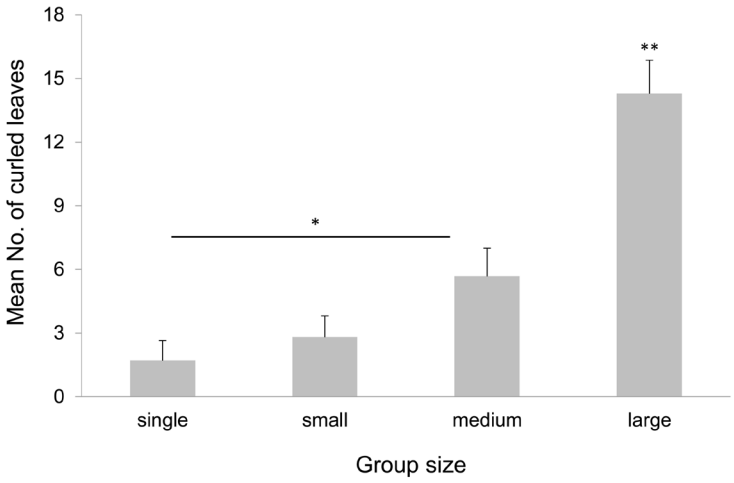

Figure 2. Nest construction activity across different groups sizes, quantified as mean number of curled leaves measured prior to the experimental period. Large groups had curled more leaves than all other groups and medium groups had curled more leaves than small groups. * $P < 0.05$ and ** $P < 0.01$ indicate a statistically significant difference between group sizes.

in singles. In medium sized groups however, mortality was greater in the presence of predators ($\chi^2_1 = 15.59$, $P < 0.0001$) but in small groups mortality was greater in the absence of predators ($\chi^2_1 = 5.17$, $P = 0.02$).

Protective retreats: Nest construction activity

Prior to the introduction of clubionids to half of the groups, there was a significant difference in nest construction activity between group sizes: large groups had curled significantly more leaves than all other groups (Wilcoxon signed-rank test: all $P < 0.01$), while medium groups had curled more leaves than singles (Wilcoxon signed-rank test: $T = 268.5$, $N = 38$, $P < 0.05$, Figure 2).

During the following two weeks, all groups added more leaves to their nests but contrary to our prediction, the presence of a predator did not lead to an increase of nest construction activity compared to predator absence (Wilcoxon signed-rank tests $T = 1143$, $N = 66$, $P = 0.14$).

Hunt or being hunted? Foraging in presence of a predator

We calculated the proportion of spiderlings outside the nest to estimate whether spiderlings would leave the nest and forage or stay inside the protective retreat. Group size significantly affected the proportion of spiderlings outside the nest. A lower proportion of spiderlings in large groups was found to be outside the nest compared to medium and small groups (Table 3, Table 5, Figure 3). Moreover, spiderlings were less frequently outside the nest in later feeding trials (Table 3, Figure 3). There was a trend that spiderlings were more frequently outside the nest when a predator was present, albeit not significant (Table 3).

Group size had an effect on the latency to attack (Table 3, Table 5). Although large groups were found to have a lower proportion of spiderlings outside the nest, they attacked the flies earlier than small groups (post-hoc Tukey*:* $P = 0.001$).

The presence of a predator significantly affected the proportion of the provided flies being eaten (Table 3, Table 5, Figure 4). Across all groups, spiderlings fed on a larger proportion

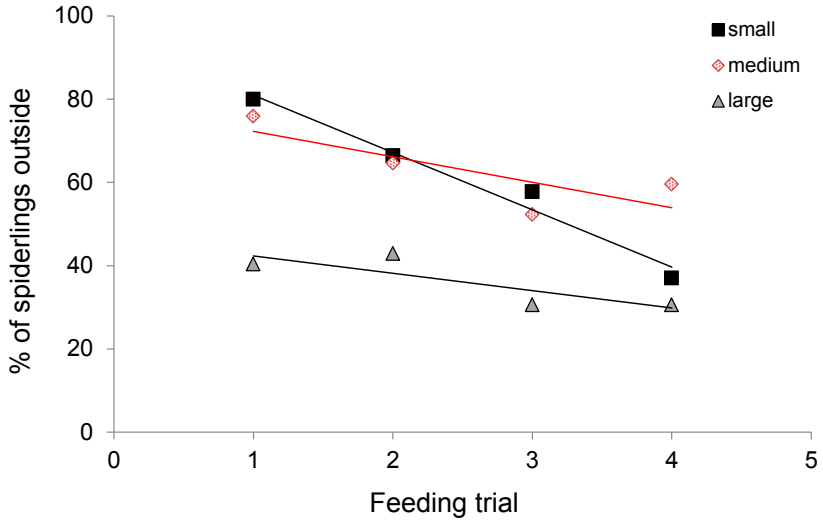

Figure 3. Percentage of *D. ergandros* outside the nest across feeding trials for the three group sizes. A higher percentage of spiderlings of medium and small groups was found outside the nest compared to spiderlings of large groups.

of flies when a predator was present. Group size moreover had a marginal effect on the proportion of eaten flies with spiderlings of small groups eating less flies than large groups (Table 3, Table 5).

Even though spiderlings ate more of the flies in the presence of a predator, they gained less weight than those spiderlings feeding in the absence of a predator (ANOVA: $F_{1,44} = 8.75$, $P = 0.005$, Table 5). Group size had a significant effect on weight gain as well ($F_{2,44} = 4.16$, $P = 0.02$) with spiderlings of large groups gaining more weight than spiderlings of medium groups (post-hoc Tukey: $P < 0.05$; Table 5). There was a significant interaction of treatment and group size ($F_{2,44} = 3.95$, $P = 0.03$), showing that within experimental groups, i.e. in predator presence, large groups also gained more weight than small groups (post-hoc Tukey: $P < 0.05$).

For the entire experiment, 16 attacks of *Clubiona* spp. were observed during the feeding trials, nine and seven of which were directed at *B. tryoni* and *D. ergandros* respectively across all group sizes. On average, clubionids attacked after 17.25 ± 3.86 minutes.

Discussion

We investigated the roles of and the relationship between group size and predation risk in the Australian subsocial crab spider *Diaea ergandros*. Our central hypothesis was that predation risk is

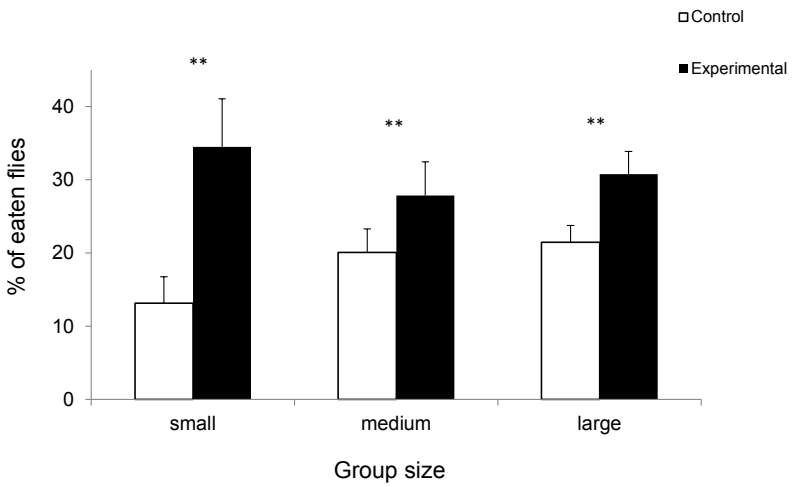

Figure 4. Percentage of flies eaten by *D. ergandros* according to treatment and group size. Experimental groups (with the predator present) of all group sizes consumed significantly more flies within the observation period compared to the control groups. ** $P < 0.01$ indicates a statistically significant difference between experimental conditions.

reduced by group living and that large groups are less vulnerable to predation.

We found that group size affected mortality with large groups having an overall lower mortality risk compared to small and medium sized groups. Large groups moreover built significantly larger nests (protective retreats) and gained more weight than smaller groups. Clubionids did attack *D. ergandros* and we found a significant effect on mortality in medium sized groups, however the overall effect of predator presence on mortality was not significant in this study. In the presence of a predator, spiderlings fed on a higher proportion of flies but gained less weight than the control groups without a predator, indicating that the clubionids not only act as predators but also as food competitors. According to the protective-retreats hypothesis, the construction of a nest could be a selective advantage for group living in *D. ergandros* (Evans, 1998a).

Even though spiderlings did not increase their construction activity in the presence of a predator in this study, large groups built significantly larger nests compared to all other groups and had a very low overall mortality. This indicates that the size and complexity of the nest may enhance protection from predation. Large groups had a very low overall mortality while medium groups with less complex nests suffered from a higher mortality when a predator was present. This is further supported by the finding that a lower proportion of individuals in large groups was found to be outside the nest during observation periods: predators may not have significantly affected mortality because most of the spiderlings were inside the protective nests (Evans, 1998a).

Small groups and singles hardly built nests and had the highest mortality rates, albeit independent of predator presence. Thus, we did not find support for the dilution effect in our laboratory experiment; however a potential effect may have been obscured by the extremely high overall mortality in small groups and singles and the differences in constructing protective retreats. The importance of nests as protective retreats was also shown in other spider species, including *Stegodyphus dumicola* (Henschel, 1998; Seibt and Wickler, 1990), *Stegodyphus mimosarum* (Seibt and Wickler, 1990), *Cyrtophora hirta*,

Table 5. Summary of the main effects of predator presence and group size on the response variables (latency to attack, % spiders outside the nest, % of eaten flies and relative weight gain/day (%), % mortality).

	Predator presence	Group sizes (small, medium and large)
Latency to first attack	no effect	lowest in large groups, highest in small groups
% of spiderlings outside the nest	no effect (trend for a higher percentage outside in predator presence)	lower percentage outside in large groups compared to medium and small groups
% of flies eaten	higher percentage of flies eaten	no effect (trend that large groups ate a higher percentage)
Average weight gain/ day [%]	lower weight gain	higher in large groups compared to medium groups *in predator presence:* higher in large groups compared to small groups
% Mortality	no effect	lowest in large groups, highest for single individuals

Phonognatha graeffei and *Theridion* spp. (Manicom et al., 2008) but also in birds. In the sociable weaver, individuals escaped from predators by hiding in their complex communal nest (Brown et al., 2003). Moreover, caterpillars constructed a retreat into which they moved back when encountering a predator (Mega and de Araujo, 2008).

The idea of spiderlings altering their risk-taking behaviour and being less active in the presence of a predator was not supported in our study. Contrary to our prediction, there was a trend that spiders were even more frequently outside the nest in the presence of a predator. A possible explanation for this trend could be that spiderlings were more vigilant because a predator was present. A larger proportion of spiderlings being outside would increase the likelihood of detecting a predator. However, we never observed spiderlings reacting to the predator, even when a group member was attacked and eaten (also see Evans, 1998a), suggesting that vigilance in this system plays a minor role compared to for example birds (Beauchamp and Ruxton, 2008; McNamara and Houston, 1992).

Interestingly, spiderlings fed on a larger proportion of flies during the observation period when a predator was present but still gained less weight compared to the groups without a predator present. Since we found clubionids attacking spiderlings as well as flies, we suggest that they might simultaneously be food competitors and predators. This makes sense when considering the overlap of prey groups between *Diaea ergandros* and *Clubiona robusta* with Hymenoptera and Coleoptera comprising main prey groups for both (Austin, 1984; Evans, 1998a). Thus, competition might even play a greater role than expected. Specifically, this interaction between clubionids and *D. ergandros* may be termed as asymmetric intraguild competition (predation) where one predator kills the other that has a similar food resource (Polis and Holt, 1992), although we do not have data on fitness gains for the clubionids used in this experiment. The idea of clubionids being competitors is further supported by our field study. Whenever clubionids were present, the nests contained heavier spiderlings. We found that larger nests were not more likely of being infested by a predator, so there was no linear effect between increasing group size and being detected by a clubionid. Clubionids may however stay in those *D. ergandros* nests that are more profitable in terms of overall prey availability but also those that are inhabited by larger, particularly well-nourished spiderlings (Evans, 1998a) while they might quickly move on when conditions are less profitable. Thus, clubionids might profit from and hence prefer environmental conditions that are generally beneficial for *D. ergandros* as well. Consequently, clubionids might constitute food competitors for *D. ergandros*, rather than exclusively predators – an effect that is absent in non-predatory animals.

As predicted, groups with a predator present gained less weight than groups without a predator in our experiment. We moreover found an effect of group size on weight gain with large groups gaining more weight than smaller groups. *D. ergandros* in large groups also attacked earlier and caught proportional more flies than smaller groups. This is an interesting finding because increasing group size often negatively affects individual feeding efficiency in group-living animals (Packer and Ruttan, 1988). Although larger groups may have a reduced variance in

prey capture and hunt more efficiently compared to smaller groups, for example in webbuilding social spiders (Buskirk, 1981; Rypstra, 1989; Spiller, 1992), the individual gains may differ. Increasing group size for example negatively affected feeding efficiency and growth in African social and subsocial spiders, indicating a cost of living in large groups (Ruch et al., 2009; Whitehouse and Lubin, 1999).

The opposite pattern found in our study indicates increased direct fitness benefits for individuals living in large groups. *D. ergandros* hunt without a web, so larger groups might be more efficient in hunting, for example by attacking as a group (Ruch, personal observation). Spiderlings in smaller groups may be unable to catch a sufficient amount of prey, which would also explain the overall high mortality in small groups and singles independent of predator presence.

An interesting addition to the effect of group size on mortality is the effect of a caring female. Our field data showed that the presence of a predator in *D. ergandros* is lower when the mother is present. This suggests an additional important role for maternal care. Evans (1998b) has already shown that in *D. ergandros* maternal care is essential for spiderling survival due to a number of reasons. For example, mothers carry out most of the early nest construction. They also capture large prey for their young and eventually may serve as a nutritious meal themselves (Evans et al., 1995). The current results however suggest that mothers could also play an important adaptive role by directly minimizing predation risk. Similarly, Yip and Rayor (2011) found that in another spider species, *Delena cancerides,* mothers are far more effective in predator defence than any other spider in the colony.

In conclusion, the results from the field and the laboratory suggest that large groups have a survival advantage over smaller groups, mainly due to the formation of larger protective retreats. Different from other subsocial spiders, large groups grew better than small groups, indicating a direct benefit of living in large groups. Moreover, maternal care seems to be important to lower predation risk. Clubionids may not only be predators but also food competitors, thereby constituting a two-fold cost for *Diaea ergandros*. Hence, even though some of the general anti-predatory adaptations promoting group-living in herbi- or insectivores may apply to predators as well, their effects will be different when predators not only threaten survival directly but also indirectly through competing over the same prey.

Author contributions statement

BU, JMS, JR and MEH conceived and designed the experiments. BU and JR conducted the experiments. JR and BU analysed the data. JR, BU, JMS and MEH wrote the manuscript. BU and JR have contributed equally to the presented work.

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Appendix 1

Table 6. GEE analyses of the foraging experiment

Response variable	Analyses	Explanatory variables	χ^2_1 GEE	*df* GEE	*P* GEE	N_{groups}
Proportion of spiderlings outside the nest	GEE Error function: binomial Association structure: AR1 ID: group ID	Predator (present/absent)	3.6	1	0.058	48
		Group size (small, medium, large)	12.8	2	**0.0017**	
		Predator * Group size	0.6	2	0.74	
		Feeding trial	7.9	3	**0.048**	
Latency to attack	GEE Error function: normal Association structure: AR1 ID: group ID	Predator (present/absent)	0.72	1	0.4	57
		Group size (small, medium, large)	13.1	2	**0.0014**	
		Predator * Group size	3.4	2	0.18	
		Feeding trial	1.46	3	0.69	
Proportion of flies being eaten	GEE Error function: binomial Association structure: AR1 ID: group ID	Predator (present/absent)	12.9	1	**0.0003**	62
		Group size (small, medium, large)	10.8	2	**0.0045**	
		Predator * Group size	0.57	2	0.75	
		Feeding trial	6.03	3	0.11	

We tested the explanatory variables predator, group size as well as their interaction (*) and feeding trial for each response variable. '*df*' represents the degrees of freedom for each variable obtained from the model comparison with Wald tests.

Chapter 8

General Discussion

Differences and similarities between Thomisidae with social behaviour and other group-living spiders

Sociality in spiders has evolved multiple times independently across six to eight families (depending on the definition of permanent sociality) (Agnarsson et al., 2006; Lubin and Bilde, 2007). A detailed knowledge of natural history and taxonomy of solitary, subsocial and social species is required to understand the selective agents driving the evolution of sociality across families. Yet, this knowledge is often scarce, which hinders broader comparative studies (Agnarsson, 2012; Yip and Rayor, 2013). Like in all spider families, social behaviour is rare in Thomisidae with only four subsocial or social species of the roughly 2100 thomisids described worldwide (Platnick, 2014), of which 126 species (25 genera) can be found in Australia (Szymkowiak, 2007).

The discovery of a subsocial thomisid outside the genus *Diaea* (**chapter 2**) indicates that subsociality may have evolved multiple times independently within Thomisidae. Alternatively, classificatory and taxonomic uncertainties may have masked a common origin of sociality in Thomisidae (compare e.g. Agnarsson & Rayor (2013) for Sparassidae), since thomisid taxonomy is still poorly understood (Benjamin et al., 2008; Szymkowiak, 2007). A molecular-phylogenetic approach (**chapter 3**) demonstrated that social behaviour has evolved at least twice independently in thomisids and that the subsocial *Xysticus bimaculatus* is not closely related to any of the subsocial or social species classified as *Diaea*. Deeper-level phylogenetics of Thomisidae could not be resolved, which however was not the aim of this study.

Multiple origins of sociality can be found in two other families: Eresidae and Theridiidae (Lubin and Bilde, 2007). These have in common that the permanently social clades lack diversification (Agnarsson et al., 2006; Johannesen et al., 2007). Diversification is probably restricted by inbreeding, which causes a loss of genetic variability in social spiders (Agnarsson et al., 2013; Avilés, 1997; Lubin and Bilde, 2007). In Thomisidae, only a single species, *Diaea socialis*, is described as permanently social and intraspecific genetic variability of its subclades was dated to be young and low compared with two of the subsocial thomisids (*Diaea ergandros, Xysticus bimaculatus,* **chapter 3**) indicating a pattern similar to social eresids and theridiids. While a genetic distinction between two populations of the subsocial *D. ergandros* could be demonstrated, a diversification between sampling areas of the permanently social *D. socialis* was not detected (**chapter 3**). This may be the result of the rather limited distribution of *D. socialis* (Evans, 1997) and the young age

of its subclades (**chapter 3**). Although there is limited evidence, this finding is consistent with the hypothesis that permanently social spider lineages fail to diversify due to high extinction rates (Agnarsson et al., 2006; Agnarsson et al., 2013). Alternatively, social lineages may need more time to diversify (Lubin and Bilde, 2007). However, the discovery of the social behaviour of *X. bimaculatus* has exemplified that the arachnofauna of Australia is far from well known, meaning that the results need to be interpreted with caution. In the relatively well-studied Theridiidae, eight to nine independent origins of sociality in eleven to twelve different species have been documented until 2006 (Agnarsson, 2006; Agnarsson et al., 2006). Their social behaviour was mainly studied in North and South America, but new subsocial species from Australasia were discovered only recently (Agnarsson, 2012). Additional species of crab spiders with social behaviour may thus be discovered in the future.

Subsocial and social crab spiders differ from most other group-living spiders because they lack a capture web. The construction of a large capture web in most subsocial and social spiders allows cooperative capture of relatively large prey items (Avilés, 1997; Lubin and Bilde, 2007) and facilitates communication between group members via web vibration, for example in prey-capture events (Vakanas and Krafft, 2001). However, it has been suggested that these benefits do not apply to non-webbuilding subsocial spiders and raised the question which other factors may promote group living (Evans, 1998a; Yip and Rayor, 2011). Although subsocial crab spiders lack a capture web, field observations and a laboratory experiment revealed that spiderlings do attack prey as a group (**chapter 6**). This behaviour had not been described before and it was assumed that subsocial and social crab spiders hunt solitarily but allow group members to join foraging (Evans and Main, 1993; Main, 1988). By communally attacking large prey items, group-living crab spiders and webbuilding social spiders may have similar hunting benefits. It may thus be the capability of spiderlings to overwhelm large prey that facilitates cooperative behaviour in these spiders. The non-webbuilding *Delena cancerides* on the other hand captures prey solitarily, but also differs from subsocial crab spiders in actively searching for prey items outside the communal retreat (Yip and Rayor, 2011). In this huntsman spider, adult females hunt and commonly share the prey with their offspring (Yip and Rayor, 2011). The same may be true in *X. bimaculatus* (**chapter 2**). Similarly, females of the subsocial crab spider *D. ergandros* capture and share prey items (Evans, 1998a; **chapter 5**). Adult females often have greater prey-capture efficiency compared to juveniles and play a major role in defending offspring from predators or building protective nests (Evans, 1998a, b; Schneider, 2002; Yip and Rayor, 2011; **chapter 7**). Thus, like in all other subsocial and social spiders studied to date, maternal care is a key characteristic of subsocial behaviour in Thomisidae (Evans, 1998a; Evans et al., 1995; Lubin and Bilde, 2007; Salomon et al., 2011; Salomon et al., 2005; Schneider, 2002; **chapter 2**). However, females and offspring may have conflicts over food provisioning (Mock and Parker, 1997; Trivers, 1974). Such conflicts may arise over food provisioning between female and offspring as well as between offspring groups.

The acceptance of unrelated spiders

A major difference between *D. ergandros* and most subsocial and social spiders of other spider families is the regular acceptance of unrelated conspecifics, which may further increase these female-offspring conflicts but also conflicts between offspring (Evans, 1999; Evans and Goodisman, 2002; **chapter 4).** To understand effects and consequences of such conflicts, it is critical to reveal whether females or offspring have more influence over food sharing in situations where unrelated spiderlings are present and whether the latter negatively affect group dynamics. In this thesis I demonstrated that groups consisting of a mixture of own and foster offspring had a lower growth and higher mortality compared to sibling groups, no matter whether the caring female was an own or foster mother (**chapter 5**). At first sight, this suggests that females provide food indiscriminately and food distribution is negotiated between offspring, with siblings having an advantage over mixed groups. In subsocial *Anelosimus* spiders, females indiscriminately provide food to own and foster offspring (Samuk and Avilés, 2013). The same holds true for *Stegodyphus lineatus,* but allomaternal care depends on the reproductive state of the females (Schneider, 2002). For *D. ergandros*, however, it could be shown that females lost mass when caring for either own or foster sibling broods but gained mass when caring for broods containing a mixture of own and foster offspring (**chapter 5**). Female mass development moreover mirrored offspring-mortality, meaning that offspring of those females that lost weight had a low mortality and vice versa. This suggests that females may still have influence on resource distribution since they seem to be willing to share more prey when the brood contains siblings. In great tits (*Parus major*), where both males and females provide care, offspring dynamics affect the conflict over food provisioning (Royle et al., 2012). The amount of care differs between the sexes depending on the social network structure of offspring. While females provide more food to smaller sized offspring groups with a strong social network structure, males provide more food when caring for large groups with a low social network structure (Royle et al., 2012). Thus, sibling broods in *D. ergandros* may emit a stronger signal due to a higher network structure, which may furthermore lead to lower foraging rates of the caring female and thus more food for offspring (**chapter 5**). The potentially higher network structure may be the result of less direct competitive interactions between spiderlings. In mixed broods on the other hand, competition may have led to the exclusion of unfamiliar or unrelated individuals from foraging.

To study the foraging dynamics in groups consisting of either sibling or unrelated individuals in more detail, individual hunting and foraging behaviour in the absence of a caring female was observed (**chapter 6**). Foraging groups consisting of siblings grew better compared with unrelated groups. This corroborates the findings of **chapter 5** and studies on other communally foraging subsocial spiders (Ruch et al., 2009b; Schneider and Bilde, 2008). Furthermore, family groups attacked more frequently as a group (**chapter 6**), which may increase the hunting success when targeting very large prey items and thus broaden the spectrum of available prey (Rypstra, 1993). Even though group attacks were rare in groups of unrelated

individuals (**chapter 6**), these spiderlings regularly shared prey. Even though the flies were large, they were small enough to be consumed by a single individual. A likely explanation for food-sharing might be that fighting over a prey item is costlier than sharing (Stevens and Gilby, 2004). Moreover, these individuals may obtain food more efficiently by sharing, in which case groups can be described as 'foraging societies' (Whitehouse and Lubin, 2005). This may also explain the acceptance of immigrants in *D. ergandros*.

Duration of cooperative activities

Subsocial crab spiders cooperate for a very long period and only leave the nest after mating (Evans, 1995). Dispersal after mating is however a typical characteristic of permanently social spiders (Avilés and Purcell, 2012; Lubin and Bilde, 2007; Lubin et al., 2009), and this late dispersal in *D. ergandros* is thus different from dispersal strategies observed in some other subsocial spiders. In *Stegodyphus lineatus* (Schneider, 1995) and some Theridiidae (Yip and Rayor, 2013), juveniles disperse after around four weeks.

Other subsocial spiders, such as *Delena cancerides* and *Anelosimus arizona* are associated for nine months to up to one year, which is very similar to *D. ergandros* (Yip and Rayor, 2013). The late dispersal in *D. ergandros* could be explained by the benefits gained from inhabiting protective retreats (Evans, 1998a; **chapter 7**). The importance of nests as protective retreats was demonstrated in other spider species such as *Stegodyphus dumicola* and *S. mimosarum* (Seibt and Wickler, 1990), *Cyrtophora hirta*, *Phonognatha graeffei* and *Theridion* spp. (Manicom et al., 2008). In *D. ergandros,* individuals in large groups built relatively large nests. Evans (1998a) found that large groups have an advantage over small groups and suggested that spiders suffer from predatory attacks of another spider (Clubionidae) that can be frequently found inside the nests (Evans, 1998a). Whether they preferentially use the nest as a shelter or as a food resource is unknown. Monitoring survival probability, nest construction activity and feeding behaviour of differently sized groups in the absence or presence of a higher-order predator (Clubionidae) (**chapter 7)** revealed that large groups generally survived better than smaller groups, which was independent of predator presence. Clubionids attacked *D. ergandros*, which negatively affected survival in medium sized groups, however, the overall effect of predator presence on mortality was not significant in this study. Individuals of large groups were mostly hiding inside the protective retreats and clubionids may not have negatively affected survival because of the effective shelter (**chapter 7**). In contrast to the subsocial huntsmen (Yip and Rayor, 2011), *D. ergandros* did not actively defend themselves from predators. *D. ergandros* spiderlings fed on a higher proportion of flies but gained less weight in predator presence than in predator absence, which indicates that the clubionids not only act as predators but also as food competitors (**chapter 7**). Hymenoptera and Coleoptera are the main prey groups for *D. ergandros* and *Clubiona robusta* (Austin, 1984; Evans, 1998a). Considering the overlap of prey, competition seems to play a greater role than expected and may represent a form of asymmetric

intraguild competition (predation), where one predator kills the other (Polis and Holt, 1992). Intraguild predation can also be found in other spiders, for example the web-building spider *Grammonota trivitatta* and the wolf spider *Pardosa littoralis* (Denno et al. 2004). Both species feed on planthoppers in the same habitat. In an experiment, *Pardosa* significantly reduced the survival of *Grammonota.* Similar to our observations, the rate of intraguild predation was relatively low (Denno et al., 2004). Clubionids may also act as kleptoparasites of *D. ergandros* and benefit from the long-lasting nest as a shelter.

The relatively long period of group-living *D. ergandros* seems beneficial in terms of survival (**chapter 7**), but likely has implications for the mating system. In most group-living taxa, one sex disperses prior to maturity, which secures outbreeding (Greenwood, 1980). In *D. ergandros,* late-instar juveniles may move between nests, which may secure outbreeding to some extent (Evans, 1999), but spiders were inbred within sampling patches (Evans and Goodisman, 2002), indicating frequent mating among relatives. Inbreeding is usually maladaptive because of an accumulation of deleterious recessive alleles (Charlesworth and Charlesworth, 1987), but regular inbreeding may also purge these deleterious alleles (Bilde et al., 2005; Pusey and Wolf, 1996). In this case, the short-term costs of inbreeding may be lower than the costs of inbreeding-avoidance mechanisms and inbreeding may be favoured when the costs of an inbreeding depression are low (Waser et al., 1986).

Incestuous mating is in fact common in permanently social spiders and increases cooperation between highly related group members (Lubin and Bilde, 2007). Inbreeding leads to a female-biased sex ratio in permanently social spiders (Avilés, 1997; Lubin and Bilde, 2007). Intra-colony mating moreover results in highly structured subpopulations and large genetic differentiation between colonies. The sex-ratio bias found in the social spider *Anelosimus eximius,* for example, allows a rapid colony growth (Avilés, 1986). In these spiders, colonies can be described as 'isolated entities', where some colonies grow rapidly, while others go extinct. It has been suggested that natural selection should favour any heritable trait that increases the survival of the entity, such as increased production of females (Avilés, 1986). While this approach of multilevel selection (group selection) may be valid in highly inbred and thus highly structured populations, it probably has no implication for the evolution of subsociality in crab spiders because the requirements for multilevel selection are not met. Firstly, nests are no isolated entities because migration between *D. ergandros* nests happens frequently. Secondly, I could demonstrate that populations are not significantly differentiated, meaning that there is gene flow between different populations (**chapter 4**).

Subsocial spiders usually have an outbred mating system, but genetic relatedness is also an important factor promoting cooperative and social behaviour, such as in *Stegodyphus* spiders (Ruch et al., 2009b; Schneider and Bilde, 2008). In subsocial spiders, high genetic relatedness can be the result of low female mating rates, which are thought to facilitate the transition from a solitary to a social lifestyle (monogamy hypothesis) (Boomsma, 2009; Boomsma et al.,

2011; Cornwallis et al., 2010). Considering the long period of cooperation in *D. ergandros*, females may not resist inbreeding or even seek incestuous matings to further increase relatedness in their brood.

Detailed data on natural mating rates have been collected for subsocial and social *Stegodyphus* (Bilde et al., 2005; Lubin et al., 2009; Ruch et al., 2009a; Schneider, 1997; Tuni and Berger-Tal, 2012) as well as *Anelosimus* spiders (Avilés and Purcell, 2012; Corcobado et al., 2012; Klein et al., 2005) and natural mating rates seem to be relatively low with females usually mating with one, two or three mating partners. Natural mating rates are difficult to observe in subsocial and social *Diaea* because mating takes place inside the leaf-nests in tall *Eucalyptus* trees. A previous population-genetic study indicated very low mating rates in *D. ergandros*, but the power to detect multiple mating was relatively limited due to low allozyme-marker variability (Evans and Goodisman, 2002). Reinvestigating the natural mating rates of *D. ergandros* using microsatellite markers, predictions of the monogamy hypothesis were tested (**chapter 4**). Consistent with Evans and Goodisman (2002), the natural mating rates were low, with half of the analysed nests being sired by a single mating pair. The power to detect polyandry was higher and revealed that half of the broods was sired by at least two different fathers. As predicted, females did not avoid mating with siblings but did not facilitate inbreeding either, which is consistent with other subsocial spiders (Ruch et al., 2009a; Tuni et al., 2012). Considering that *D. ergandros* has an inbred population structure (Evans and Goodisman, 2002) that may negatively affect fitness if recessive deleterious alleles have not been purged in the course of their evolutionary history, females may secure outbreeding by mating with more than one male (Arnqvist and Nilsson, 2000), although this reduces offspring relatedness. Since siblings generally showed relatively high levels of cooperation (**chapters 5, 6, 7**), mating with two males may not necessarily reduce offspring cooperation in *D. ergandros*.

Conclusions and outlook

The dynamics in group-living crab spiders are shaped by interactions between family members and foreigners. Communal activities such as hunting and foraging seem to be negatively affected by immigrating spiderlings. The results of **chapter 5** and **6** as well as previous findings on the nest construction activity (Evans, 1999) suggest that related groups have an advantage over unrelated groups in *D. ergandros*. Although group hunting and food sharing seems to be negatively affected by immigrating unrelated spiderlings in this species, these may have valuable effects as well such as benefits from increased group size (**chapter 7**) or potentially benefits from outbreeding (**chapter 4**).

While living in large groups can be beneficial (**chapter 7**), individual fitness can decline beyond a group-size optimum (Higashi and Yamamura, 1993). Consequently, individuals are predicted to abandon a group at a certain point (Higashi and Yamamura, 1993). Experimental manipulation of group size to detect the optimum and the study of interactions between relatives and immigrants when the optimal group size is exceeded are promising next steps to investigate the limits of cooperative behaviour in these spiders.

The differences and similarities between

D. ergandros and the other group-living thomisids remain to be studied. For example, *X. bimaculatus* (**chapter 2**) seems very similar in lifestyle and behaviour to the comparably well-studied subsocial crab spider *D. ergandros* (Evans, 1995). Whether or not *X. bimaculatus* offspring migrate between colonies as well and how this could affect communal activities are compelling questions for future research.

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Appendix 1:

Cuticular Antifungals in Spiders: Density- and Condition Dependence

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Cuticular Antifungals in Spiders: Density- and Condition Dependence

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Abstract

Animals living in groups face a high risk of disease contagion. In many arthropod species, cuticular antimicrobials constitute the first protective barrier that prevents infections. Here we report that group-living spiders produce cuticular chemicals which inhibit fungal growth. Given that cuticular antifungals may be costly to produce, we explored whether they can be modulated according to the risk of contagion (i.e. under high densities). For this purpose, we quantified cuticular antifungal activity in the subsocial crab spider *Diaea ergandros* in both natural nests and experimentally manipulated nests of varying density. We quantified the body-condition of spiders to test whether antifungal activity is condition dependent, as well as the effect of spider density on body-condition. We predicted cuticular antifungal activity to increase and body-condition to decrease with high spider densities, and that antifungal activity would be inversely related to body-condition. Contrary to our predictions, antifungal activity was neither density- nor condition-dependent. However, body-condition decreased with density in natural nests, but increased in experimental nests. We suggest that pathogen pressure is so important in nature that it maintains high levels of cuticular antifungal activity in spiders, impacting negatively on individual energetic condition. Future studies should identify the chemical structure of the isolated antifungal compounds in order to understand the physiological basis of a trade-off between disease prevention and energetic condition caused by group living, and its consequences in the evolution of sociality in spiders.

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Introduction

Living in groups is widespread and found in insects, spiders, birds and mammals, among other animals. Individuals that live in groups obtain benefits such as predator avoidance [1–4], foraging efficiency [5,6], and enhanced reproductive success [5]. However, group living has associated costs: compared to solitary individuals or small groups, individuals in large groups can incur costs such as increased competition for resources [7–9]. Moreover, group-living animals are faced with the potential risk of accumulating pathogens that can spread more easily between group members [10,11]. Therefore, group living can not only be costly in terms of competition between individuals but also in terms of pathogen defense and disease contagion [12–14].

One important cost derived from contagious diseases is the activation and use of immune responses. Immunity can be costly because of toxic byproducts of immune reactions or because it requires resources that are spent at the expense of other functions [15,16]. To decrease these costs, some group-living animals modulate their investment in immune response according to the risk of infection [17]. Under crowded conditions (i. e., when contagion risk is high), some insects show more active immune system compared to organisms living in low densities [18,19], this might allow them to be more resistant than individuals kept solitarily [19,20]. Such density-dependent activation of immune responses can be interpreted as an adaptive strategy to decrease the costs associated with the maintenance and activation of immune defenses. A simpler strategy to deal with microorganisms is to avoid contagion, either via behavioural avoidance of infected individuals or places [21,22], hygienic behaviour in the nest [23] or via chemical avoidance with antimicrobials on the skin or cuticle [24–26]. Despite incurring some cost, both behavioural and chemical protections can reduce the cost of activating the immune system once the pathogen has infected the host.

The subsocial crab spider *Diaea ergandros* lives in nests built from *Eucalyptus* leaves. Nests contain up to 70 spiderlings that are usually the offspring of a single female [27]. These nests persist several months and all spiders of the group communally enlarge the nest by attaching more leaves. The inside of these nests can be quite sealed, moldy and contain food debris [27], and therefore favors the development of pathogens with the risk of pathogenesis being elevated at increased conspecific density. Furthermore, infections can be particularly dangerous because group members are close relatives and there is thus low genetic variability that could result in more susceptible groups [14,28]. Previous experimental research on *D. ergandros* shows that individuals in large groups build larger and more protective nests and survive better in the presence of a predator compared to small groups or singly kept

spiders [29]. However, the influence of pathogen pressure on large spider groups might be higher but has not been explored yet.

The main aim of this study was to investigate whether *D. ergandros* spiders have developed density-dependent polyphenism pathogen defenses. Antifungal cuticular response in both natural nests and artificial nests of varying density was measured. Despite previous descriptions in different taxa, cuticular antifungal activity has not been described in spiders yet. Costs involved in the maintenance of cuticular antifungal activity were examined by measuring spiders' lipid body reserves. This study represents the first exploration of density dependence in preventive antifungal production within a spider species and evaluates its possible dependence on physiological condition (lipid reserves). We predict that antifungal protective activity will be a) present in crab spiders, b) a costly trait, dependent on physiological condition, and c) more intense with increasing nest density.

Materials and Methods

Ethics

No permits were required for the described study, which complied with all relevant regulations. The species used in these experiments (*Diaea ergandros*) is not an endangered or protected species under CITES regulations.

Study species

The present study was carried out with the subsocial crab spider *Diaea ergandros* Evans, 1995 (Araneae: Thomisidae). Unlike other social and subsocial spiders, these spiders do not build webs and instead live in nests built from *Eucalyptus* leaves [30,31]. Each nest consists mainly of a single mature female and her offspring, although migration between nests can occur [31,32]. Even though spiders may migrate between nests, relatedness between nest mates is relatively high [31,32]. Juveniles develop during 8–9 months after which they leave the nest [30]. For the present study we only used juvenile spiders of the instars 4 and 5 (see below). Spider nests were collected from *Eucalyptus* trees in June 2012 along the Lachlan Valley Way (34°47′5.02″S, 148°51′16.72″E; 34°32′37.32″S, 148°44′9.66″E) between Yass and Boorowa, NSW, Australia.

Antifungal activity measurements

Antifungal activity was measured from the cuticle of spiders following a procedure modified from [24]. Since one spider did not provide enough sample of cuticular antifungal measurements (unpublished data), we used groups of five spiders for each sample. Spiders were anesthetized with carbon dioxide and washed with 2 mL of Ethanol 90% for five minutes to remove cuticular antifungals. Ethanol was evaporated from the sample with a rotary evaporator (25 mbar, 25°C). Under sterile conditions in a fume hood, each dry sample of spider extract was re-suspended in 125 μL of Luria Bertani (LB) broth and 100 μL of a culture of *Cordyceps bassiana* spores (2000–3000 spores/μL) in LB broth were added. Prior exploratory analysis varying spore concentration from 965–3580 spores/μL show no significant correlation with optical density after 24 h of fungal growth in LB broth (Pearson $r = 0.16$, $P = 0.38$, $N = 15$), showing that our assay is not sensitive to initial spore concentration. From each sample, 200 μL were placed in 96-well plates for measuring fungal growth as increments in optical density (OD) with time using a spectrophotometer (405 nm). Antifungal activity was measured as inhibition of spore germination after 24 hours in comparison with a positive control that consisted of a mixture of 100 μL of the *C. bassiana* culture and 100 μL of sterile LB broth. As a negative control (with no fungi, used to assure sterility during the assay), we used a mixture of 100 μL sample of spider extract in LB broth with 100 μL of sterile LB broth. At least two positive and one negative controls were used for each plate. The OD after 24 hours was considered the value of antifungal activity. Large OD values represent high fungi growth.

Antifungal activity and energetic reserves under natural densities

Cuticular antifungal activity was measured in samples from 14 nests (LB broth with fungi and cuticular extracts; see below), 11 positive controls (LB broth with fungi) and 5 negative controls (LB broth without fungi). To examine the relationship between the level of antifungal activity in spider cuticles and the energetic body condition (lipid body reserves) under different densities, 20 nests that contained 12–61 spiders were used. Nest size was estimated by measuring length and width of each nest (±0.1 mm). Nest density was calculated as number of spiders/nest size. Due to contamination, antifungals could not be measured in 6 nests, and so sample size was reduced to 14 nests when antifungal activity was analyzed. For this part of the study, we only used juvenile spiders of 4th and 5th instars.

Antifungal activity and energetic reserves under manipulated densities

In this experiment we tested if solitary spiders differed in their antifungal activity and body energetic reserves from their siblings kept in groups. For such purpose, individuals from selected nests were randomly allocated to one of two treatments: solitary and grouped spiders. Solitary spiders were kept individually and grouped spiders were kept in groups of 16 individuals in plastic transparent cups (100 mL) for 10 days under natural light and darkness regime. This controls for environmental and sanitary conditions that could be variable in natural nests that are probably exposed to different pathogens. Spiders were starved seven days before the experiment to get individuals with similar initial body condition at the start of the experiment. Once the experiment started, spiders were offered three meals consisting of one male and one female living *Drosophila melanogaster* per individual. After the 10 day period, five solitary or five grouped spiders from each nest were washed together in 90% Ethanol and antifungal activities were compared (see above). In this experiment we used a total of 10 nests of juveniles (ranging 27–85 spiders per nest) at the 4th instar when antifungal activity and lipids were measured. Due to contamination, antifungals could not be measured in two of the 10 nests, leaving a total of eight nests for the analyses of antifungal activity.

As a measurement of individual body condition in both natural and artificial nests, we measured lipid body reserves [33]. Lipids were quantified as the difference in body dry weight before and after three 24 hour submersions in chloroform. We found that lipid reserves ranged from 0–6 mg in natural nests and 0–1.5 mg in artificial nests (see results). This can be explained by the age of the studied animals, considering that spiderlings grow with age: while natural nests comprised individuals of 4th and 5th instars, artificial nests included only individuals at the beginning of the 4th instar.

Statistical analyses

The relationship between nest density (number of spiders/cm^2), antifungal activity and lipid reserves in nests taken directly from the field was examined using linear regressions. To test for the effect of density manipulation on antifungal activity and lipid

contents, we used general linear mixed models with treatment (solitary or crowded) and original nest density (number of spiders) as explanatory variables as well as their interaction. The interaction between both covariates was tested but it was removed from the analysis for being non-significant (antifungals: $P = 0.695$, lipids: $P = 0.634$). Given that solitary and crowded treatments came from the same nest, nest ID was included as a random variable in the models. Relationships between antifungal activity levels and lipids were analyzed with Pearson correlations. The presence of outliers was examined with Cook's distances and variance homogeneity was tested with Fligner-Killeen tests [34]. All analyses were performed in R 2.10.0 [35].

Results

A washing of *D. ergandros* cuticles efficiently reduced fungal growth in a culture medium after 24 hours, showing that spiders possess antifungal activity in the cuticle that is effective against the fungal pathogen *Cordyceps bassiana* (ANOVA $F_{2,27} = 9.416$, $P<0.001$; Figure 1). A priori contrasts show that OD measurements of fungal growth in media with cuticle washings were significantly lower than OD of positive controls with *C. bassiana* ($t = 2.744$, $P = 0.011$) and significantly higher than negative controls ($t = 2.228$, $P = 0.034$); positive controls showed higher OD than negative controls ($t = 4.202$, $P<0.001$), confirming that there was no contamination in the culture medium.

In nests taken from the field, there was no relationship between nest density and cuticular antifungal activity ($R^2 = 0.035$, $P = 0.524$, $N = 14$; Figure 2). In addition, the intensity of cuticular antifungal activity was not correlated with the amount of lipid body reserves in individual spiders (Pearson $R = -0.303$, $P = 0.293$, $N = 14$). However, nest density and individual lipid content were negatively related ($R^2 = 0.229$, $P = 0.032$, $N = 20$; Figure 3), meaning that spiders from high-density nests had a lower lipid content compared to spiders from low-density nests.

In artificial nests, grouped spiders did not differ in antifungal activity from their solitary siblings (Mixed model $F = 3.211$, $P = 0.116$, $N = 8$; Figure 4). Initial spider density was controlled but was omitted from the final analysis for being non significant ($F = 0.644$, $P = 0.453$, $N = 8$). However, grouped spiders showed higher lipid contents than their solitary siblings (Mixed model $F = 5.303$, $P = 0.047$, $N = 10$; Figure 5). Initial spider density was marginally positively related to lipid reserves ($F = 4.692$, $P = 0.062$, $N = 10$). The intensity of cuticular antifungal activity was not correlated with the amount of lipid reserves in either solitary (Pearson $R = -0.011$, $P = 0.980$, $N = 8$) or grouped spiders ($R = 0.196$, $P = 0.641$, $N = 8$).

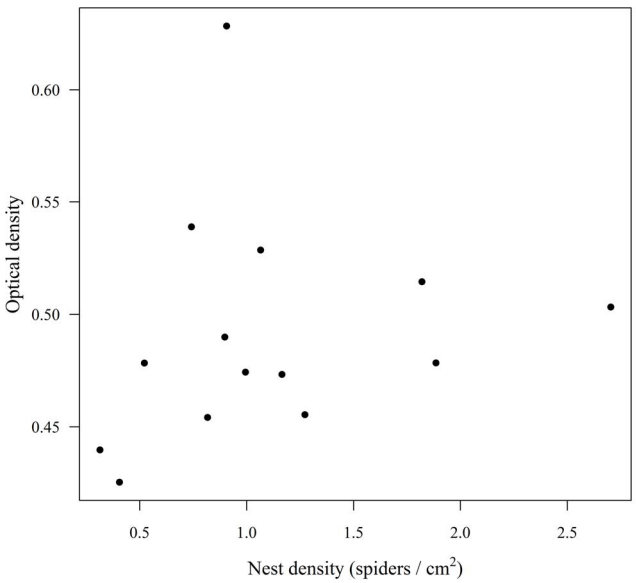

Figure 2. Relationship between nest density and antifungal activity in juvenile *Diaea ergandros* from natural nests (non-significant $R^2 = 0.035$).
doi:10.1371/journal.pone.0091785.g002

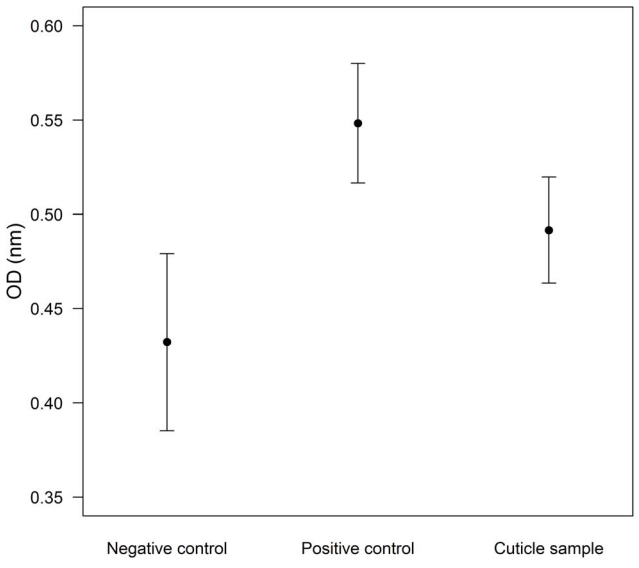

Figure 1. Effect of cuticular antifungals of *Diaea ergandros* spiders on fungal growth. Negative controls are cuticular samples without fungi, whereas positive controls are fungal cultures without cuticular samples. Bars represent means ±95% confidence intervals.
doi:10.1371/journal.pone.0091785.g001

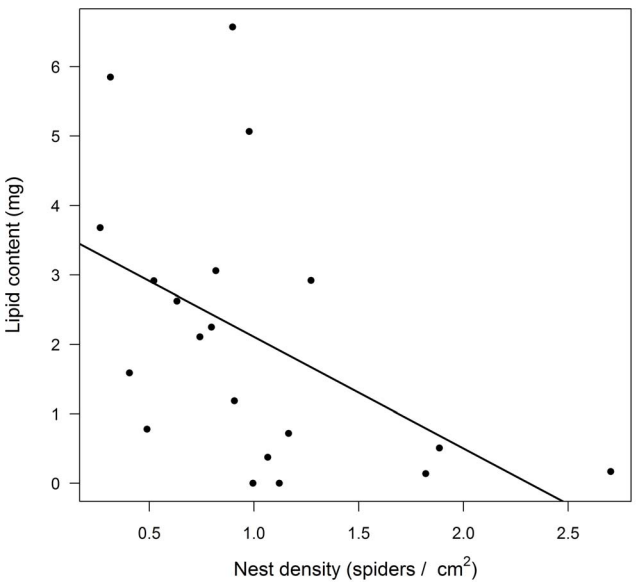

Figure 3. Relationship between nest density and lipid reserves in juvenile *Diaea ergandros* from natural nests ($R^2 = 0.229$).
doi:10.1371/journal.pone.0091785.g003

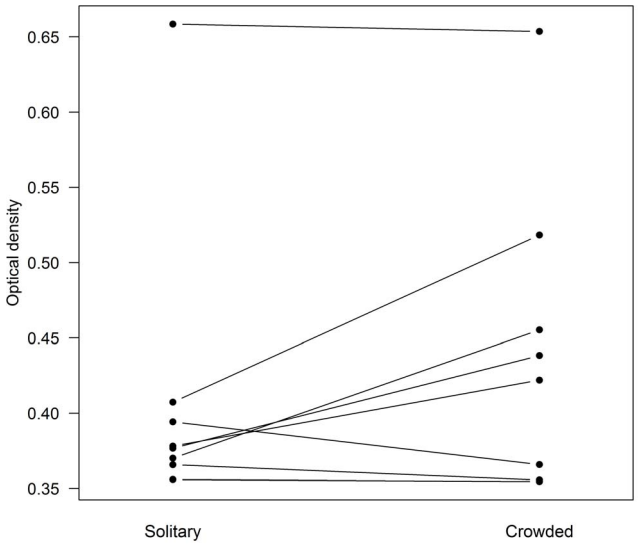

Figure 4. Antifungal activity in *Diaea ergandros* individuals that were experimentally kept crowded or solitary. Lines link individuals coming from the same nest.
doi:10.1371/journal.pone.0091785.g004

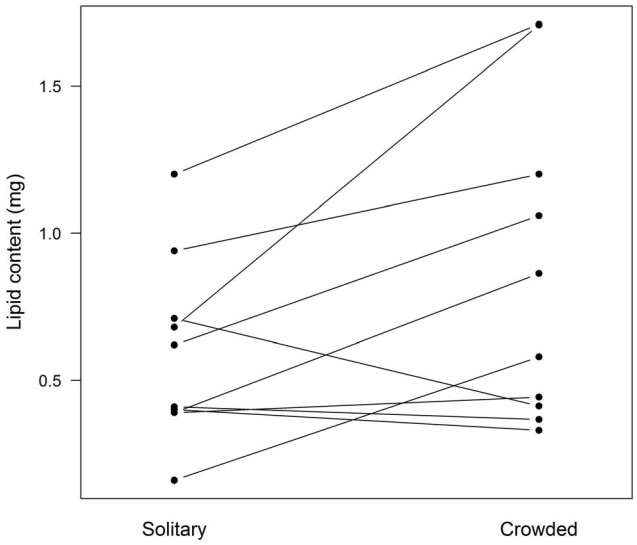

Figure 5. Lipid reserves in *Diaea ergandros* individuals that were experimentally kept crowded or solitary. Lines link individuals coming from the same nest.
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Discussion

The present study represents the first evidence that spiders produce cuticular compounds that can reduce potential fungal infections. Cuticular antimicrobials have been described in insects such as ants, termites, wasps, bees, moths, thrips and whiteflies [24–26,36,37], and represent a first line defense against pathogens and parasites [38]. Our finding supports the idea that antimicrobial defense accompanies the evolution of sociality in animals living in large aggregations [13,24].

A variety of antifungal compounds have been found in arthropod (particularly insect) cuticles, and they can be either secreted by the host itself or produced by symbiotic microorganisms [39]. These compounds can include free fatty acids, proteins (defensins), amides, aldehydes, terpenes, glucanase enzymes, chitinase and protease inhibitors, alkaloids and quinones [39,40] which, together with cuticular melanization [41,42], inhibit spore germination, hyphae growth or penetration into the body. In particular, melanization response [41], caprylic, valeric and nonanoic acid [26], monoterpenes [43] and salicylaldehyde [44] have shown to be effective against the same fungus used in the present study (*Cordyceps bassiana*) in lepidopterans and coleopterans. To our knowledge, there is no description of specific cuticular antifungals in spiders, but many of the compounds present in insects have ancient evolutionary origins and find homologies in other insects, nematodes and mammals [40,45–47]; hence, similar compounds can also be present in spiders, contributing to the inhibition of fungal growth in our assays and probably against natural pathogens. Given that many of these compounds are soluble in ethanol, which was the solvent used for their extraction in the present study, our measure of antifungal activity probably includes the effect of several of these or related compounds. As a perspective of this work, investigating the chemical nature and the action spectrum of the cuticular compounds isolated in *D. ergandros*, as well as addressing to what extent they are synthesized by the host itself or by symbiotic microorganisms would give further insight into the study of antifungals in spiders.

The synthesis of the abovementioned cuticular antifungals [26,36] certainly requires resources that are obtained from the host diet, such as amino-acids and fatty acids, and thus we predicted that only animals in good physiological condition would be able to produce effective antifungals. However, we found no relationship between energetic body-condition (lipid reserves) and intensity of antifungal activity. There are two possible explanations for this finding: (a) that these antifungals are not costly to produce and individuals in a range of conditions can maintain high levels, which seems unlikely given their chemical composition, or (b) that cuticular antifungals are so important in infection avoidance that individuals cannot allow to reduce their production. Given that living solitary can be costly in terms of foraging efficiency and predation risk [29], the latter interpretation can also explain why experimentally isolated individuals lost energetic condition compared to individuals kept in a group in our laboratory experiment.

Isolated individuals paid an energetic cost and not a cost in antifungal activity, probably because dietary restriction can be overcome [48] whereas loss of antifungals is too risky. If the same resources (e. g. amino-acids, fatty acids) are shared between cuticular antifungals and metabolic function, a trade-off between disease prevention and physiological condition may result [49]. As we ignore the plasticity of up- and down regulation of cuticular antifungals, we cannot discard the possibility that differences in antifungal activity could be detected in a long-term experiment.

We predicted that investment in antifungal protective activity would be higher in crowded nests, especially given the potentially higher risk of contagion that exists when there is high genetic relatedness among nest members [24], as in *D. ergandros*. However, we found no relationship between nest density and cuticular antifungal activity, neither in nature nor under laboratory conditions, suggesting that spiders constitutively express cuticular antifungal activity against the tested fungus. It is possible that cuticular antifungal activity, unlike immune response [17,18,50], is not a dynamic trait that can be regulated under varying risk of infection [21]. Other unmeasured components of spider defense, such as haemolymph immune response or melanization, might be adjusted under different densities, as occurs in other arthropods [17,19], but this remains to be tested.

Despite the benefits of group living in terms of foraging, reproduction or predator avoidance [1,4,5], our results show that

living at high densities is costly in terms of reducing energetic reserves. However, we only found this pattern under natural conditions, where food was not artificially supplemented and under natural predation risk. On the other hand, when density was manipulated in the laboratory, food was provided and predators were excluded, grouped spiders maintained higher lipid reserves than their solitary siblings. These contrasting results suggest that when animals are stressed (i. e. under natural conditions), living in large groups can be energetically disadvantageous, while when animals are not stressed (i. e. under laboratory conditions) living in groups is energetically beneficial. For example, nutrient availability under laboratory conditions could reduce cannibalism within nests affecting nest density [51,52], but this idea remains to be tested.

In the present study we showed that living in large groups impacts on physiological condition of group members depending on the environmental conditions, presumably food availability. Despite energetic body condition being highly sensitive to group size, this was not the case for cuticular antifungal activity, which was not affected by nest density. The permanent pressure of pathogens on spider nests is likely to be responsible for the low plasticity of cuticular antifungal expression; if investment in pathogen protection needs to be constant, it can explain why energetic condition is compromised if resources are scarce. Future studies should formally evaluate the physiological basis of a potential trade off between lipid reserves and cuticular antifungals, and evaluate the importance of protective defense in the evolution of sociality.

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Author Contributions

Conceived and designed the experiments: DGT JR TP FP. Performed the experiments: DGT JR TP FP. Analyzed the data: DGT JR TP FP. Contributed reagents/materials/analysis tools: DGT JR TP FP. Wrote the paper: DGT JR TP FP.

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