Evolution of Sociality: mechanisms and dynamics of social behavior in spiders

(Evolution von Sozialität: Mechanismen und Dynamik des Sozialverhaltens bei Spinnen)

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“Willst du mit mir hausen, so laß die Bestie draußen!”

--- Johann Wolfgang von Goethe

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1 INTRODUCTION

1.1 Animal communities and cooperation

Most animal species live solitarily, as the potential for conflict with conspecifics over limited environmental resources or mating partners is high and thus group formation is often associated with costs for the individual (Krause & Ruxton, 2002; Schmid-Hempel, 1995). Simple forms of social grouping are linked to parental care, which might have facilitated the first steps towards sociality in the course of evolution (Korb & Heinze, 2016). After the care period, ecological factors may constrain offspring from dispersing when the costs of dispersal are high due to high mortality or low chances of successful reproduction (Emlen, 1994). Consequently, for a variety of animal species, ranging from vertebrates like mammals, birds and fish (Krause & Ruxton, 2002) to invertebrates like crustaceans (Thiel, 2011), insects and arachnids (Choe & Crespi, 1997; Lubin & Bilde, 2007), the benefits of group living outweigh the costs and a permanently social lifestyle developed. The origins of these animal societies became a major study interest of evolutionary biologists, trying to understand under which circumstances group living evolves. Especially intriguing are highly specialized communities in which individuals cooperate in communal tasks such as nest maintenance, predator defense, foraging and breeding (Clutton-Brock, 2002; Cockburn, 2006; Keller & Reeve, 1994; Lubin & Bilde, 2007). In particular, cooperative breeding that involves alloparental care, in which adult individuals in addition to the genetic parents participate in rearing offspring (Riedmann, 1982), sparked the interest of many scientists.

Helping behaviors and alloparental care are facilitated by group living and at the same time further promote sociality when benefits of helping exceed its costs (Lacey & Sherman, 1997; Ligon & Burt, 2004). Comprehending the underlying mechanisms of cooperative breeding is thus viewed to be essential for understanding how social groups form and are maintained (Alexander, 1974; Hatchwell, 2009; West-Eberhard, 1987). Cooperative breeding influences choices and possibilities of indiviudals, as it offers a different set of options to increase fitness, despite the costs of group living. The optimal reproductive strategy for an individual living in a social group might be different from the optimal reproductive strategy available to a solitarily breeding individual (Komdeur, 1992; Emlen, 1991). Sharing tasks and apportioning the work load can free

resources that are now available for reproduction (Mumme, Koenig & Ratnieks, 1989). Thus, as a consequence of social living and cooperation, the life history of individuals can be altered substantially (Trumbo, 2013). However, how reproductive role in cooperative breeders is determined and which internal and external processes are involved is poorly understood for many social species.

In the present work I investigated mechanisms and dynamics of sociality in the case study of a social, cooperatively breeding spider with skewed reproduction in females. In particular, I focussed on various aspects of reproductive and brood care behavior. The comparison with a solitarly breeding congener allows me to discuss basic prerequesites and consequences of sociality in spiders. To this aim, I explored behavioral and morphological aspects of cooperation and allomaternal care. The results provide information on task participation of females with different reproductive backgrounds as well as on morphological mechanisms which enable virgin females to help. The resulting information allows to visualize the benefits virgin females provide to the group and whether there are particular evolutionary adaptations that allow for it. Understanding how reproductive role in females is determined is essential for understanding the evolution of helping behavior by virgin females. To this aim, I investigated the relevance of the characteristic male scarcity in spider communities on the determination of the skewed reproduction in females. Furthermore, I examined the consequences of the cooperative social lifestyle on the life history of breeding individuals and discuss the results in in the context of social evolution.

1.2 Parental and alloparental care

Parental care includes a wide range of behavioral and physiological adaptations that involve costs for the parent, but at the same time increase the fitness of a parent's offspring (Clutton-Brock, 1991; Royle, Smiseth & Kölliker, 2012; Trivers, 1972). In terrestrial arthropods, parental care is relatively rare, as ecological constraints often favor the production of a large number of eggs and low investment in the protection of the individual egg or offspring (Clutton-Brock, 1991; Costa,

2006; Trumbo, 2012). Nevertheless, parental care in a broad sense has evolved in many taxonomic groups – including arthropods – and comprises all forms of care, from choosing or creating a suitable oviposition site, to egg care and provisioning of young (Clutton-Brock, 1991; Klug, Alonso & Bonsall, 2012). Parental care is most commonly performed by females, and in the most extreme cases results in extended offspring care, involving regurgitation feeding to the offspring for a prolonged period (Fetherston, Scott & Traniello, 1990; Kullmann, 1969; Malm & Jensen, 1993; Staerkle & Kölliger, 2008) and matriphagy, in which the female´s body serves as a terminal investment in her offspring (Smiseth, Kölliker & Royle, 2012; Suzuki, Kitamura & Matsubayashi, 2005; Tizo-Pedroso & Del-Claro, 2005), resulting in her death. It is hypothesized that extended brood care in non-social species may have facilitated the evolution of helping and thereby cooperative breeding (Field & Brace, 2004; Queller, 1994), and extended brood care is widely thought to be a precondition for the evolution of eusociality (Alexander, 1974; West-Eberhard, 1987). This is commonly referred to as the "subsocial route to sociality" (Wheeler, 1928).

In cooperatively breeding species, the offspring is usually provisioned by closely related helpers in addition to or even instead of the mother (Cant, 2012; Choe & Crespi, 1997; Wilson, 1971). There are two types of cooperative breeding: those in which there is some degree of shared parentage of offspring, and more intriguingly, those in which adult non-breeders help raising the young. The latter indicates the common occurrence of division of reproductive behavior in cooperatively breeding societies, with one or a few individuals exclusively reproducing, while others act as helpers at the nest, (e.g. in insects Wilson, 1971; birds Cockburn, 2006; mammals Lukas & Clutton-Brock, 2012; fish Taborsky, 2009; and arachnids Avilés, 1997; Lubin & Bilde, 2007). Helping efforts are linked to higher fitness for the reproducer and can entail an increased future reproductive success for the helper (Woolfenden & Fitzpatrick, 1978). However, in some cases helping restricts or even eliminates the possibility of own reproductive success in helpers (Heinsohn & Legge, 1999), but the proximate causes that determine which individuals within a group become reproducers and which remain unmated helpers are not always well understood. The evolutionary causes underlying decisions to forego own reproduction and take on the helping role is often ascribed to kin selection (Boomsma, 2009; Hamilton, 1964a, 1964b; Ratnieks, Foster

& Wenseleers, 2006), which favors helping behavior by means of inclusive fitness benefits. When dispersal is linked to high mortality and the chance of successful reproduction is low, the costs of independent breeding might exceed the costs of helping (Du Plessis & Williams, 1994; Hatchwell & Komdeur, 2000). The degree of helping is shaped by relatedness between helper and recipient, the fitness costs of helping, and the benefit of helping to the recipient (Field, Croning & Bridge, 2006). In addition to kinship (Boomsma, 2009), behavioral variation and the determination of reproductive role may be influenced by proximate factors such as body size, age, place in dominance hierarchy, or behavioral type (Carter, English & Clutton-Brock, 2014; Clutton-Brock, 2002; Emlen & Wrege, 1991; Monnin & Peeters, 1999; Pruitt, Oufiero, Avilés & Riechert, 2012; Ratnieks & Anderson, 1999). Furthermore, individuals in cooperatively breeding societies often show differential task participation, leading to task differentiation or even to the evolution of specialized castes as in eusocial insects (Jeanson & Weidenmüller, 2014; Oster & Wilson, 1978; Trumbo, 2012). One aspect that is not well understood is whether and how the reproductive state of individuals shapes their propensity to provide different types of help (Hatchwell, 2009), i.e. whether being mated or virgin influences their absolute and relative engagement in i) alloparental care and ii) other helping tasks within cooperatively breeding societies. Especially when alloparental care entails intensive tasks like food provisioning or matriphagy, physiological adaptation to brood care can be expected. In many eusocial species, such as in Hymenoptera, virgin female helpers develop morphological and physiological adaptations to provide extended brood care (Adkins-Regan, 2005; Fénéron, Durand & Jaisson, 1996; Wilson, 1971). However, the kind and extent of morphological adaptations to maternal and virgin allomaternal care are not intensively investigated, especially when it comes to arthropods.

To the aim of understanding the role of virgin helpers in a group and especially their role during brood care, I investigated the participation of reproducing females and virgin females in different tasks of brood care and in a non-reproductive task in the study case of social spiders. Furthermore, I examined the morphological dynamics of maternal and possibly virgin allomaternal care in reproducing females and virgin helpers.

1.3 Sex ratios and reproductive skew in animal communities

Sex ratio is defined as the proportion of males relative to the proportion of females in a group (Ancona, Dénes, Krüger, Székely & Beissinger, 2017). Fisher (1930) proposed that frequency-dependent selection leads to an equilibrium of sexes (Futuyma, 2009) in natural populations, as in a population with skewed sex ratio parents that produce more of the minority sex would have an advantage as their offspring would have more mating possibilities. However, significant departures from this Fisherian sex ratio can be observed in nature with sex ratios being skewed either in favor of the males (Donald, 2007; Hurd et al., 1994; Torres & Drummond, 1999) – or more commonly – in favor of the females (Colwell, 1981; Nunney, 1985; Olsent & Cockburn, 1991). This is due to the fact that random, population-wide competition for mates is unusual for most animal societies (Hamilton, 1967). Instead, mate selection might occur within local groups with increased relatedness among group members (Vollrath, 1986), a process termed local mate competition (Hamilton, 1967). In local mate competition, the female's genes are proliferated most successfully if most of her progeny is female, and just the number of sons that are needed to fertilize all females are produced (Taylor & Bulmer, 1980). In this way, competition between closely related males is minimized. Furthermore, according to the repayment model by Emlen et al. (1986) sex ratio should be skewed towards the most helpful sex – usually females – in cooperative breeding societies.

The sex ratio might differ within an individual population depending on when it is evaluated (Jennions & Fromhage, 2017). The primary sex ratio refers to the distribution of sexes at fertilization of the eggs and biases indicate a differential investment of the parents in one of the sexes. Even if sex ratios are equal at this stage, sex ratio at birth (secondary sex ratio) and at maturation (MSR, tertiary sex ratio) might be biased due to differential mortality of the sexes during development (Székely, Weissing & Komdeur, 2014). The MSR does not necessarily translate directly into the adult sex ratio (ASR), which refers to the proportion of males in the adult population at any given time (Ancona et al., 2017), as it can be influenced by unequal maturation times, mortality and migration patterns between the adult sexes (Székely et al.,

2014). Similarly, the relationship between ASR and the operational sex ratio (OSR) might vary (Kokko & Jennions, 2008), for example depending on mating and remating patterns of the different sexes, leading to a sex-biased OSR even when the ASR is is unbiased. Natural cases in which ASR and OSR are identical indeed are probably exceptional (Székely et al., 2014). ASR as well as OSR are relevant factors for understanding the intensity of sexual selection within a population (Emlen & Oring, 1977; Kvarnemo & Anhesjö, 1996; Székely et al, 2014), as the intensity of intra- and intersexual selection is expected to increase when the ASR and/or OSR is biased in favor of one sex. When one sex is more rare than the other, the costs of being choosy and and the benefits of investing in competitive traits which increase mating rates might be altered (Jennions & Fromhage, 2017). Choosiness by one sex could lead to a situation in which not all individuals of the other sex are mated, and this can result in a reproductive skew within this sex.

Reproductive skew is a widespread phenomenon in social animal communities that can be found in many vertebrates as well as invertebrates (Clutton-Brock, 1998; Keller & Reeve, 1994). Reproductive skew comprises a continuum between egalitarian breeding societies and societies in which one or few individuals monopolize reproduction within a group (Keller & Reeve, 1994). Especially when resources are limited, with a low variance in individual foraging success and high costs of reproduction, reproductive skew minimizes conflict over reproduction among related group members (Poethke & Liebig, 2008). At the same time, reproductive skew can increase individual reproductive output in cooperative breeding groups (Poethke & Liebig, 2008). Through alloparental care, individuals that do not meet the threshold for own successful reproduction can invest their surplus of resources not needed for survival into offspring of close relatives. In this way they increase not only the fitness of reproducers but also their own due to kin selection (Hamilton, 1964a; Poethke & Liebig, 2008). The breeding role can be determined by a dominance hierarchy as found in many mammals (Cant & Johnstone, 2000; Reeve, Emlen & Keller, 1998), by lack of resources (e.g. mating partners or breeding territory; Komdeur, 1992) or by a

morphologically distinct caste system (Beekman at al., 2006). However, the exact processes that determine which females reproduce and which remain unmated are not well understood.

Understanding the implications of ASR is essential for the study of social evolution (Ancona, et al., 2017), as it can indicate an impact of mate competition on determining reproductive roles. But estimating the ASR and subsequently the OSR in wild animal populations is often challenging due to different likelihoods of observing either of the sexes (Ancona et al., 2017). I attempted to estimate the influence of MSR/ASR on the reproductive skew in social spiders, in which the nests resemble self-contained population units. This will provide insights on how the reproducing and helping roles in social animal communities are determined.

1.4 The relation between group living and reproductive strategies

Reproductive strategies are influenced by resource availability for reproduction and group living can alter this factor. Natural selection acts on maximizing the reproductive success of living organisms (Young, 2010), forcing them to allocate restricted resources optimally. For this reason, animals face a trade-off between investing their energy budget in reproduction or non-reproductive efforts like growth and survival (Gadgil & Bossert, 1970), or differently put between present and future reproduction (Williams, 1966). The extent to which a parent should invest in parental care relative to somatic maintenance or growth is strongly influenced by life history and ecology (Stearns, 1992). Thus, two contrasting reproductive strategies have evolved. Most animals pursue an iteroparous strategy (Cole, 1954; Fritz, Stamp & Halverson, 1982; Singer, 2016), which is favored when environmental conditions are variable and juvenile survival to maturity is uncertain (Murphy, 1968). Iteroparous individuals invest only part of their resources in a single reproductive event while the other part of the energy budget is directed to survival and future reproduction, allowing for multiple reproductive events distributed in time and space. The trade-offs between current and future reproduction are expected to lead to progressive increase in reproductive effort with age as residual reproductive value declines (Clutton-Brock, 1991; Pianka, 1976). In contrast, following the definition by Fritz et al. (1982), semelparous species reproduce a single time only and allocate all available energy into the single reproductive

event with death as a by-product (Alonso-Alvarez & Velando, 2012; Cole, 1954; Stearns 1992). This strategy is also sometimes termed “big bang reproduction” (Gadgil & Bossert, 1970; Pianka, 1976), “uniseasonal-uniparous reproduction” (Kirkendall & Stenseth, 1985) or “suicidal reproduction” (Smith & Charnov, 2001), indicating a strategy in which all resources are invested in one reproductive event at the expense of own survival. Semelparity is exceedingly rare in vertebrates (Braithwaite & Lee, 1979; Crespi & Teo, 2002; Smith & Charnov, 2001), but more commonly observed in a minority of invertebrates (Fritz et al., 1982; Trumbo, 2013). The demographic model suggests that semelparity is favored under restrictive ecological conditions in which adult mortality is high (Murphy, 1968; Tallamy & Denno, 1981), while juveniles face comparably higher chances of survival (Bell, 1980). Under these conditions, fitness output can be increased by investing maximally in a single bout and thus increasing chances of offspring survival, instead of investing in an uncertain second reproductive event (Stearns, 1992; Roff, 2002). Consistently, the subsequent death is assumed to be a consequence of intense investment in the brood and not a result of voluntary sacrificial behavior (Bonnet, 2011).

Tallamy and Brown (1999) proposed that semelparity in insects is linked to the evolution of maternal care. When there are few opportunities for repeated reproduction, maternal care might have evolved due to reduced costs instead of significantly increased benefits of care behaviors. However, care-giving insects seem to be predominantly iteroparous, especially when parental care takes place in a sheltered nest (Trumbo, 2013). Food provisioning in a safe retreat can lead to delayed dispersal of the offspring (Wyatt & Foster, 1989), entailing sibling competition and dependence of offspring on their mother’s survival (Trumbo, 2013). This dependence leads to an increased risk of complete failure of reproduction in case the mother dies. To solve this problem, nests could be established by co-foundresses which provide allomaternal care and increase the chance that at least one caring individual survives to the full extent of the care period (Queller, 1994). The spread of parenting costs between multiple individuals and thus the possible increase in maternal survival might lead to a within-nest iteroparity, in which mothers might be able to produce more than one clutch (Trumbo, 2013). Especially when mothers can feed while

caring for their brood, they might be able to gather sufficient resources for multiple reproductive events (Tallamy, Walsh & Peck., 2004) despite extreme brood care. The present work investigates aspects of life history and reproductive strategy in social spiders as a study case for social animal communities, to evaluate the consequences of cooperative care.

1.5 Sociality in spiders

Sociality is a rare phenomenon in spiders and an especially interesting case of animal communities. Most spiders are predominantly or exclusively predatory, generalist hunters with conspecifics being on the menu as well, besides other small arthropods (Wise, 2006). Cannibalism is known to occur in many species (Wise, 2006), leading to a situation in which the evolution of sociality appears to be very improbable. However, about 25 spider species are known to permanently live in cooperating groups (Avilés, 1997; Bilde & Lubin, 2007; Viera & Agnarsson, 2017).

The arachnologist Eugène Simon was among the first naturalists to document spiders living peacefully in societies in South America (1891) as well as in oriental Africa and India (1892). But doubts about the accuracy of these observations were immediately raised, as the existence of social behavior in spiders, beyond the necessary interactions during courtship and mating behavior, seemed to be highly unlikely in such an aggressive and solitary animal group (McCook after Scientific American, 67/12, page 186). But in fact, communally living spiders had been reported before by Darwin (1845) and Cambridge (1889), and soon published reports on spiders living in aggregation accumulated (Bolivar, 1892; Marshall, 1898; Schwarz, 1904; Jambunathan, 1905), dispelling those doubts. However, the existence of spider communities remained puzzling and it comes as no surprise that to this day, social spider communities still inspire scientists around the world to investigate that phenomenon in detail. As the evolution of sociality appears to be so unlikely in spiders, they depict an interesting model organism to investigate the question of which internal preconditions and external circumstances promote social living and what consequences arise from it.

Today, besides the solitary life style, three different modes of sociality in spiders are commonly classified based on the territorial behavior displayed by the individuals and on whether group living is maintained throughout the whole life or not (Avilés, 1997; Agnarsson, Avilés, Coddington & Maddison, 2006; Kullmann, 1968):

1) **Solitarily living spiders** are most common, with individuals dispersing immediately after emerging from the egg sac to lead an **independent life** and aggressively claiming individual territories. Typically, interaction between adult individuals is limited to courtship and mating, in several species even putting the male at risk of being eaten by the female (Johnson & Sih, 2005; Newman & Elgar, 1991; Schneider & Elgar, 2001). Females often leave or die before the offspring emerges from the egg sac.
2) In **subsocial species,** adult spiders are solitary, but the female shows an extended phase of brood care, leading to cohabitation and cooperation between the mother and her brood for several weeks. Juvenile spiders ("spiderlings") share the web and cooperate for days or weeks after the mother's death. However, before spiderlings mature they disperse to henceforth lead a solitary life, leading to a lifestyle that is only **periodically social**.
3) **Colonial spiders** live in large aggregations throughout their lifetime. Within these aggregations each spider maintains her own territory and if brood care occurs it is performed solitarily. Interactions between the spiders are limited and cooperative execution of tasks does not occur. Because of this **non-cooperation** these species are sometimes labeled as **parasocial**.
4) Only few **permanently social spiders** live truly socially in large colonies throughout their whole life. They **cooperate in all tasks** within the nest and show no territorial behavior. They hunt and feed communally. Communities of permanently social spiders are generally characterized by a sex ratio with fewer males than females within a colony, a reproductive skew with only part of the females reproducing and a lack of premating dispersal, leading to extreme inbreeding.

The present work refers to the permanently social spiders of the fourth mode whenever social spiders and sociality in spiders is mentioned.

Permanent sociality is rare in spiders but found in such phylogenetically distant families as Tangle web spiders (Theridiidae), Crab spiders (Thomisidae), Meshweaver spiders (Dictynidae), Velvet spiders (Eresidae) and others (Avilés, 1997). It is thus widely assumed that sociality in spiders has evolved at least 20 times independently (Agnarsson et al., 2006; Avilés, 1997). Even within genera in which several social species occur, an independent origin of sociality is assumed for each species (Avilés et al., 1997; Johannesen, Lubin, Smith, Bilde & Schneider, 2007; Kraus & Kraus, 1989). As closely related sister clades often lack social species, a solitary lifestyle is suggested to be the primitive condition (Avilés, 1997). This multiple origin of sociality suggests a common basis within spiders that facilitates the evolution of sociality, despite their generally aggressive and solitary behavior. In many species spiderlings remain together in their first days after hatch (Foelix, 2011), requiring a suppression of aggression among them. This behavior, prolonged and modified via maternal care, is assumed to be this common basis (Burgess, 1878; Buskirk, 1975).

Almost all spider families that include social species are characterized by a maternal care period in which mothers and offspring live in close proximity for a while and interact peacefully (Avilés 1997, exception: dictynid spiders). These interactions require a social plasticity that allows for mutual tolerance between mother and offspring as well as a suppression of cannibalistic behaviors (Wise, 2006). It is thus commonly assumed that sociality in spiders is closely linked to brood care and is derived from a subsocial primary stage (Agnarsson et al., 2006; Bowden, 1991; Buskirk, 1981; Krafft & Horel, 1980; Kraus & Kraus 1989; Kullmann 1972; Settepani, Bechsgaard & Bilde, 2016) by elimination of pre-mating dispersal due to extension of maternal care and thus extended juvenile aggregation (Toyoma, 1999) which subsequently lead to the formation of family groups. Some authors claim that neoteny, e.g. the achievement of sexual maturity at an earlier developmental stage and the retention of juvenile behaviors (Kraus & Kraus, 1989; Seibt & Wickler, 1993), might be the origin of permanent sociality (Burgess, 1978; Kraus & Kraus, 1989 and 1990). The inhibition of cannibalistic behavior during adolescence might have been the origin

for the likewise inhibition in adults (Buskirk, 1975). To comprehend how a permanently social lifestyle has evolved in spiders it is therefore essential to understand the underlying mechanisms of reproductive and brood care behavior displayed in social and related subsocial species (Assi Bessékon & Horel, 1996).

The present work focuses on the life history, reproductive system and brood care behavior in the social eresid spider *Stegodyphus dumicola,* to investigate the dynamics and mechanisms of sociality and cooperative breeding in a social spider. *Stegodyphus dumicola* is one of three social spiders within its genus, which also includes 18 subsocial species and thus allows for comparison of closely related species with different social levels. Social *Stegodyphus* live in colonies of several hundreds of spiders, but usually only a minority of females reproduce (Salomon & Lubin, 2007). It is not well understood how the reproductive role of a female is determined or how the non-reproducing females contribute to colony life. To obtain an insight into the evolution of permanent sociality in these spiders and to understand how social living is shaped by and in turn effects brood care behavior, it is essential to explore the causes and consequences of why presumably many females within a colony forego their own reproduction. By comparing the situation in the cooperative breeding *S. dumicola* to the closely related solitarily breeding species *Stegodyphus lineatus*, similarities and differences between them allowed us to construct a scenario of social evolution. I aimed to discover possible causes and consequences of cooperative brood care by investigating how reproductive role is determined and how – if at all – virgin females are able to provide brood care. Furthermore, I examined the effects of the social and cooperative lifestyle in *S. dumicola* on different life history traits.

1.6 I) TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES

Social spiders are cooperative breeders that share a communal nest and collaborate in prey capture, nest defense, and brood care (Avilés, 1997; Lubin & Bilde, 2007). Mating and reproduction take place in the nest among related group members, which results in extreme inbreeding and high genetic relatedness among the individuals of a colony (Agnarsson et al., 2013; Settepani, Bechsgaard & Bilde, 2014; Settepani, Schou, Greve, Grinsted, Bechsgaard &

Bilde, 2017). Only a proportion of females within a colony reproduces (Avilés, 1997; Lubin & Bilde, 2007; Salomo, Mayntz & Lubin, 2008) and the unmated females are assumed to take on the role of helpers in brood care (allomothering) and other tasks in the nest (Bilde & Lubin, 2007; Salomon & Lubin, 2007). Previous studies showed that reproductive females or females of uncertain reproductive state direct care to offspring of other group members (Christenson, 1984; Kraus, 1988; Kullmann, Nawabi & Zimmermann, 1972; Samuk & Avilés, 2013). Notably, in the context of cooperative breeding and alloparental care, the role of mating status has not been investigated. It is not known whether all females care for egg sacs and regurgitate food or whether this behavior is exclusively displayed by females that have oviposited before, as observed females were either mated (Salomon & Lubin, 2007; Kullmann et al., 1972) or mating status was not known (Christenson, 1984). Thus, we currently do not know whether virgin females actually provide allomaternal care. This study aimed to fill this gap by assessing the role of virgin females in cooperative breeding, and to investigate whether mating state shapes patterns of allomaternal care.

In the genus *Stegodyphus*, females show extreme and suicidal maternal care that includes egg sac construction, tending and guarding of egg sacs, regurgitation feeding of the hatched spiderlings, and matriphagy at which point females are consumed by the offspring (Schneider, 2002; Seibt & Wickler, 1987, 1988a). In the social *S. dumicola*, brood size and offspring growth rate increases in the presence of mated females that have lost their own brood, suggesting that helpers acquire indirect benefits and promote group productivity (Salomon & Lubin, 2007). However, since about 60 percent of females in a nest of the social *S. dumicola* remain unmated (Salomon et al., 2008), it is essential to investigate the contribution of these females to various tasks to understand group organization. To this aim, we asked whether virgin females perform allomaternal care to the offspring of reproducing females, and whether the brood care provided includes all activities from egg sac care and regurgitation feeding, to matriphagy. Interestingly, maternal behaviors in spiders, such as tolerance towards offspring and provisioning of prey, have been found to be closely linked to reproductive status of the female (Assi Bessékon, Horel & Gundermann, 1992; Assi Bessékon & Horel, 1996; Eason, 1964). Only females that oviposited and

previously cared for their own young accept foster offspring and provide brood care, while females before oviposition react aggressively and do not provide for foreign offspring (Assi Bessékon et al., 1992; Assi Bessékon & Horel, 1996). The same is true for the subsocial solitarily breeding *Stegodyphus lineatus*, in which only mated females that produced an egg sac provide maternal care to cross-fostered offspring. Even in the social *Stegodyphus sarasinorum* from India, non-gravid females do not seem to provide maternal care but might accept an egg sac for some time (Bradoo, 1975). These observations suggest that virgin females in many spider species lack the requisite internal state to provide brood care and that brood care and regurgitation feeding are triggered by a preceding reproductive event (Schneider, 2002). Maternal care behaviors in virgin *S. dumicola* females would therefore indicate a remarkable adaptation to cooperative breeding in social species (Jones, Riechert, Dalrymple & Parker, 2007; Schneider, 2002).

An additional or alternative mechanism to allomaternal care, by which virgin females might benefit the reproductive females and their offspring, could be the specialization for certain tasks in the nest, which would allow mothers to focus on reproduction. Accumulating evidence for behavioral specialization in prey capture, web construction, defense behavior, as well as brood care, shows that despite the lack of morphological differentiation, social or facultative social spiders exhibit some degree of task differentiation (Settepani, Grinsted, Granfeld, Jensen & Bilde, 2013; Pruitt & Riechert, 2011; Wright, Holbrook & Pruitt, 2014; but see Ainsworth, Slowtow, Crouch & Lubin, 2002 and Settepani, Bilde & Grinsted, 2015). Although there is some evidence for unequal task participation among subordinate and (presumably) non-reproducing helpers in different animal groups (Emlen & Wrege, 1991; Monnin & Peeters, 1999; Cant, 2003; Mooney, Filice, Douglas & Holmes, 2015), in general we have little knowledge about how the reproductive state of individuals shapes behavioral specialization. Therefore, we asked whether the relative investment in reproductive and non-reproductive tasks differs between virgin females and mothers. If there is differential task participation between virgins and mothers, we expected virgin females to specialize on prey capture. This should be especially prominent on the risky task of the “pilot spider” (Bradoo, 1980), which in cooperatively hunting *Stegodyphus* spiders is the

first to reach and attack potential prey. This is a most precarious task with a high risk for the attacking spider of getting separated from the colony (Henschel, 1992), getting injured or killed, as potential prey might turn out to be defensive (Bradoo, 1980; Junghanns & Holm, pers. obs. 2013) or a predatory foe (Griswold & Meikle, 1990; Henschel 1998). If foraging is mainly executed by virgin females, this absolves reproductive females from attacking prey and facing the increased risk of injury or death. Instead, due to virgin females taking over other tasks, maternal care could be intensified, leading to an optimized resource allocation within the colony and consequently an increase in growth and survival of offspring. Thus, if there is task specialization, we further hypothesize that mothers are predominantly occupied with brood care and participate less in prey capture, coinciding with observations in mothers of the subsocial *S. lineatus*, which stop foraging when offspring hatch (Schneider, Salomon & Lubin, 2003).

To investigate whether virgin females are indeed brood caring allomothers and whether mothers and virgins are involved to the same or a different degree in reproductive and non-reproductive tasks, we observed experimental colonies of mated and unmated females. Participation in egg sac tending by the different reproductive states was used to investigate brood care behavior in groups of mated and unmated females, as this behavior can, unlike regurgitation feeding, often be observed outside the denser parts of the nest. Regurgitation feeding is often performed within nest entrances or inside the nest and females are more susceptible to disturbances (own observations) that might arise during close examination of the groups and is therefore difficult to observe in a quantifiable way. Thus, to gather qualitative data on virgin females and the different tasks of brood care they may be involved with, continuous observations and video recordings on a subset of groups were conducted. Finally, attack behavior was examined during feeding trials in which the identity of the “pilot spider” was detected in the above described groups.

1.7 II) MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE

Spiders show maternal care, for example by wrapping their eggs in silk cases and guarding the offspring by carrying the egg case or newly hatched offspring with them (Foelix, 2011). Some species show extended maternal care by providing the offspring with captured prey (Assi Bessékon et al., 1992; Avilés, 1997; Lubin & Bilde, 2007; Toyama, 1999) or regurgitation feeding (Kullmann, 1969; Kullmann, Sitterz & Zimmermann, 1971). Regurgitation feeding is an energy demanding task, and mothers lose weight (Salomon, Schneider & Lubin, 2005) and may undergo physiological changes involving degradation of the midgut (Nawabi, 1974; Salomon, Aflalo, Coll & Lubin, 2015), which is known to function as storage organ for glycogen and fat (Alberti & Storch, 1983; Nawabi, 1974;). Females in several genera have taken this behavior to the extreme, as they are consumed by their offspring after the provisioning period in (Foelix, 2011; Kim, Roland & Horel, 2000; Kullmann, 1968; Toyama, 1999; Viera, Ghione & Costa, 2007). *Stegodyphus* is one of these spider genera with females providing extended maternal care including regurgitation feeding and matriphagy (Kraus & Kraus, 1989; Lubin & Bilde, 2007). In the solitarily breeding subsocial *Stegodyphus lineatus*, experimental cross-fostering revealed that virgin females do not adopt and care for cross-fostered brood and will never produce an egg sac (Schneider, 2002). This implies that only mated females that have produced an egg sac are physiologically able to perform extended offspring care, and suggests that mating or oviposition initiates an internal maturation process that physiologically enables mothers to provide offspring care (Fénéron et al., 1996; Kim & Horel, 1998; Krafft & Horel, 1980; Mas & Kölliker, 2008; Pinilla, Aguilar, Dieguez, Millar & Tena-Sempere, 2012; Schal, Holbrook, Bachmann & Sevala, 1997; Schneider, 2002). Indeed, massive morphological changes in the midgut tissue related to brood care and maturing oocytes in the ovaries were observed exclusively in mated but never in virgin females of *S. lineatus* (Junghanns, 2013). In cooperatively breeding *Stegodyphus* mothers as well as mated and assumedly the large proportion of unmated (Salomon et al., 2008) female helpers provide (allo)maternal care, including regurgitation feeding and matriphagy (Lubin & Bilde, 2007; Salomon & Lubin, 2007). It is currently unknown whether such allomothers have developed physiological adaptations to cooperative breeding.

A comparative investigation of morphological dynamics during brood care in solitary and social breeders will contribute to our understanding of adaptations that facilitate cooperative brood care. To this aim, I conducted a study of the physiology of extreme offspring provisioning in mothers, virgin helpers and virgin non-helpers of the cooperatively breeding *Stegodyphus dumicola*. I investigated the dynamics of internal morphological changes during the brood care period in reproducing and non-reproducing females of the social *S. dumicola* and compare it to the situation in mothers and virgins of the solitarily breeding congeners *S. lineatus* (Junghanns, 2013; Salomon et al., 2015). I focused on the spiders´ midgut tissue, for which intense structural changes and the accumulation of liquefied tissue have been documented in mothers of subsocial *Stegodyphus* species during brood care (Junghanns, 2013; Nawabi, 1974; Salomon et al., 2015). To allow for a comparison between the social and the subsocial species, histological paraffin samples of *S. lineatus*, which had been processed in the frame of my diploma thesis (Junghanns, 2013), were re-evaluated (See Figure 1) and supplemented with semithin sections of the midgut. In preparation for regurgitation feeding, progressive accumulation of extracellular material in the midgut was observed in mothers of the subsocial congener *S. lineatus* (Junghanns, 2013; Figure 1 C and E). Therefore, I expected similar changes in brood caring females of the social *S. dumicola*. However, because *S. dumicola* females share the workload during a – compared to subsocial species – extended brood care phase (Salomon & Lubin, 2007), I expected changes related to brood care to be less pronounced in brood caring females of *S. dumicola*. If virgin helpers in *S. dumicola* indeed contribute to all tasks of brood care, I expected the dynamics of their midgut tissue to be similar to those of the mothers. I also explored the degree of oocyte maturation in virgin non-helpers, mothers and virgin helpers as a proxy for reproductive maturation. I hypothesize that reproductive maturation is a prerequisite for triggering the brood care mode in unmated females – the to-be allomothers – which might be visible in the developmental state of the oocytes in the ovaries (Fénéron et al., 1996).

As in the studies conducted on mothers and virgins of the solitarily breeding *S. lineatus*, I first assessed naturally occurring morphological changes in the midgut tissue of *S. dumicola* females at different time intervals during the brood care period (control group). This allowed me to assess

whether extreme brood care in mothers of the cooperatively breeding *S. dumicola* involves similar morphological changes in the midgut to those observed in mothers of *S. lineatus*. Second, I aimed to test whether morphological changes associated with regurgitation feeding of the offspring (Junghanns, 2013; Nawabi, 1974; Salomon et al., 2015) represent permanent changes, or whether tissue can be restored if a female loses her brood, enabling her to produce and care for a replacement clutch (Schneider & Lubin, 1997a; Futami & Akimoto, 2005; Viera et al., 2007). To identify the reversibility of morphological changes I experimentally removed eggs or offspring from females at different stages during the brood care period (removal group). This design allowed me to address the question of whether physiological changes related to brood care are reversible in *S. dumicola*, and if so, until which stage(s) during the brood care period. I furthermore investigated the question of whether the evolution of allomaternal care is associated with physiological adaptations in unmated helpers, e.g. changes in the internal morphology that triggers their ability to regurgitate food and provision offspring. I expected that virgin helpers undergo similar physiological changes associated with the provisioning of young as do mothers. Additional to the samples for paraffin histology, midgut tissue samples of females of *S. dumicola* as well as of *S. lineatus* were prepared for semithin sectioning to provide further insights into morphological dynamics during brood care. Finally, if virgin helpers provide extreme brood care, I wanted to know, whether this ability is associated with oocyte maturation that would indicate an internal maturation process, which has not been observed in virgin *S. lineatus* (Junghanns, 2013).

1.7.1 Internal anatomy of a spider

The body of a spider is partitioned in the frontal prosoma and the caudal opisthosoma. The prosoma comprises mainly sensory structures like the eyes and the nervous system, but with its four pairs of walking legs also serves locomotion. The opisthosoma contains the main parts of the respiratory, excretory, circulatory, digestive and reproductive organs (Foelix, 2011). The opisthosomal part of the intestinal tract serves such functions as food transport, releasing enzymatic secretions for digestion, absorbing nutrients and storing resources in form of fat and glycogen (Nawabi, 1974). Thus, it has been termed “liver” (Beklemischew, 1960), “fatbody”

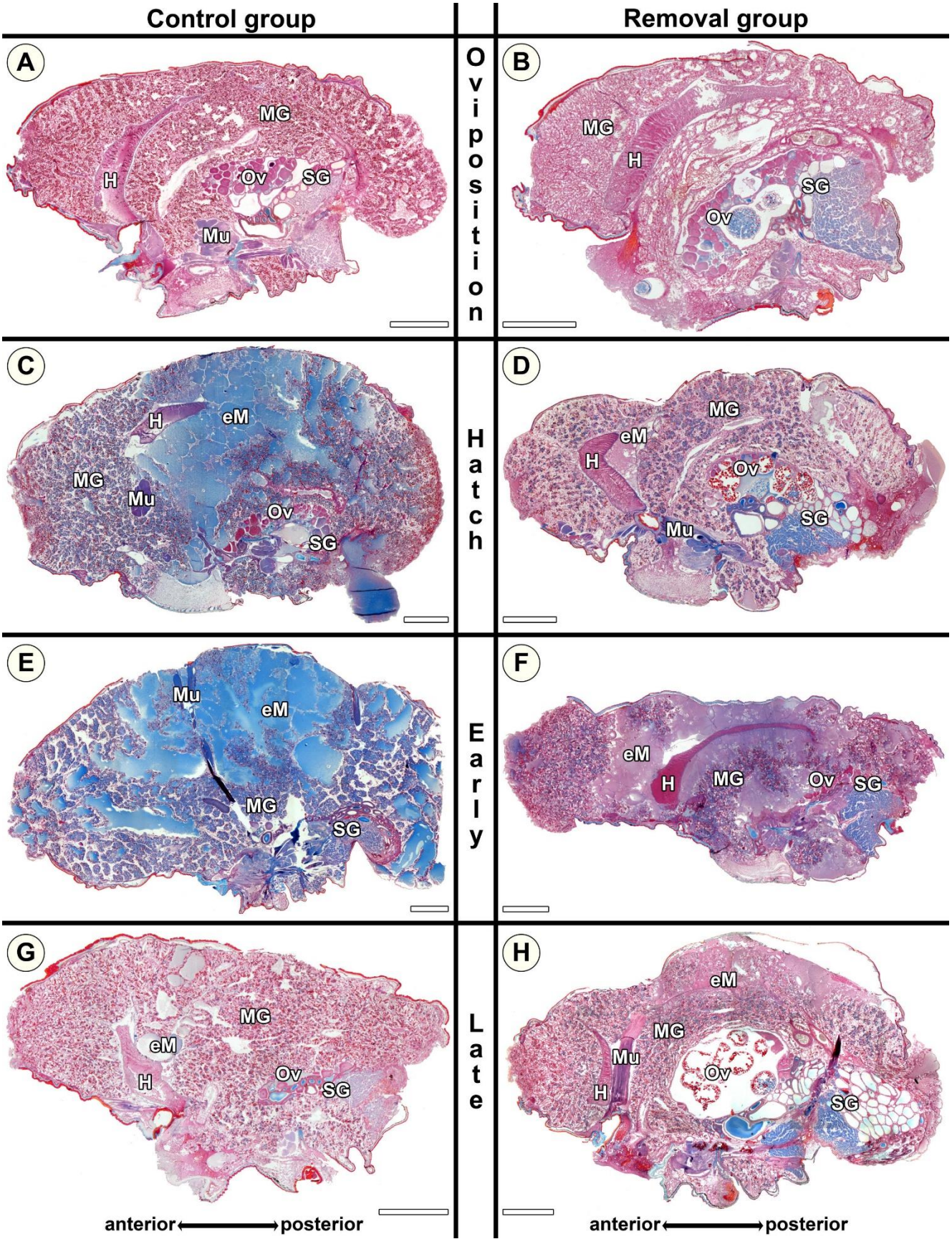
(Wasmann, 1846) or “chylus-stomach” (Bertkau, 1885) in the literature, with each of those names only capturing part of its various functions. In the present work the structure will be called midgut gland, following the designation and definition by Nawabi (1974). The midgut gland consists of the main midgut tube and a system of intensely branched diverticula as well as an interstitial tissue which interconnects the diverticula, forming altogether a macroscopically compact structure (Ludwig, 1990; Nawabi, 1974). The midgut gland takes up most of the space of the opisthosoma (Alberti & Storch, 1983), enveloping the other organs such as the heart, the book lungs, the silk glands and the ovaries. Each diverticulum is surrounded by a basal lamina and the epithelia consist of two types of cells: The resorptive / digestive cells and the secretory cells (Bertkau, 1885; Millot, 1926). The less common secretory cells often contain vacuoles (Collatz, 1987), which are light microscopic dark zymogen granules (Foelix, 2011) that can release proenzymes in the gut lumen for food digestion. The resorptive cells are most abundant and constitute three quarters of all cells of the diverticula. They are characterized by numerous inclusions (Collatz, 1987; Foelix, 2011). Resorptive cells are responsible for food uptake, processing of nutrients and their transportation to the underlying layer of interstitial cells (Foelix, 2011). The interstitial tissue serves the main storage function and contains resources in form of fat or glycogen as well as crystalline excretory products (Collatz, 1987; Foelix, 2011, Nawabi, 1974). Re-examinations of the light microscopic studies on the subsocial species *Stegodyphus lineatus* in different stages of brood care (Junghanns, 2013) revealed characteristic brood care-related dynamics in the midgut region (Figure 1), which had also been documented by Salomon et al. (2015) in the same species and by Nawabi (1974) in the subsocial *Stegodyphus pacificus*. The quantity of blue stained granules within the cells of the midgut tissue changes with massive amounts of those granules accumulating at the time when spiderlings hatch and during early regurgitation (Figure 1, C and D). The interstitial tissue appears most prominent at the time when spiderlings hatch and in early stages of the regurgitation feeding phase but degenerates in late regurgitation stages. The study further revealed that extracellular material accumulates in large amounts in the lumina of diverticula and newly forming extracellular spaces in preparation for regurgitation feeding (Figure 1, C and D). The extracellular material can be distinguished in pink or grey stained, coarse and flaky structured food remnants and presumed regurgitant, which is

more homogenous structured and often stains blue. New extracellular spaces seemed to be created by cell degradation, a process that led to progressive disappearance of diverticula with digestive and secretory cells disintegrating first and the basal lamina remaining longest. The females were able to halt the advance of the above described histological changes and reabsorb the accumulated extracellular material with their remaining intact diverticula when they were separated from their offspring until the time when spiderlings hatched (Figure 1, D). However, signs of degradation of cells remained and no evidence for cell regeneration was found.

The ovaries of spider females stretch as elongated structures mid to ventrally in the opisthosoma (Foelix, 2011) and contain oocytes in different developmental stages. Vitellogenesis of the oocytes was described by Trabalon, Bautz, Morniniere and Porcheron (1992), who distinguished between a previtellogenic, early vitellogenic and late vitellogenic successive phase. The previtellogenic phase is characterized by a homogeneous basophilic ooplasm with a disc-like "vitelline body" appearing close to the nucleus. The early vitellogenic phase features the increasing accumulation of yolk vesicles in the ooplasm, the disappearance of the vitelline body and oocytes, which increase dramatically in size. In the advanced early vitellogenic phase the yolk vesicles are as large as the nucleus. When the oocytes enter the late vitellogenic phase huge yolk vesicles appear, and the nucleus is no longer visible. Only the previtellogenic and the beginning of the early vitellogenic phase of oocyte development were found by Trabalon et al. (1992) in virgin spiders. The advanced stages of the early vitellogenic phase as well as the late vitellogenic phase only occurred in mated females in his studies. My studies on *Stegodyphus lineatus* (Junghanns, 2013) affirm these findings. Although one alleged virgin female caused some confusion, as it showed late vitellogenic oocytes, the current re-examination of the samples showed that this female was indeed mated as it had sperm in its spermatheca. All virgin *S. lineatus* females showed exclusively undeveloped previtellogenic oocytes with a size of 150 µm and a homogeneously pink stained matrix in AZAN lacking larger granules. Similarly, to virgins, mothers which cared for their brood did not show late vitellogenic oocytes. Only mated *S. lineatus* females that had lost their brood were able to mature oocytes after their first oviposition again to an early vitellogenic phase (oocyte size 200 to 300 µm) or a late vitellogenic phase (oocyte size up to 370 µm) that exhibited a grained ooplasm. These results suggested that *S.*

lineatus mothers refrain from utilizing energy to develop eggs when brood is present but maintain their ability to mature oocytes even late in the brood care period in case the brood is lost.

Figure 1: Re-examination of histological sections of opisthosomata of *Stegodyphus lineatus* mothers (Junghanns, 2013). Females were either immediately chemically fixed (left column, control group) or offspring were removed (right column, removal group) at oviposition (A, B), hatching of offspring (C, D), early regurgitation phase (5 days after hatching, E, F) and late regurgitation phase (10 days after hatching, G, H). Females in the removal experiment were chemically fixed 15 days after hatching of offspring, when matriphagy would occur under natural conditions. In the control group, by the time offspring hatch (C), massive changes have occurred compared to females at the time of oviposition (A). Blue stained secretion granules are abundant, and parts of the midgut tissue are dissolved with blue stained extracellular material (eM) accumulating especially in the mid-region of the midgut tissue while anterior parts stay intact for the longest time. At the late hatching phase, almost no extracellular material is left, and the opisthosoma has shriveled (see scale bars), but parts of the midgut tissue are still intact. When mothers were separated from offspring directly after hatching (D) they were able to terminate and reverse processes, as almost no extracellular material is visible. During regurgitation the ability to reverse the cellular disintegration diminishes, as extracellular material remains. Oocytes might mature in the removal group even until the late regurgitation phase. H = heart, MG = midgut, Mu = muscle, Ov = ovary, eM = extracellular material (regurgitant), SG = silk gland. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several individual images.


Control group
Removal group
Oviposition
Hatch
Early
Late
A
B
C
D
E
F
G
H
MG
H
Ov
SG
Mu
eM
anterior
posterior

1.8 IV) MALE SCARCITY AND ITS IMPLICATION ON REPRODUCTIVE SKEW

Social spider communities are characterized by female-biased sex ratios in all developmental stages (Avilés, Varas & Dyreson, 1999; Vanthournout, Busck, Bechsgaard, Hendrickx, Schramm & Bilde, 2018; Seibt & Wickler, 1988a) and a reproductive skew with only a minority of females reproducing (Vollrath, 1986; Salomon, 2008). It has been proposed that reproductive skew in social spiders is a product of competition over limited resources (Avilés, 1997; Whitehouse & Lubin, 2005; Ebert, 1998; Vollrath & Rohde-Arndt, 1983), which leads to early developmental trajectories that cause variation in female size and skewed maturation (Bilde & Lubin, 2011). Short-lived males mature before the females (Henschel, Lubin & Schneider, 1995), hence only the early-maturing females can mate as the probability to get fertilized decreases in late-maturing females (Ulbricht & Henschel, 2006). Females that lose out in the competition over resources during growth mature too late and run the risk of missing the narrow window of adult male presence in the colony, and thus likely remain unmated (Salomon et al., 2008). A similar mode of determining female role has been described for cooperatively breeding sweat bees, in which females differ in size but ultimately helping role is established by the timing of female emergence during or after male activity (Yanega, 1992). However, an additional sex role determining factor in social spiders might be the extremely skewed sex ratio with on average only 11 percent of individuals within a colony being male (Seibt & Wickler, 1988a). The pronounced female majority could lead to the assumption that the limited number of males might pose a constraint that causes the skewed reproduction within females (Salomon et al., 2008) due to the lack of mating partners, even among females that mature before males start to die off. Indeed, the operational consequence of the restricted male presence on female reproduction is so far unknown. It has been shown in social spiders that female-biased sex ratios are already present in variable values in male sperm (Vanthournout et al., 2018) and in embryonic stages (Avilés & Maddison, 1991; Avilés et al., 1999) and extend into adulthood (Seibt & Wickler, 1988a; Henschel et al., 1995). In *S. dumicola* the sexes mature asynchronously with adult males appearing first and subsequently females maturing gradually, which leads to changing adult sex ratio (ASR) over the course of the reproductive season. Consequently, ASR is assumed to be male-biased in the beginning of the reproductive season when males mature and changes to a female-

biased ASR when females start maturing and males gradually die off with the progressive season. Hamilton (1967) suggested that in systems in which males mature first and then mate multiple times, a female-biased sex ratio is expected to arise to minimize competition among brothers. We know that colonies of *Stegodyphus dumicola* generally include fewer males and that males mature early, but it is not known how many females can be fertilized by a single male.

When ASR is biased to one sex, sexual competition among individuals of the other sex is expected to occur (Jirotkul, 1999; Taylor & Bulmer, 1980), especially when the ability to mate is restricted in the minority sex. As males do not produce unlimited amounts of sperm (Nakatsuru & Kramer, 1982; Olsson, Madsen & Shine, 1997), they are only able to copulate a limited number of times, which might prevent them from fertilizing all available mating partners within a colony. Consequently, a proportion of females remains unmated, even if all mature in time to encounter adult males. Natural male mating rates have been investigated in few spider species, usually showing very low mating frequencies of one or two matings per male, especially when life expectancy is short (Vollrath, 1986; Schneider & Andrade, 2011; but see Ruch, Heinrich, Bilde & Schneider, 2009 and Morse, 2007). These limitations could entail mechanisms of sexual selection, one of which is mate choice (Andersson & Iwasa, 1996) in which one sex prefers mating with individuals of the opposite sex that show a certain value of a trait. Male choosiness might arise in a system in which males are only able to mate a limited number of times, but female density is high (Huber, 2005, Yip, Berner-Aharon, Smith & Lubin, 2016). However, Riechert and Roeloffs (1993) proposed that due to the high genetic similarity of individuals within social spider colonies, the benefits for competition and choosiness among mates should be low. Nevertheless, sexual competition among males has been documented in social spiders (Bradoo, 1975 in *Stegodyphus sarasinorum*; Henschel et al. 1995 in *S. dumicola*; Lubin, 1986 in *Achaearanea wau*), indicating that even in inbred species variability among mates might be high enough to favor mate choice (Henschel et al., 1995).

Females within a colony can differ quite extensively in body size, even within individuals that hatched from the same egg sac (Kraus & Kraus, 1990; Henschel et al., 1995), with the largest females being almost twice as large as the smallest females. These size differences may be due to differential access to resources during growth but also due to the fact that in *Stegodyphus* sexual maturity can be reached after a varying numbers of molts (Kullmann et al., 1972). Males might prefer large females over smaller females, as female size is known to be positively correlated with the number and size of her offspring (Kessler, 1973; Marshall & Gittleman, 1994; Schneider, 1996b). In this case, small females might thus still be doomed to remain unmated even if potential mating partners are available, and in social spiders become virgin helpers instead of reproducers. It might thus be advantageous for females to invest more time in growing before maturation, especially since in spiders, growth is not continuously like in vertebrates but punctuated by molts. Consequently, the final molt to maturity determines the final size of the female (Vollrath, 1987). However, female reproductive strategy in *Stegodyphus dumicola* might be determined by the "decision" to either grow large or mature early, as in a system with suicidal brood care, benefits should arise for both sexes from reproducing early in the reproductive season. Due to the extreme brood care behavior, which progressively drains the resources of brood caring females and eventually leads to the death of the parental generation, it should be beneficial for females to reproduce early. Early hatched offspring in a colony benefit from a full pool of colony resources for the longest possible time before resources are depleted by regurgitation feeding and matriphagy (Henschel et al, 1995). It is assumed that the oldest and thus probably the largest offspring has an advantage in competing over dwindling resources (Ulbrich & Henschel, 1999; Schneider, 1995; Whitehouse & Lubin, 1999) and therefore amplifies its dominance over time. Those well-fed spiderlings are most likely the first ones gaining all resources needed to mature in the coming reproductive season and thus will become the reproducing females of the next generation (Henschel et al., 1995). Additionally, it has been shown that male mating history can determine female fitness to some extent (Jiao et al., 2011). Thus, the females that manage to mature early enough to be able to mate with virgin males could enjoy fitness benefits. For females, timing of maturation can thus be equally as important as body size to maximize their fitness. Females are therefore trapped in the quandary of taking their time

to grow large but also to outcompete other females in being fast enough to reproduce early. For subadult females the ability to adjust the speed of moulting flexibly to the presence of an adult male could thus be beneficial by ensuring that they grow as long as possible but are ready to mate in time, before the short-lived males disappear. That the timing of molting can be determined by environmental stimuli or chemical cues emitted by conspecifics is known from a variety of invertebrates (O´Connor & Van, 2006; Kim, 2001; Fowler & Gobbi, 1988).

Here, I aimed to investigate the role of the low male numbers within a colony of *S. dumicola* in determining the breeding system and the formation of reproductive skew. Evaluating the ASR can be an adequate approach to estimate the OSR and thus provide cues on selective pressures on the sexes. However, investigating ASR in natural populations is often challenging (Ancona et al., 2017). As premating dispersal is lacking in *S. dumicola* colonies (Lubin &b Bilde, 2007; Lubin, Birkhofer, Berger-Tal et al. & Bilde, 2009), young colonies represent closed populations in which MSR and ASR can be explored to obtain more information on the breeding system. I collected data on colony composition from natural nests at the beginning of the reproductive season to complement existing knowledge on sex ratios and skewed maturation in colonies of *S. dumicola.* To investigate the implications of low male presence in the colony for the determination of reproductive role in females, I tested whether the presence of an adult male, and thus an early mating possibility, influences the decision of females to mature. For this purpose, I produced experimental colonies of juvenile females with and without adult male presence in the lab and recorded the latency between the penultimate and ultimate molt of females. If females are able to react flexibly to the presence of adult males, I expected them to mature faster when a male is present to ensure (early) mating. I further investigated in mating trials, whether males showed a mating preference for females of the large or small size category. If their mating ability is limited and males are choosy, I expected them to decide to mate with the large female which promises higher fitness benefits. Furthermore, I explored the potential rate of reproduction (PRR, Kvarnemo & Ahnesjö, 1996) a male can achieve, to investigate the implication of adult sex ratios on a) the mating system and b) the OSR in this species. In the social *Anelsoimus eximius* males

cannot inseminate all adult females (Vollrath, 1986). However, Ruch et al. (2009) found that males of the subsocial *Stegodyphus tentoriicola* visit up to nine females, although it was not clear how often males copulated. In this study I presented males of *S. dumicola* with adult virgin females *ad libitum*. I counted the number of matings and subsequently observed the reproductive output of the mated females to gain new insights into the mating system, which allows to draw conclusions on the determination of female reproductive role.

1.9 IV) LIFE HISTORY

An essential foundation for understanding dynamics and mechanisms in an animal system is basic knowledge about the life history traits of a species. A change from a solitary to a social lifestyle might entail fundamental changes in reproductive biology and life history, which could alter the fitness outcomes of an individual (Hatchwell & Komdeur, 2000). Thus (species)specific knowledge of life history traits is essential to comprehend the full scope of consequences of social living and cooperation in social spiders. Although *Stegodyphus dumicola* has been subject to a series of studies in the past, basic data is often inferred from knowledge gained on subsocial relatives such as *S. lineatus* or deduced from observations on the whole colony. For example, a semelparous lifestyle, with females producing a single egg sac during their life time, is generally assumed for mothers of the social species due to the fact that subsocial congeners reproduce only once (Grinsted, Breuker & Bilde, 2014; Salomon, Mayntz & Lubin, 2003) and the number of egg sacs in a colony is low compared to the number of females (Henschel, Lubin & Schneider, 1995). However, this assumption might underestimate the fitness effects of sociality in spiders. The lack of detailed information on certain life history traits is presumably due to the fact that systematic examinations of individual life histories in colonies of the social species are difficult to implement. In a system with several reproductive females and a closed nest in which constant observation is challenging or even impossible, it is extremely difficult to follow the life history of a single individual. However, assumptions based solely on data derived from a subsocial species hinder our understanding of how sociality has evolved, and which costs and benefits arise from a cooperative lifestyle. Similarly, data gathered from general observations of colonies instead of

individuals might give a false impression of individual reproductive success and the breeding system in social spiders. Thus, I intended to obtain information on individual life history that will contribute to understanding the consequences of sociality and cooperation. To this aim, I observed single mated females, grouped with three virgin females in experimental colonies after mating. I recorded the time until oviposition and hatching of offspring as well as the total number of egg sacs. It has been assumed that the number of egg sacs in a colony should equal the number of mated females, as virgins in the subsocial species do not oviposit and mated females only produce a second clutch when the first one is lost (Schneider, 2002). If mothers in the social *S. dumicola* can produce more than one egg sac in the presence of virgin helpers, this would suggest an evolutionary consequence of social living. The production of multiple egg sacs by mated females would depict a transition from the strictly semelparous life history to an iteroparous reproductive strategy that is able to increase a female's reproductive success. My goal was, to provide reliable basic data on reproductive traits to enable a direct comparison between a social and a subsocial species, thus allowing a discussion of the effects of sociality and cooperative brood care.

1.10 Study questions

I) TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES

Does the investment in reproductive and non-reproductive tasks differ between virgin females and mated females or do females of different reproductive backgrounds participate to the same degree in both types of tasks, including brood care?

II) MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE

Are differences in lifestyle and brood care between solitarily and cooperatively breeding mothers reflected in morphological changes that go along with brood care? Are there morphological indications for adaptations to allomaternal care in virgin females?

III) MALE SCARCITY AND ITS IMPLICATION ON REPRODUCTIVE SKEW

Are sex ratio and timing of maturation skewed between males and females in natural colonies? What are the implications of the low male presence in the colony for reproductive skew? Is sex ratio a factor that might drive reproductive skew?

IV) LIFE HISTORY

What are the similarities and differences in life history and reproductive traits between the subsocial and social species? Do reproductive life history traits indicate adaptations to social living?

2 METHODS

2.1 Study species

2.1.1 The genus *Stegodyphus*

The genus *Stegodyphus* Simon 1873 belongs to the velvet spiders (Eresidae) with 21 known species (Platnick 2014). They typically do not live on the ground, like many other Eresidae, but built their retreats and webs in shrubs and trees (Simon, 1892). *Stegodyphus* is distributed in Africa, Asia and Europe as well as in South America with one species known from Brazil. Like all other spiders in the eresid family, most *Stegodyphus* species are known to be subsocial (Yip & Rayor, 2014). However, a permanently social lifestyle has evolved three times independently within the genus: in *S. sarasinorum* Karsch 1891 in Asia as well as in *S. dumicola* Pocock 1898 and *S. mimosarum* Pavesi 1883 in southern Africa. Each of those social species have their own subsocial sibling species (Johannesen, 2007; Kraus & Kraus, 1989, 1990; Settepani et al, 2016) suggesting independent origins of sociality in all three cases. Mothers of all *Stegodyphus* species show an extreme form of brood care behavior. After oviposition they tend to the egg sac by exposing it to the optimal microclimatic conditions (Millot & Bourgin, 1942). When the offspring are ready to leave the egg sac the mother opens it, as her offspring are not able to leave the egg sac independently. The emerging offspring are helpless and depend on the care of their mother, as they are not able to hunt and feed on prey until after their fourth molt (Millot & Bourgin, 1942). Instead, the offspring feed on a nourishing fluid produced and regurgitated by the mother (Kullmann et al., 1971). This regurgitation feeding is performed over the course of several weeks (Seibt & Wickler, 1988) and the length of the regurgitation phase depends on the species. Studies of subsocial species have shown that the female undergoes massive physiological changes during the brood care process, with parts of her midgut tissue liquefying (Junghanns, 2013; Nawabi, 1974; Salomon, 2015), which she is presumably regurgitating to the offspring. At the end of brood care, the rest of maternal resources is acquired by the offspring directly via a process called matriphagy, during which the offspring will suck the female dry within a few hours (Schneider, 1996a), resulting in the mother´s death. During brood care, the female transfers up to 96 percent of her bodily resources to her offspring (Salomon et al., 2005) and the weight of offspring increases up to 15 times (Kullmann et al., 1971) in just a few weeks. This extreme brood care

behavior leads to a semelparous life with females raising a single successful brood (Salomon et al., 2005; Schneider et al., 2003). In subsocial species a replacement clutch may be produced, if the first brood is lost (Schneider, 1999; own observations). However, the probability of successfully raising the second clutch is low due to high female mortality (Schneider, 1999).

2.1.2 The subsocial *Stegodyphus lineatus*

Stegodyphus lineatus Simon 1873 shows a circum-mediterranean distribution occurring from southern Europe to northern Africa and central Asia and is the only species of this genus that occurs in Europe (Millot & Bourgin, 1942). It occupies dry and hot habitats where it builds its funnel-like retreat and catching web in lower vegetation (Millot & Bourgin, 1942). During the mating season between March and April (Schneider, 1995) adult males roam around to find receptive females (Schneider, 1997a). They will cohabit with the females for some days, sharing retreat and prey (Schneider, 1995). If the male finds a female already guarding an egg sac, he might try to discard it to trigger her willingness to remate (Schneider, 1997b). Thus, mated females often react aggressively to the mating advances of further males. About two weeks after mating, the female will produce an egg sac containing between 40 and 140 eggs (Schneider, 1996b) and ceases from foraging (Schneider et al., 2003). For the next month she tends to the egg sac before opening it to release the offspring (Millot & Bourgin, 1942). Regurgitation feeding usually last for two to three weeks (Schneider, 1995). After matriphagy, the offspring share their natal nest and prey for some more weeks (Millot & Bourgin, 1942) and then disperse to start a solitary life before they mature. Maturation takes place after nine to eleven moltings in males and after eleven or more moltings in females (Kullmann et al., 1972). Post-adult moltings have been documented in females (Kullmann et al., 1972; own observations 2013) and males (Kraus & Kraus, 1989).

2.1.3 The social *Stegodyphus dumicola*

The permanently social *Stegodyphus dumicola* Pocock 1898 is distributed in southern Africa where its colonies can be found in local patches (Seibt & Wickler, 1988a) of dry savanna regions. Colonies inhabit a sponge-like nest which provides protection from predators (Henschel, 1998)

and unfavorable climatic conditions (Seibt & Wickler, 1988a). For prey capture the colony maintains a two-dimensional capturing web that can reach sizes of more than 1m² (Seibt & Wickler, 1988a). Nest maintenance, prey capture and brood care are executed cooperatively by all spiders. In the social species individuals do not disperse as juveniles but mate and reproduce within the parental colony, leading to an inbred mating system (Johannesen, Hennig, Dommermuth & Schneider, 2002) and nest mates that are genetically homogenous (Settepani et al., 2017). The sex ratio is extremely skewed with, on average, only 17 percent of eggs produced (Avilés et al., 1999) and 11 percent of adult spiders (Seibt & Wickler, 1988a) being male. Before the first females are mature, males mature synchronously within about a week (Henschel et al., 1995) and reach maturity after the sixth or seventh molting (Kraus & Kraus, 1990). Their mature life extends over four to six weeks (Henschel et al., 1995) and dispersal events are mostly limited to adjacent nearby nests (Lubin et al., 2009), which are interconnected by threads or capture web. Females mature asynchronously (Salomon et al., 2008) over the course of weeks or months, and mature after eight or nine moltings (Kraus & Kraus, 1990). Their adult life expectancy is several months (Seit & Wickler, 1988). Colonies within a population do not necessarily mature synchronously (Henschel et al, 1995), but adjacent nest are usually in a similar developmental stage (Salomon et al., 2008; own observations). On average, only 40 percent of females reproduce (Salomon et al., 2008), leading to a pronounced reproductive skew within the colony. Oviposition takes place between February and March (Marshall, 1898), about XX days after mating. Approximately 30 to 100 eggs are produced (Griswold & Meikle, 1990; Avilés, 1999, Salomon & Lubin, 2007). Females can regularly be observed transporting the egg sac outside the nest for cooling (Henschel, 1992; Seibt & Wickler, 1988a) or back inside when weather conditions change. After hatching, regurgitation feeding lasts for about 7 weeks in groups of females (Salomon & Lubin, 2007), which are known to cooperate during brood care (Kullmann et al., 1971). There is no overlap of adult generations (Marshall, 1898; Seibt & Wickler, 1988a), as at the end of summer between April and June gradual death of the adult females due to matriphagy occurs (Henschel et al., 1995) before the new generation matures.

Colonies are inhabited by consecutive generations for up to seven or eight years (Miller, Griswold, Scharff, Řezáč, Szűts & Marhabaie, 2012). New colonies are established in two ways: via fission

and/or propagule dispersal (Bilde & Lubin, 2007). During fission of the main colony spiders disperse individually or in small groups over short distances (Henschel, 1998) to establish a new nest in a nearby branch. These new nests are often still connected with the main colony via threads or a shared capture web and an exchange of individuals between the nests may happen frequently. Propagule dispersal may lead to dispersal over longer distances, with a single mated female releasing hundreds of silk threads to balloon away (Crouch, Lubin & Bodasing, 1998; Schneider, Roos, Lubin & Henschel, 2001). Predation risk for dispersing spiders and single spider colonies is extremely high (Henschel, 1998; Ulbrich & Henschel, 2006), but if the ballooning female survives, she and her offspring will establish a new colony.

2.2 Collection sites and general animal maintenance

The samples for ultrastructural studies of the subsocial *Stegodyphus lineatus* were provided by Aarhus University. Adult females were collected in Israel in April 2012 at Mt. Amasa (31.31N, 35.12E) and Lehavim (31.36N, 34.83E) before oviposition and transferred to a climate chamber at Aarhus university with a constant 25°C and a 12:12 light:dark cycle. They were kept within their natural retreat in plastic containers (90 x 70 mm) that allowed them to build a capturing web. Food was provided two to three times per week in form of house flies or crickets until the time of oviposition. As in nature females will stop feeding during brood care (Schneider et al., 2003), food provisioning was stopped as soon as an egg sac was present. Samples of midgut tissue of females in different life stages (see below) were chemically fixed and stored in glutaraldehyde and sent to Greifswald University for further preparation.

57 natural nests of the social *Stegodyphus dumicola* were collected in three consecutive years 2013 (N = 23), 2014 (N = 20) and 2015 (N = 14) between the end of October and mid-November in South Africa before egg sacs appeared. Nests from 2013 were collected in the region of Shingwedzi (-22.98, 31.30), Middelfontein (-24.68, 28.55) and Mokopane (-24.40, 28.78), nests from 2014 at Shingwedzi (-22.98, 31.30) and Skukuza (-24.93, 31.69) and nests from 2015 at northern Kwa-Zulu-Natal (-26.93, 32.82). Spiders collected in 2013 were kept at constant 25°C and a 13:11 light:dark cycle at Aarhus University. Spiders collected in 2014 and 2015 were kept at Greifswald University in a climate chamber with a 12:12 light:dark cycle and a temperature

ramp of 27°C (5h) : 30°C (2h) : 27°C (5h) : 19°C (12h). I dissected all nests within a few days after collection, and we separated the sexes, as well as juvenile and subadult (penultimate instar) females from adult females. Subsequently males and immature females were reared separately. To be able to control the mating status of the spiders, only spiders that matured in the lab were used for experimental designs. Females that never encountered a male after maturation were used as either "virgin non-helping females" or, when cohoused with a mated female and her offspring, as "virgin helpers" in the experimental designs. Likewise, males that matured in the lab and never encountered an adult female were used as virgin males in some of the experiments. To obtain mated females a male and a virgin female from the same natural nest (unless stated otherwise) were united in a separate container overnight. Trials in which the copulation was observed, showed that females often exhibit secretions on their genital area, whereas those were never seen in virgin females. Thus, a female that spent a night with a male was subsequently considered to be mated if these secretions were observed and will henceforth be termed "mother". However, as lack of those secretions is not a sure sign for virginity, females that had spent a night with a male but did not show those secretions were excluded from any further experiments, as mating status could not be verified. Any orphaned offspring resulting from experiments was transferred to an adoptive nursery group consisting of spiders which were not used for experiments and reared henceforth under favorable conditions. Unless stated otherwise, all spiders were fed once or twice every week with one fly (*Calliphora*) for every two or three adult spider females. Water was provided every day during breeding season and off breeding season two to three times a week.

2.3 I) TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES

2.3.1 Group composition

To investigate participation of virgin helpers and reproductive females in brood care and foraging, 192 experimental colonies of *S. dumicola* were created, with two just-mated ("mothers") and three virgin helpers from 23 natural nests collected in 2013. This resembles the reproductive situation in colonies in the wild, where about 60 percent of females remain

unmated (Salomon et al., 2008). The set-up with two mothers increased the likelihood that cooperative brood care would be observed, even if virgin helpers would not provide brood care, as cooperative brood care is known from reproducing females (Kullmann et al., 1971). All females received an individual marking with a water based acrylic color. Mothers were marked in either red or orange and virgin helpers were marked in blue, green or yellow. As females in a colony mature asynchronously, the establishment of groups stretched over a period of 9 weeks between November 2013 and January 2014. Experimental colonies were kept in transparent plastic containers (122 x 82 x 52 mm), of which two sides were covered with mesh to ensure sufficient ventilation. A plastic ring (diameter 53 mm) in the center of the container provided a frame for nest and web construction. Water was provided every second day. Groups were checked daily for oviposition events and only groups in which at least two egg sacs were produced (N = 171 including 860 individuals) were used for the experiments to increase the likelihood that both mated females had oviposited and were thus indeed reproducing females/mothers.

2.3.2 Brood care observations

Observations on egg sac care were conducted in 2014 between January, when the first egg sac was produced, and March, over the course of 18 weeks. During checks once or twice per day the reproductive identity (mother or virgin helper) of the female tending the egg sac was recorded. Tending the egg sac was defined as any contact with the egg sac, ranging from touching it e.g. with a leg, sitting on it, handling it or transporting it. Cases in which no spider was caring for the egg sac were recorded as well. During 217 observations, more than one female was observed during egg sac care. This includes instances in which more than one female was tending a single egg sac as well as cases in which more than one egg sac was cared for by different females in parallel. Only groups that were observed at least 20 times for egg sac care behavior were considered for statistical analyses. Thus 825 individuals from 165 experimental groups were observed a total of 3719 times during egg sac care and included in the statistical evaluation.

To obtain qualitative data on brood care beyond egg sac care, eight groups were recorded 24/7 between March and June 2014 with four network cameras (Vivotek ip8332, 1280x800) that

recorded footage in MPEG-4 and used infrared to enable recording in the dark. As regurgitation behavior is more likely to be detected at close range and a frontal angle two groups containing offspring were directly observed continuously for 3 hours.

2.3.3 Feeding trials

During feeding bouts, houseflies (*Musca domestica*) or crickets (*Gryllus bimaculatus*) were presented to the 171 experimental colonies that contained at least two egg sacs, two or three times a week and prey attack behavior of the spiders was recorded. The reproductive state of the first attacking spider in each experimental colony was noted. If no attack occurred within an hour after presenting the prey, no attack was scored. To investigate whether the participation by the mothers and virgin helpers in the task of the initial attack changes when an egg sac is present, only groups that were observed at least five times before and after oviposition each were considered for statistical evaluation (N = 113 groups including 565 individuals). All groups were observed 24 times during feeding trials conducted within ten weeks between January and April 2014. Consequently, a total of 4128 trials including 2483 trials in which an attack was observed, and 1645 trials were no attack occurred were included in the statistical analyses.

2.3.4 Statisticical analyses

Generalised linear mixed models (GLMM) were used to analyse the data on egg sac care and attack behavior in the R-package lme4 (Bates, Maechler, Bolker & Walker, 2015) in R (R Core Team R, 2016). To investigate whether there was an effect of reproductive state on egg sac care, a mixed logistic regression was performed. The response variable was the number of observations on egg sac care behavior out of the total number of observations per individual spider (probability of caring for an egg sac). The reproductive state (mother or virgin helper) was incorporated as a fixed effect. Natural nest, experimental group and establishment date of the group were included as random effects. Minor but unproblematic levels of overdispersion were detected in the model (Zuur, Ieno, Walker, Saveliev & Smith, 2009). To estimate the *P*-value of

reproductive state, a likelihood ratio test was used to compare the full model with a reduced model only containing the random effects.

To address the question of whether reproductive state affected attack behavior, a mixed logistic regression was performed in which the response variable was the number of first attacks out of the total number of feeding trials per individual spider (probability of being the first attacker). As above, reproductive state was included as a fixed effect, while the natural nest, the experimental group and establishment date of the group were included as random effects. Since experimental group and the establishment date did not contribute to explaining any variance of the full model (estimated variance = 0), these factors were removed from the model. Minor but unproblematic levels of overdispersion were detected in the model (Zuur, et al., 2009). To estimate the *P*-value of reproductive state, a likelihood ratio test was used to compare the full model with a reduced model only containing the random effect of source nest.

Another mixed logistic regression was run to test if the presence of an egg sac affected the attack behavior of mothers and virgin helpers. As only groups that were observed at least five times before as well as after oviposition were used, the number of groups was reduced to 113 and the 24 trials of each group were split up into two unevenly sized sets (one with egg sac presence and one without). The response variable was the number of attacks out of the total number of feeding trials per individual spider (probability of being the first attacker before oviposition versus probability of being the first attacker after oviposition for each individual). The fixed effects were reproductive state and presence of an egg sac (yes or no) as well as their two-way interaction. natural nest, experimental group, establishment date of the group and individual (as each individual has two counts) were included as random factors, but as in the previous model, experimental group and establishment date did not explain any variance and were removed from the full model. No overdispersion was detected in the model. Sequential model reduction was performed, and models were compared with likelihood ratio tests to obtain *P*-values for model components related to effects of egg sac (egg sac*reproductive state and egg sac).

2.4 II) MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE

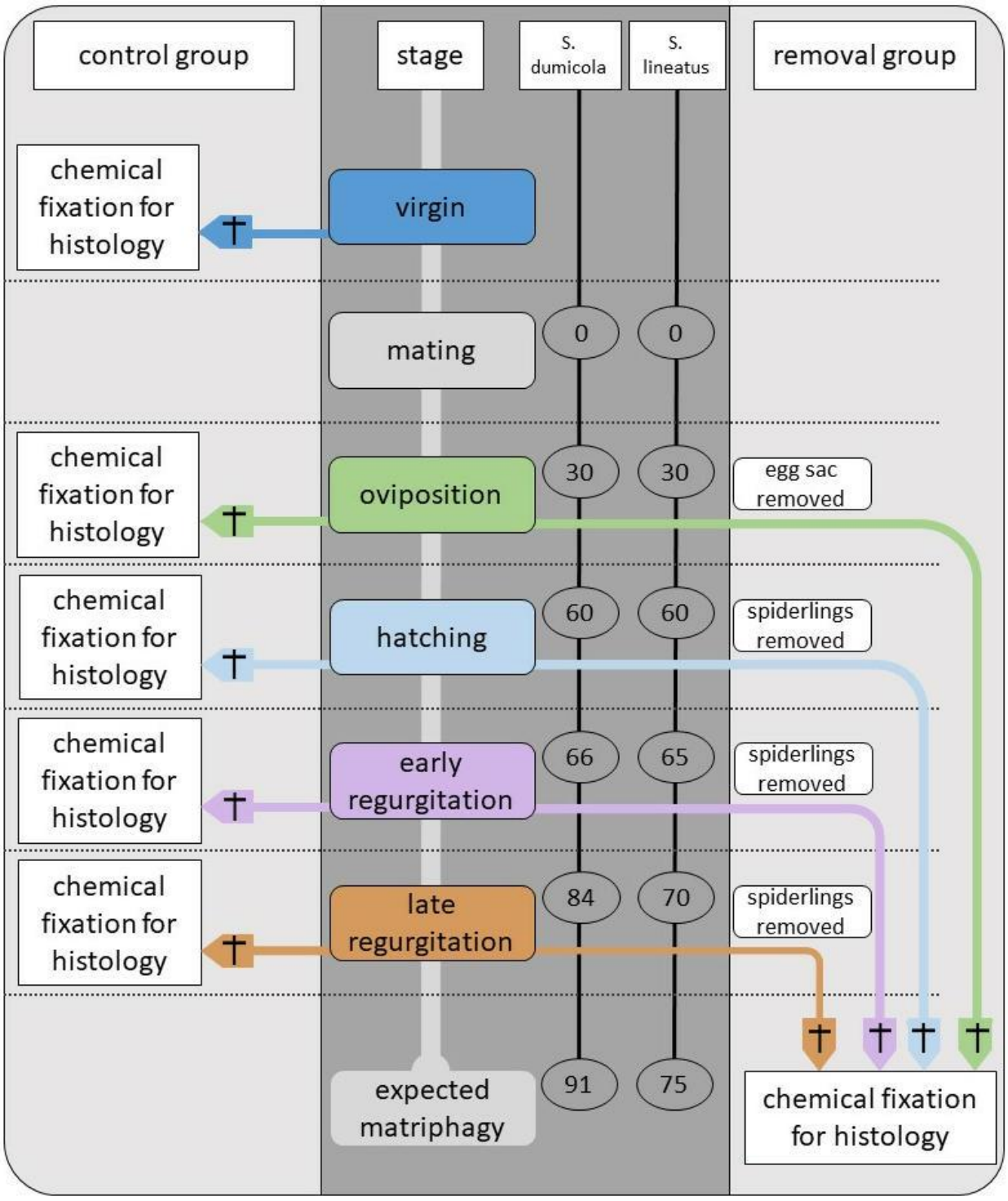
2.4.1 Group composition and Generation of samples of *S. dumicola*

For the histological examination of morphological changes associated to brood care, 16 natural colonies of *S. dumicola* collected in 2013 and 5 collected in 2014 were used to set up 33 experimental colonies and 14 virgin non-helping females for paraffin sectioning. Another 34 experimental colonies and 4 virgin non-helping females were used for semithin sectioning.

The experimental protocol is outlined in Figure 2. The studies on *S. dumicola* followed a similar experimental design as was used for the studies on *S. lineatus* (Junghanns, 2013), which facilitated the greatest possible comparability between the social and the subsocial species. We created experimental colonies of *S. dumicola* including two (2013) or one (2014) mothers and three virgin helpers – each from the same colony and individually marked with acrylic water-based color. They were kept in transparent plastic containers (122 x 82 x 52 mm) of which two sides were covered with mesh to ensure sufficient ventilation. A plastic ring (diameter 53 mm) in the center of the container provided a frame for nest and web construction. Experimental colonies were fed twice or three times per week and water was provided every second day. They were checked every day for oviposition, but it was not possible to ascribe the egg sacs that were produced to a specific female. After oviposition, the experimental colonies were randomly assigned either to the control group or the removal group. The control group for assessing morphological changes during brood care in the midgut tissue and the ovaries was allowed to follow a natural course of brood care behavior and all individuals of an experimental colony were chemically fixed for either histology or ultrastructural examinations at one of the following stages in their reproductive cycle: i) oviposition, ii) the day when offspring hatched (“hatching”), iii) in an early phase of regurgitation feeding (“early”, 6 days after hatching), and iv) in a late phase of regurgitation feeding (“late”, 24 days after hatching) (Figure 2; Table 1). The removal group was manipulated by removing either the egg sac or offspring at one of the four stages given above to examine the effect of offspring removal on midgut integrity and oocyte stage. After removing the egg sac or offspring, females were kept alive until the time when matriphagy was expected to occur under natural conditions at 31 days after hatching (Henschel et al., 1995; Reut Berger-Tal pers. comm.) and females were then chemically fixed for paraffin sectioning or semithin sectioning (Figure 2;

Table 1). If replacement clutches were produced, they were removed. Virgin non-helping females that never had any contact to males or mated females and their offspring were examined to allow for an assessment of brood care related changes. All females used for sample generation were anesthetized by CO2 before processing them for the designated method.

Figure 2. Experimental design for examining morphological effects of brood care in mothers of *S. lineatus* and mothers and virgin helpers of *S. dumicola* as well as virgins from both species. Experimental colonies of *S. dumicola* consisted of one to two mothers and three virgin helpers. The dark grey box in the center depicts significant stages of the reproductive cycle and a representative time frame in days for *S. dumicola* and *S. lineatus*, starting at mating. Females were scrutinized for histological examinations before mating (virgin, dark blue) or after mating when they reached one of the following stages of brood care: at oviposition by the mated female (green), at hatching of offspring (light blue), at an early stage of regurgitation feeding (6 days after hatching, purple) or at a late stage of regurgitation feeding (24 days after hatching, brown). The control group (left light grey box) was chemically fixed for histological analyses on the exact day they reached one of the stages. In the removal group (right light grey box) the egg sac or offspring (spiderlings) were removed at one of the above-mentioned stages (oviposition, hatching, early or late regurgitation), and females were maintained until the time of expected matriphagy (31 days after hatching) before they were chemically fixed for histological analyses.


control group
stage
S. dumicola
S. lineatus
removal group
chemical fixation for histology
virgin
mating
0
0
chemical fixation for histology
oviposition
30
30
egg sac removed
chemical fixation for histology
hatching
60
60
spiderlings removed
chemical fixation for histology
early regurgitation
66
65
spiderlings removed
chemical fixation for histology
late regurgitation
84
70
spiderlings removed
expected matriphagy
91
75
chemical fixation for histology

2.4.2 Generation of samples of *S. lineatus*

In addition to the re-examination of the 32 paraffin samples processed for my diploma thesis (Junghanns, 2013; pp. 30 – 33, this study, Table 1), 25 females of *S. lineatus* were scrutinized to obtain midgut tissue samples for morphological examinations via semithin sectioning, following the same scheme as described in above (Figure 2). Samples of six virgin helpers as well as nine maternal females for the control group and ten maternal females for the removal group (Table 1) were generated at the different stages during brood care that were described above: at i) oviposition (control N = 3), ii) the day when offspring hatched ("hatching"), iii) in an early phase of regurgitation feeding ("early", 5 days after hatching), and iv) in a late phase of regurgitation feeding ("late", 10 days after hatching) (Figure 2).

2.4.3 Paraffin sectioning of *S. dumicola*

The opisthosomata of the 131 females used for light microscopic examinations were separated from the prosoma and a second penetration site for the fixative solution was created by cutting off the spinnerets on the rear end of the opisthosoma. Samples were transferred to an alcoholic picric acid after Bouin (1897, Duboscq-Brasil) and stored for at least one week. Before embedding the opisthosoma in paraffin (Rodiplast), the samples were dehydrated in a graded alcohol series and washed in the intermedium Tetrahydrofuran (THF), which allows paraffin to infiltrate the sample evenly. Sections of 5 µm were produced with a rotation microtome HM 360 and then stained with AZAN (Geidies, 1954). AZAN stains basophilic structures in red while acidophilic structures are stained blue. As a result, the nuclei are stained red, connective tissue light blue, secretion blue and granules of the cells blue, red or yellow (Burck, 1988). The samples were analysed and photographed using an Olympus BX60 System Microscope and Zeiss Axio Vision 4.8. To avoid interpretation bias, the histological sections were analysed blind with regard to the identity of the samples. As correlates for the changes during brood care the following morphological traits were analysed: the abundance of secretion granules (blue stained granules) and the abundance and location of extracellular material, both of which are considered to accumulate for regurgitation purposes (Junghanns, 2013; Nawabi, 1974; Salomon et al., 2015), as

well as the relative abundance of the interstitial tissue. As fat gets dissolved during the embedding process, with this method it is not possible to make statements about fat content of tissues. The ovaries were investigated with regard to the developmental stage of the oocytes (Trabalon et al., 1992). The decisive factor for classification of the developmental stage of the ovary was the most developed state of oocytes observed. Nevertheless, ovaries of females that show early or late vitellogenic oocytes usually also contain less matured oocyte stages (Trabalon et al., 1992).

2.4.4 Semithin sectioning of *S. dumicola* and *S. lineatus*

The midgut tissue of 138 *S. dumicola* females and 25 *S. lineatus* was dissected for semithin sectioning, which complement the results obtained from the paraffin sections by allowing to make statements about fat content of cells. The midgut tissue was cut in pieces and chemically fixed in 2,5% glutaraldehyde in phosphate buffer for at least one week and post-fixated in 1% osmium tetraoxide, which stabilizes proteins and binds lipid molecules. After dehydration in a graded alcohol series and washing in propylenoxyd, which serves as transition fluid, samples were embedded in resin (AGAR R1078, medium-hard). Resin blocks were trimmed with a razor blade and subsequently cut at a Leica Ultracut microtome with a Diatome diamond knife at 500 to 700 nm. Semithin sections were stained with Richardson solution (Richardson, 1960) and examined and photographed at the Olympus BX60 System Microscope and Zeiss Axio Vision 4.8.

	Stegodyphus lineatus				*Stegodyphus dumicola*							
Histological method	Paraffin sectioning		Semithin sectioning		Paraffin sectioning				Semithin sectioning			
Experimental group	mothers		mothers		mothers		virgin helper		mother		virgin helper	
	control	removal	control	removal	control	removal	control	removal	control	removal	control	removal
Oviposition	3	2	3	2	7	8	21	24	5	4	15	12
Hatch	2	3	2	3	2	4	6	12	4	3	12	9
Early	2	4	2	3	4	3	12	9	6	4	17	12
Late	2	2	2	2	1	4	3	12	4	4	12	12
virgin (non-helper)	5		6		14				3			

Table 1: Overview on sample sizes of the solitarily breeding *S. lineatus* and the cooperatively breeding *S. dumicola* used for paraffin sectioning and semithin sectioning in the different experimental groups and life stages. Paraffin sections for *S. lineatus* (grey background) have been processed during my diploma thesis (Junghanns, 2013) but have been re-evaluated for the present work.

2.5 IV) MALE SCARCITY AND ITS IMPLICATION ON REPRODUCTIVE SKEW

2.5.1 Observation on natural nests

All 57 natural nests collected in South Africa in 2013, 2014 and 2015 were dissected in November of the respective year before egg sacs appeared in the colonies. The natural nests were not selected randomly in the field. To gain a sufficient number of individuals per colony but at the same time avoid a large surplus of spiders, the aim was to collect medium-sized colonies, which was estimated in the field from nest size and spider activity. At dissection, the total number of spiders, as well as the number of non-adult and adult females and males was registered to obtain data on the overall sex ratio at maturation (MSR) and the adult sex ratio (ASR) at dissection. Subadult and adult males can easily be distinguished from females by their inflated palps. However, males cannot be reliably distinguished at earlier juvenile stages. Thus, juvenile spiders were sexed retroactively by following the colonies until all were subadult or had matured. The MSR was determined by calculating the percentage of all males relative to that of all females in a natural nest. To determine the ASR, the percentage of adult males versus adult females within a colony at the time of dissection of the natural nests was take into account. To investigate whether MSR differed between the years we performed an ANOVA. Checking the diagnostic plots revealed a normal distribution of the data so that there was no need for a transformation. A spearman´s rank correlation was performed to test whether the sex ratio was correlated to colony size.

2.5.2 Maturation experiments

I aimed to investigate whether the time between the penultimate and ultimate molt of a female depends on the presence of an adult male in the group. Seven colonies collected in 2014 were used to randomly separate juvenile females of the same colony into groups of ten. To avoid a biased selection of females or an effect of timing, two groups from one colony were composed at the same time and only one of the groups was provided with an adult male. A total of 220 females were arranged in 11 experimental groups with a male and 11 experimental groups without a male between December 2014 and January 2015. Experimental groups were checked

every day and when a female molted, the date was noted, and she was marked with a water based color for individual recognition. The date of every further molt of the females was noted and the color-mark was renewed. In this way it was possible to record the time between the penultimate and ultimate molt of a single female. When more than one female molted on the same date and the identity could not be clarified, the females were excluded. When a female died before the end of the observation period (see below) it was replaced by a juvenile or subadult female of the same colony to ensure a consistent number of spiders in a group. However, this replacement female was not considered for the maturation experiment. It was not necessary to replace any males, as all males survived until the end of the observation period. First oviposition events were recorded for all groups. Observations in experimental groups with a male were carried out until all females of a group were adult or until an egg sac existed in the group for at least one month. In nature this would mark the time when the first egg sacs hatch (Ulbrich & Henschel, 1999) and most males in the colony are supposedly dead (Henschel et al., 1995). Thus, females that mature just then would presumably be less likely to mate and reproduce successfully due to the lack of mating partners and the existence of resource demanding offspring of other females in the colony. Experimental groups without a male were terminated as soon as all females reached adulthood or after at least 14 weeks after the start of the experiment, which was six or more weeks after an egg sac had been produced in the corresponding group with a male.

Statistical evaluation was performed by using the median latency between the penultimate and ultimate molt of all individuals per experimental colony and henceforth we treated the experimental colony as statistical individual to avoid pseudoreplication. Since varying numbers of individuals per experimental colony were used to calculate this median, we weighted each median by the number of individuals contributing to it and tested the difference with a weight ANOVA. Whether females had been assigned randomly to the different treatments was tested by performing a U-test on the latency between establishment of the experimental groups and the first maturation event.

2.5.3 Mate choice trials

To investigate whether size differences of females determine male mating preferences, 16 mating trials were conducted with adult virgin spiders from seven colonies collected in 2014. Females that matured within a week were assigned to the large or small category using the patella-tibia-length as a proxy for overall size, which was measured using a Zeiss Discovery.V20 Stereoscope and Zeiss Axio Vision 4.8. As maximum female size depended on the source colony, with females of some colonies growing considerably larger than those of other colonies, assignment to the large or small category was not based on an absolute measure but on the relative measures between two females. The difference of the patella-tibia-length between the large female and the small female it was paired with was usually 200 µm or more. For identification, large females received a red dot and small females a green dot of water-based acrylic on the opisthosoma. A large and a small female from the same source colony were paired in a plastic container (100 mm high and 50 mm in diameter) for two nights to allow them to build a web. To assure a similar feeding condition of the two females and thus minimize an influence of the current feeding situation on female behavior or male decision, a fly (*Calliphora*) was presented as prey to the females on the second day. On the third day, a virgin male taken from the same colony was introduced to the females and the identity of the female with which the male copulated first was recorded. A copulation was scored as soon as the couple engaged in a copulatory position and the male inserted one of his pedipalps. The end of a single copulation was marked by the male leaving the copulatory position. If no copulation occurred within two hours (N = 2), the trial was interrupted and repeated with the same individuals the next day, which in both cases resulted in copulations. Males were allowed to stay with the females for three hours after the first copulation started to observe instant remating behavior of the male and the females. After the trial was terminated both females were weighted using a micro-scale (Satorius). Data was evaluated using a binominal test.

2.5.4 Potential rate of reproduction in males

Sixteen males from eight colonies collected in 2015 were used to estimate whether males engage in multiple matings over the course of several days, with one to three females in succession being presented on a single day. Two of those males were mated *ad libitum* to investigate the number of successful matings a male can achieve, with females being presented every two to four days until the male died. For a mating, a virgin male was placed with a single virgin female. To minimize possible age effects of the female, only females that matured within the week before the trial were used. Over the course of three to four weeks the male was presented with further virgin females until the male died. As in the mating trials, a mating was scored as soon as the couple engaged in a copulatory position and the male inserted one of his pedipalps. Due to obscured visibility caused by the mating position and the web, it was not possible to score the number of insertions per mating. The male was separated from the female immediately after the copulatory position was abandoned. Unsuccessful trials in which the male did not copulate were not recorded. After the mating, the female was co-housed with three virgin females and the first oviposition event was recorded as proxy for a successful mating. Rearing egg sacs until hatching might be delicate under laboratory conditions (own observations) and thus hatching success alone might not be a reliable measurement of mating success. However, hatching success was recorded as well to provide some unquestionable additional data for fertilisation success of the male.

2.6 III) LIFE HISTORY

2.6.1 Observations on reproductive traits in experimental colonies

Life history data were collected from 55 mothers from seven different colonies collected in 2014 and 32 mothers from eight different colonies collected in 2015 (total N = 87) which were followed from mating until death. Each mother was grouped with three virgin helpers, resulting in an experimental group including only one mother, and thus allowed to assign reproductive success to a single female. The size of the experimental groups allowed cooperation among the females but also enabled observation. All experimental groups that were included featured a hatching

event, thus indicating a successful mating. The latency between the mating of the mother and the first oviposition event in the group as well as between the first oviposition and hatching of the offspring was recorded. For 51 experimental groups from 2014 any second oviposition events before offspring hatched were recorded as well. At the end of the brood care period, when most adult females were dead, the total number of egg sacs and the number of offspring that survived was recorded in those 51 groups. T-tests were performed to compare the latency between mating and oviposition as well as between oviposition and hatching of offspring between the years.

3 RESULTS

3.1 I) TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES

3.1.1 Brood care observations

The observations on egg sac care behavior revealed that not only mothers but also virgin helper tended the egg sacs. In 157 of 165 observed groups (95%), both mothers and virgin helpers, took part in egg sac care. In turn, only in 4 of 165 groups (2%) virgin helpers were never observed at the egg sac, and in 5 groups (3%) mothers were never observed during egg sac care. During our daily observations a virgin helper was in one case observed to close an egg sac, which a mother had produced by covering the eggs with layers of silk. The number of individual egg sac care observations differed between mothers and virgins, with virgin helpers tending the egg sac significantly less than mated females (X_1^2 = 135.94, P < 0.001, Fig. 1A). This outcome did not change if cases in which more than one female in a group performed egg sac care (N = 217 of 3719 observations), were excluded (X_1^2= 139.33, P < 0.001).

The qualitative observations by video recordings revealed that virgin helpers do not only turn (Tab. 1A) and transport (Tab. 1B) egg sacs but do also regurgitate (Tab. 1C) for the offspring and get eaten by the offspring in the end of the brood care period (Tab. 1D) like mothers do.

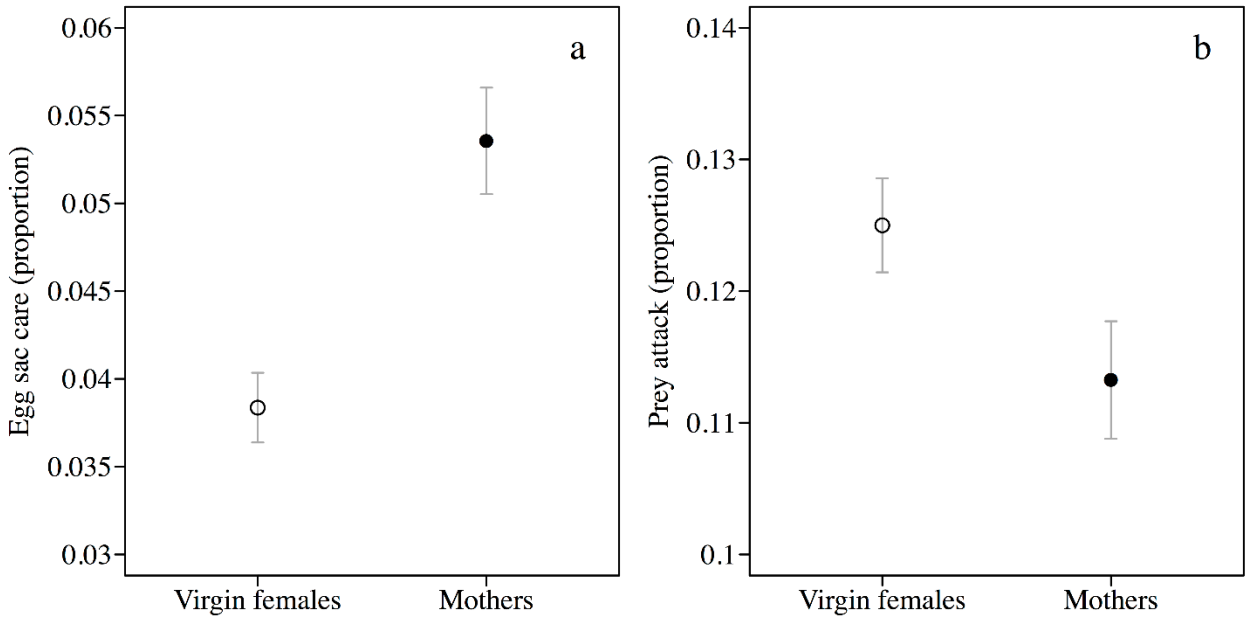


Figure 3: Task differentiation in *S. dumicola*. A) Probability of egg sac care in virgin helpers and mothers. B) Probability of virgin helpers and mothers being the first to attack prey during feeding trials. The individual probabilities represent the number of observed behaviors out of the total number of observations per spider. Error bars show standard error of the mean.

Video	Observable behavior	Link
A	Egg sac care by two virgin helpers	https://vimeo.com/287083203
B	Egg sac reallocation by a virgin helper	https://vimeo.com/287082368
C	Regurgitation feeding by a virgin helper	https://vimeo.com/287083387
D	Allo-matriphagy of a virgin helper	https://vimeo.com/287083512

Table 2: Video footage of allomaternal behaviors of virgin helpers in experimental colonies composed of two individually marked mothers (red and orange) and virgin helpers (blue, green and yellow). A, B and D taken by network cameras (Vivotek) with infrared lights, C was taken with a handheld Samsung I9100 galaxy SII with LED flash. All footage in MPEG-4 format. Password for access: Stegodyphus dumicola.

3.1.2 Feeding trials

During feeding trials, virgins were more likely to be the first to attack prey than mothers (X_1^2 = 6.51, *P* = 0.01, Fig. 1B). The presence of an egg sac did not significantly alter the probability of being the first attacker (X_1^2 = 1.08, *P* = 0.30) any more than the interaction between the reproductive state of the female and the egg sac (X_1^2 = 1.15, *P* = 0.28).

3.2 II) MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE

3.2.1 Brood care-related dynamics of the midgut tissue in *S. dumicola* control females

S. dumicola mothers at the stage of oviposition never showed blue stained secretion granules in their midgut tissue (Figure 5A). In contrast, they were massively abundant at the time of hatching of the offspring (Figure 5C). During regurgitation, mothers showed less secretion granules in their midgut tissue (Figure 5E and G). Mothers of the control group showed small- to medium-sized lacunae with extracellular material in the anterior parts of the midgut from the hatching stage onwards (Figure 7C, E and G). The lacunae never occurred in the posterior parts of the midgut but sometimes lumina filled with extracellular material were found in the mid-regions of the midgut. In the late regurgitation stage, only small amounts of extracellular material were observed (Figure 7G).

In the control groups, the progressive tissue alteration of the midgut over the course of the brood care period observed in virgin helpers (Figure 5, right column) was similar to those observed in mothers, with blue stained granules accumulating at the hatching stage (Figure 5D). Small to medium-sized lacunae with extracellular material in the anterior parts of the midgut were found in virgin helpers from early regurgitation stage onwards (Figure 5, eM in F and H).

In comparison to mothers and virgin helpers, virgin non-helpers did not show secretion granules or accumulation of extracellular material (Figure 4B).

The morphological changes in mothers and virgin helpers of *S. dumicola* were similar to those observed in *S. lineatus* mothers (see 1.7.1, pp. 30 ff. for details on *S. lineatus,* Figure 1). However,

changes in *S. dumicola* were often less pronounced than in *S. lineatus*. The size of the lacunae in *S. dumicola* midgut tissue never reached the same extent as in *S. lineatus* (compare Figure 1C and E to Figure 5C, E, D and F). Where the paraffin sections of control and removal mothers in *S. lineatus* regularly showed signs of cell disintegration (Figure 7A) in all stages from hatching onwards, such signs were rarely observed in *S. dumicola* mothers or virgin helpers and were never as profound (Figure 7B). In the early regurgitation stage, the amount of secretion granules was lower in *S. dumicola* than in *S. lineatus* (compare Figure 1E to Figure 5E and F). However, at the late regurgitation stage the midgut of *S. dumicola* mothers and virgin helpers contained more secretion granules, as compared to *S. lineatus* mothers at the same stage (compare Figure 1G to Figure 5G and H).

3.2.2 Brood care related dynamics of the midgut tissue in *S. dumicola* removal females

Mothers from which offspring had been removed at hatching showed low amounts of secretion granules and almost no extracellular material accumulation (Figure 6C). In the early and late regurgitation removal groups (Figure 6E and G), mothers showed high levels of secretion granules and moderate to large amounts of extracellular material, which was present in natural lumina of the diverticula and medium-sized lacunae in the anterior to the central parts of the midgut (Figure 6E and G). The morphological dynamics in the midgut of virgin helpers was comparable to mothers (Figure 6, right column). However, in contrast to mothers, medium-sized lacunae with accumulated extracellular material were already present in virgin helpers from which offspring had been removed at hatching (Figure 6D).

The comparison of *S. dumicola* control mothers and virgin helpers to females of the removal group shows that at hatching, the amount of secretion granules was lower in the removal group (compare Figure 5 C and D to Figure 6 C and D). However, compared to the control group, the amount of extracellular material was higher in mothers from the removal group at early and late regurgitation (compare Figure 5 G and E to Figure 6 G and E) and in virgin helpers from hatching onwards (compare Figure 5 D, F and H to Figure 6 D, F and H). These data suggest that in mothers

of *S. dumicola,* disintegration of the midgut tissue is reversible until the hatching stage as in *S. lineatus* mothers. In contrast, virgin helpers of *S. dumicola* were not able to halt and reverse processes when offspring were removed at hatching.

3.2.3 Dynamics of the midgut tissue in females of *S. lineatus* on the semithin scale

The semithin sections of midgut tissue of *S. lineatus* females revealed that four of six virgin females did not contain any lipids either in the cells of the diverticula or the cells of the interstitial tissue (Figure 9A). Only two virgin females showed fat in the interstitial tissue, one of which also possessed lipids in the cells of the diverticula.

In *S. lineatus*, seven mothers of the control group (N = 9) had lipids in varying amounts in their diverticular cells, while at the same time in six cases no lipids were observed in the interstitial tissue. One mother of the control group at the late regurgitation stage showed lipids neither in the diverticula cells nor in the interstitial tissue.

Mothers of the removal groups in *S. lineatus* (N =10) showed a similar variability in lipids content compared to the control group, with 7 females having lipids in the diverticula cells but only 4 having lipids in the interstitial tissue. Three mothers from which offspring were removed at oviposition, early regurgitation or late regurgitation did not show any lipids. One showed the highest amount of lipids observed in *S. lineatus* control mothers (Figure 9C). The results from the females from which offspring had been removed when offspring just hatched proved to be most consistent, with high amounts of fat in the cells of the diverticula as well as fat in the interstitial tissue in all 3 females. In the late regurgitation stage interstitial tissue was sometimes not visible and had probably dissolved (Figure 9 G).

3.2.4 Dynamics of the midgut tissue in females of *S. dumicola* on the semithin scale

Virgin non-helpers of *S. dumicola* (N = 3) exhibited hugely varying amounts of lipids in the diverticula cells as well as in the interstitial tissue.

In the control group, mothers from the oviposition stage (N = 5) showed consistently massive amounts of lipids in their interstitial tissue, visible as conspicuous strands of fat (Figure 9D), whereas the amount of lipid in the diverticular cells varied from moderate amounts to none. The

amount of lipids in the interstitial tissue dramatically dropped in mothers when offspring hatched (N = 4, Figure 9F), and stayed consistently low in later stages (Figure 9H). At hatching, lipid content in the diverticular cells was consistently high (Figure 9F) and, until the late stages of regurgitation feeding, were – in most mothers – higher than the amount of lipids in the interstitial tissue (Figure 9F). In two mothers at the late stage of regurgitation feeding (N = 4) no lipids were found outside of diverticular cells (Figure 9H). In one mother (of four) from the hatching stage, two mothers (of six) from the early regurgitation feeding stage and two mothers (of four) from the late regurgitation feeding stage, the interstitial tissue was not visible but appeared to have dissolved. In virgin helpers, the dynamics relating to fat are generally similar to those observed in mothers, thus not separate figure plate for virgin helpers has been created. However, virgin helpers seemed to be more variable with regard to the lipid content in their midgut tissues, which was especially apparent at the stage of oviposition. At this stage, the tissues of seven virgin helpers (of 15) did not contain any or only low amounts of lipids, which was in stark contrast to the consistently high amount of lipids in mothers at the same stage. However, at the hatching stage, tissues from virgin helpers were less variable and comparable to mid gut tissue of mothers. Interstitial tissue was dissolved in five of 12 virgin helpers upon hatching, in six of 17 virgin helpers during early regurgitation feeding and in five of 12 virgin helpers at the late regurgitation feeding stage.
Mothers in the removal group showed variable lipid contents in their midgut tissue. Two of three mothers from which offspring were removed at hatching and two of four mothers from which offspring were removed at early regurgitation feeding had, compared to mothers in the control group, low amounts of lipids in the diverticular cells. Mothers from which offspring were separated late in the regurgitation feeding process had high amounts of lipids in the diverticular cells, but no lipid in the interstitial tissue. As in the control, group the dynamic in virgin helpers was comparable to that in mothers, but variability between individuals was higher.

In comparison to mothers in *S. lineatus*, mothers and virgin helpers of *S. dumicola* store massive amounts of lipids in their interstitial tissue in preparation for brood care (compare Figure 9C and D). When offspring hatch, lipids have been transported from the storage site to the diverticula (Figure 9F), where it is available in large amounts. In contrast to *S. lineatus,* lipid content in the

diverticular cells of *S. dumicola* decreases when offspring are removed at the time of hatching, while lipid content in the interstitial tissue increases.

3.2.5 Ovarian development in mothers, virgin helpers and virgin non-helpers of *S. dumicola*

Pre-vitellogenic oocytes of *S. dumicola* (Figure 8, pvO) with a diameter of 100 µm, are considerably smaller than in the subsocial species (150 µm in *S. lineatus*). Similarly, the late vitellogenic oocytes of *S. dumicola* (Figure 8B, lvO) remain smaller with a size of up to 270 µm, which is about 100 µm smaller than late vitellogenic oocytes in *S. lineatus*. The ovaries of all control mothers in *S. dumicola* (N = 14) contained pre-vitellogenic (N = 5, e.g. Figure 5A) or early vitellogenic oocytes (N = 6, e.g. Figure 5C), except for three mothers from the oviposition stage that exhibited late vitellogenic oocytes in their ovaries. In contrast, virgin helpers of the control group (N = 35) of all stages had early vitellogenic (N = 27, e.g. Figure 5B) or late vitellogenic oocytes (N = 5, e.g. Figures 5F and 8A) in their ovaries, except for two virgin helpers from the oviposition stage and one virgin helper from the early regurgitation stage that showed exclusively pre-vitellogenic oocytes. In the removal groups, ten mothers showed late vitellogenic oocytes in their ovaries (Figure 6A and G) whereas seven showed early vitellogenic oocytes (Figure 6E) and one from early and late regurgitation stage each had pre-vitellogenic oocytes in their ovaries. Virgin helpers from all removal groups showed early vitellogenic oocytes in most cases (37 of 49, Figure 6D). From the remaining virgin helpers, eight had late vitellogenic oocytes and four showed exclusively pre-vitellogenic oocytes.

The virgin non-helpers, which did not have contact with males or mothers and their brood exhibited pre-vitellogenic oocytes (N = 2), early vitellogenic oocytes (N = 8) or late vitellogenic oocytes (N = 4, Figure 5B) in their ovaries (Figure 8B).

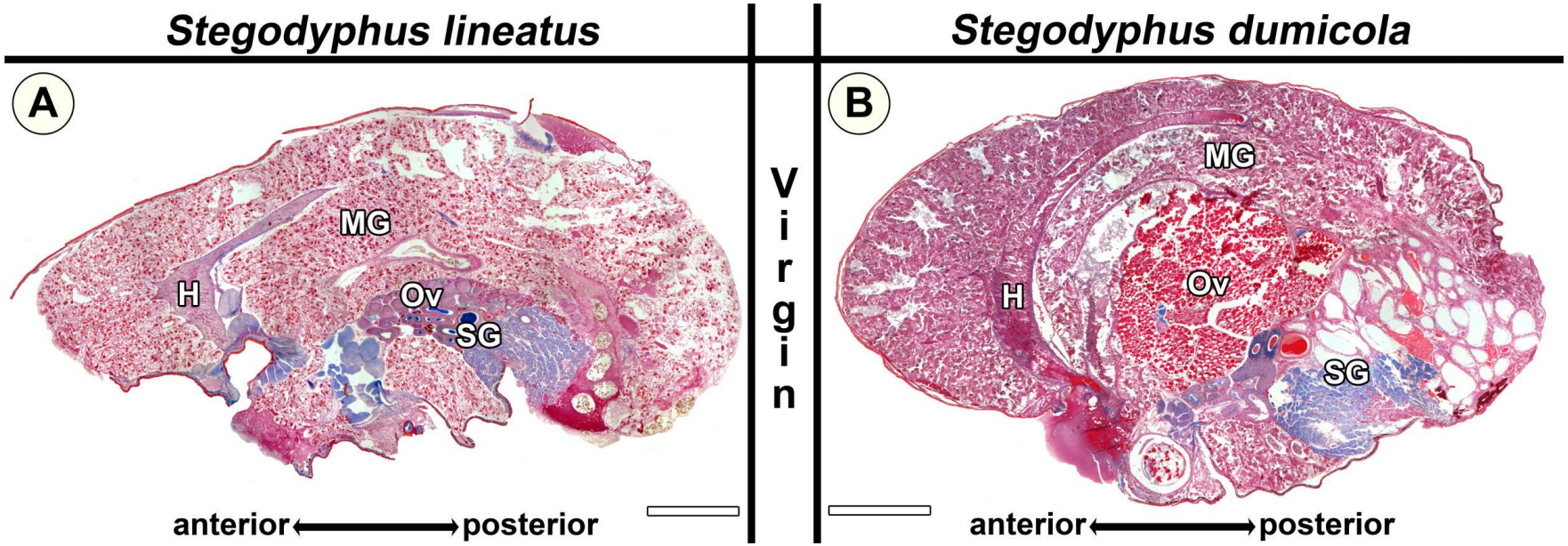


Figure 4. Virgin females of *Stegodyphus lineatus* (A) and *S. dumicola* (B). The midgut tissue (MG) consists of diverticula embedded in storage tissue and surrounds the heart (H), reproductive organs (Ov) and the silk glands (SG). No blue stained secretion granules or regurgitant are visible. The ovary of *S. lineatus* contains exclusively pre-vitellogenic oocytes while the ovary from *S. dumicola* shows far matured oocytes. (A) is based on samples examined for Junghanns, 2013. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several individual images.

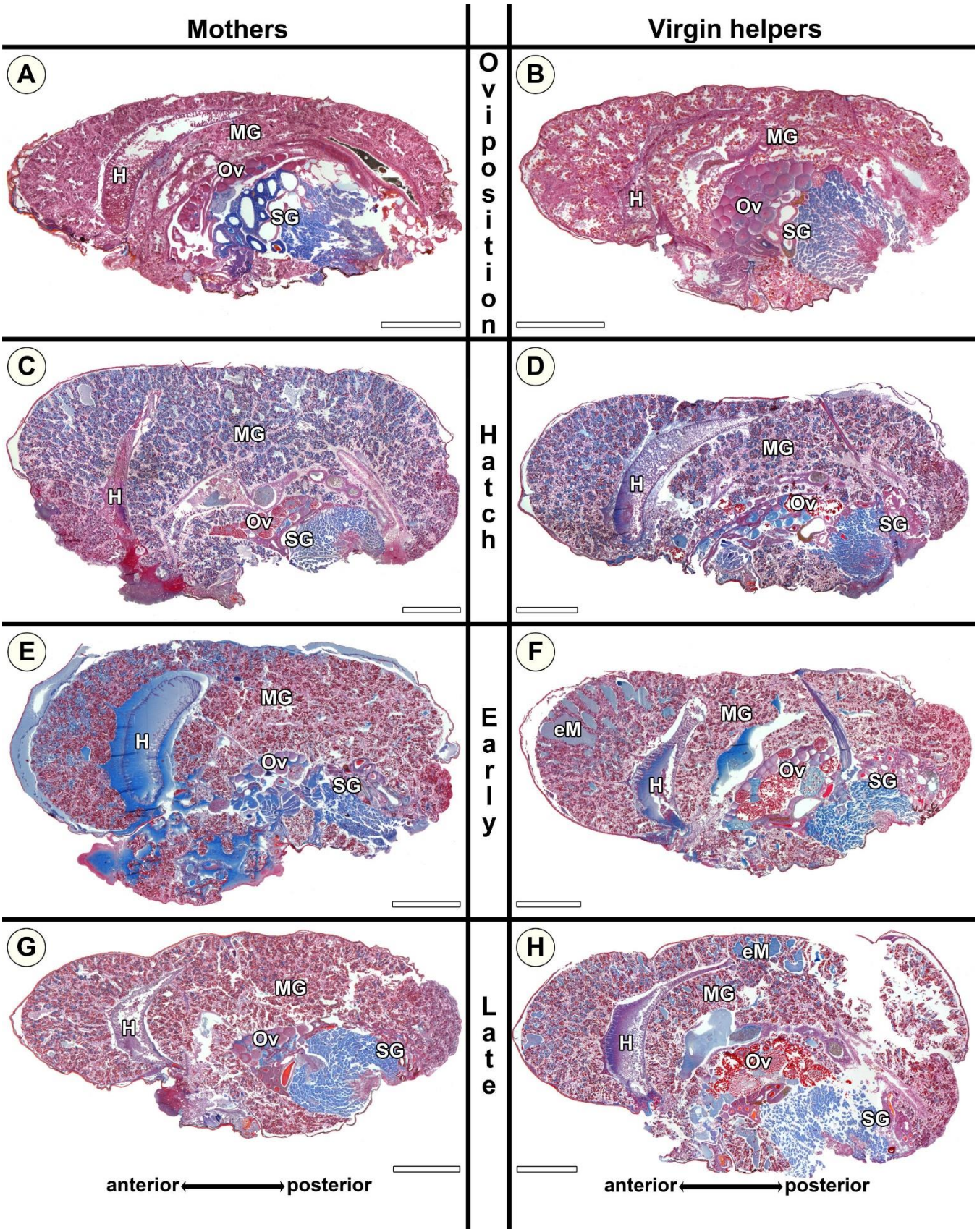
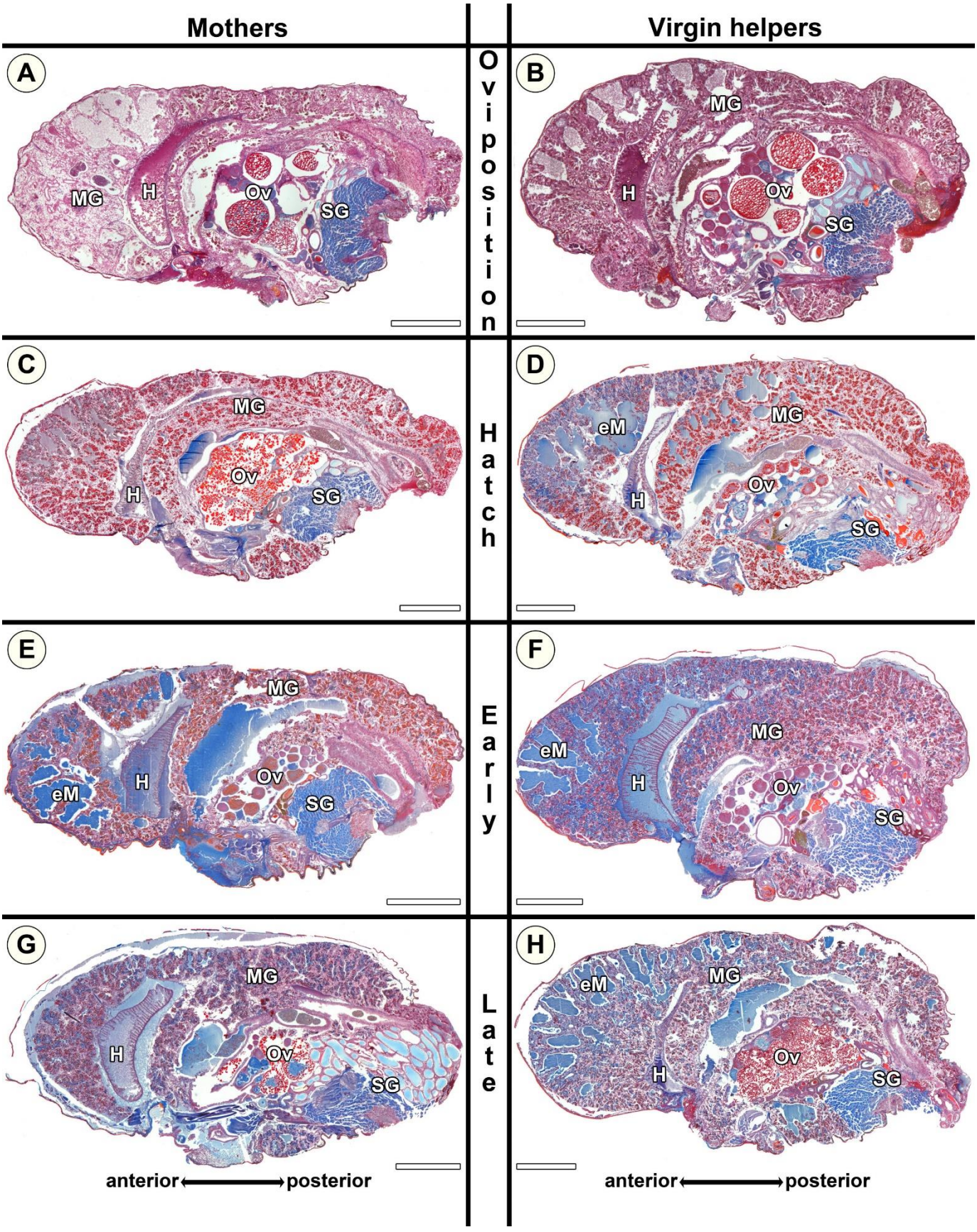

Mothers
Virgin helpers
Oviposition
Hatch
Early
Late
A
B
C
D
E
F
G
H
MG
H
Ov
SG
eM
anterior
posterior

Figure 5 (above). Histological sections of opisthosomata of *Stegodyphus dumicola* mothers (left column) and virgin helpers (right column) of the control group. Females were chemically fixed at oviposition (A, B), hatching of offspring (C, D), early regurgitation phase (6 days after hatching, E, F) and late regurgitation phase (24 days after hatching, G, H). Mothers as well as virgin helpers show similar changes with blue stained granules accumulating in the cells at the time of hatching (C, D). Extracellular material is only visible in small amounts often in anterior parts of the midgut tissue (e.g. E, F). Oocytes may undergo maturation in mothers (C) and virgin helpers (F, H). H = heart, MG = midgut, Ov = ovary, eM = extracellular material (regurgitant), SG = silk gland. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several individual images.

Figure 6 (below). Histological sections of opisthosomata of *Stegodyphus dumicola* mothers (left column) and virgin helpers (right column) of the removal group. Offspring were removed at oviposition (A, B), hatching of offspring (C, D), early regurgitation phase (6 days after hatching, E, F) and late regurgitation phase (24 days after hatching, G, H). Females were chemically fixed 31 days after hatching when matriphagy would occur under natural conditions. Mothers and virgin helpers show almost no blue stained secretion granules when offspring were removed at time of hatching (C, D) compared to controls. Extracellular material is accumulating in mothers from early regurgitation and in virgin helpers from hatching onwards more massively than in control groups, suggesting a runaway process in the formation of regurgitant. Ovaries of mothers and virgin helpers frequently show late-vitellogenic oocytes (A, B, C, G, H). H = heart, MG = midgut, Ov = ovary, eM = extracellular material (regurgitant), SG = silk gland. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several individual images.


Mothers
Virgin helpers
Oviposition
Hatch
Early
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Ov
SG
eM
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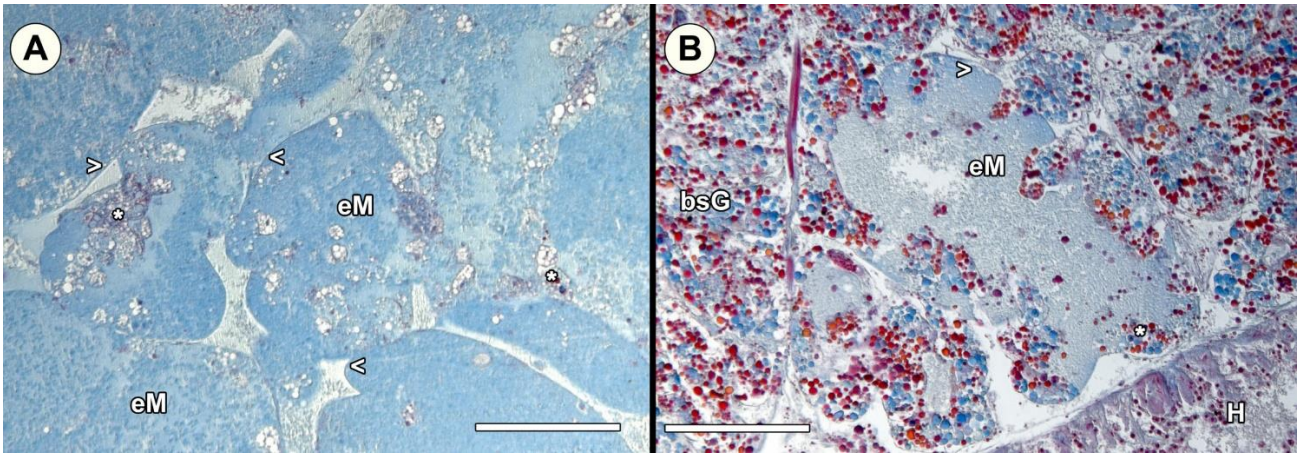

Figure 7: Brood caring female of *Stegodyphus lineatus* (A; control mother at early regurgitation) and *S. dumicola* (B; control mother at hatching). In *S. lineatus* tissue disintegration can be massive with huge lacuna filled with extracellular material (eM) arising where midgut tissue was present before. The basal lamina (arrows) remain longest but finally dissolve as well, leading to merged diverticula. In *S. dumicola* cell degradation was rarely seen and was limited to single diverticula, usually close to the heart. Asterisks (*) mark degraded cells. H = heart; bsG = blue stained granules. (A) is based on samples examined for Junghanns, 2013. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several images.

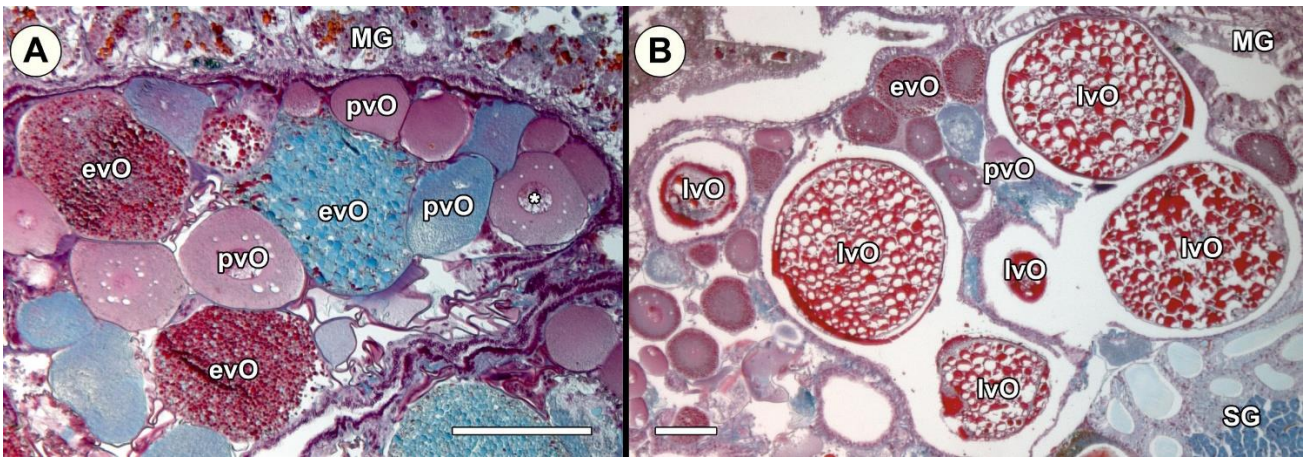

Figure 8: Oocytes in different phases of vitellogenesis in females of *Stegodyphus dumicola.* A) Ovary of a virgin helper in the removal group, when offspring were removed at oviposition. The ovary contains early vitellogenic oocytes (evO), which contain accumulating yolk vesicles in contrast to the smaller pre-vitellogenic oocytes (pvO) that contain homogenous material. B) Ovary of a virgin non-helper that contains late vitellogenic oocytes (lvO), cut on different levels. Earlier stages of vitellogenesis are present in the ovary as well. MG = midgut gland, Asterisk (*) = vitelline body; SG = silk gland. All scale bars are 2000 µm. Pictures of the opisthosomata were stitched from several images.

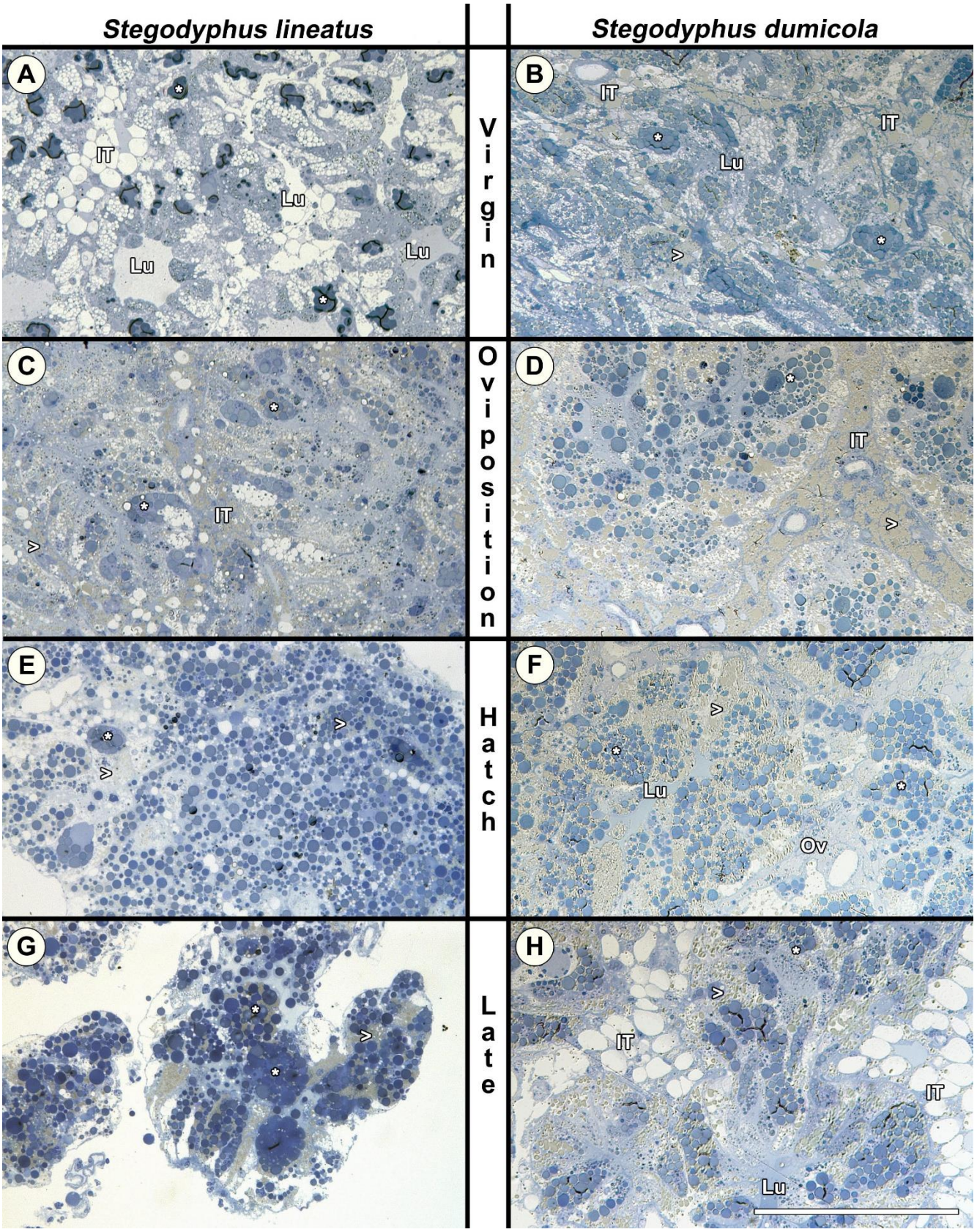
Stegodyphus lineatus
Stegodyphus dumicola
A
B
C
D
E
F
G
H
Virgin
Oviposition
Hatch
Late
IT
Lu
Ov

Figure 9 (above): Semi thin sections of midgut tissue of mothers of *Stegodyphus lineatus* (left column) and *Stegodyphus dumicola* (right column) of the control group. Virgin females in *S. lineatus (*A) often do not show any lipids (Arrows, unstained material that appears yellow in the pictures), whereas lipids can be abundant and often concentrated in the interstitial tissue (IT) in virgin non-helpers in *S. dumicola* (B). In mothers of *S. lineatus* the extent of lipid occurrence is generally variable. Few mothers show high amounts of lipids (C) in the interstitial tissue, whereas in most mothers, lipids occur in low to moderate in the diverticular cells (E, G). In late regurgitation state the interstitial tissue is sometimes not visible (E). Mothers of S. *dumicola* accumulate huge amounts of lipids in the interstitial tissue at oviposition (D) and at regurgitation feeding (F, H) most of lipids are found in the diverticular cells. At late regurgitation the interstitial tissue is visible in most cases but is comprised of cells without content. Asterisks (*) mark secretion cells of the diverticula, containing secretion granules. Lu = lumen of the diverticula. Scale bars is 200 µm.

3.3 III) MALE SCARCITY AND ITS IMPLICATION ON REPRODUCTIVE SKEW

3.3.1 Observations of natural nests

The 57 natural nests that were dissected contained between 19 and 2743 spiders (see Tables 3, 4 and 5). 30 nests included under 100 individuals, twelve between 100 and 200, eleven between 200 and 300 and the remaining four nests contained 309, 522, 738 and 2743 spiders. Thirteen colonies ranging from 23 to 2743 individuals contained no adult spiders at the time of dissecting. All colonies contained males and there was no colony that contained adult females but no adult males. However, the opposite case, with adult males but no adult females in the colony was observed in six cases. In a total of eleven colonies all males were adult at the time of dissection of the nest. In one of those nests all females were adult as well (Table 4, 14/15 KSK). Nine nests were excluded from further analyses, as an incorrect MSR might have been recorded due to mortality during the maturation of the colony. This was due to a very young age of the spiders (all juvenile, not yet subadult) at the time of dissection and subsequently a maturation time of several months in the laboratory during which several not-yet-sexed spiders died. In the 48 remaining nests, the mean MSR was 12 ± 5 %, with no significant differences between the years (2013: 11 ± 4 %; 2014: 13 ± 6 %; 2015: 12 ± 6 %; ANOVA, $F_{1,46}$ = 0.8109, p = 0.37). The highest MSR of 28 % was recorded in a colony of 93 spiders including 26 males in 2014. The lowest MSR of 2 % was found in 2015 in a colony of 109 spiders with only 2 males. The differences in the MSR were not correlated to the size of the colony (rho = - 0. 24, S = 22789, p = 0.11). The ASR in

colonies that contained at least one adult spider (N = 44) varied between 7 % and 100 % and was even or female-biased in 25 colonies. In some of those colonies adult females outnumbered adult males by up to 1:14 (e.g. 13/15 K, Table 3; 14/15 KSK, Table 4; 15/07 KZN, Table 5). In 37 colonies at least 50 % of all males had matured, and in 34 of those, mature females were present as well. In contrast, in some colonies only few males had matured when some adult females were already present (e.g. 15/05 KZN, 15/06 KZN, 15/11 KZN; Table 5).

Colony ID	*Collection date*	*Total number of spiders*	*Total number of females (adult/premature)*	*Total number of males (adult/ premature)*	*MSR (percent)*	*ASR (percent)*
13/01 M	03.11.2013	309	284 (57/227)	25 (19/6)	8	24
13/02 M	03.11.2013	738	653 (51/602)	85 (85/0)	12	63
13/03 MF	03.11.2013	51	44 (0/44)	7 (0/7)	14	-
13/04 MF	03.11.2013	93	76 (10/66)	17 (11/6)	18	52
13/05 K	09.11.2013	39	33 (8 / 25)	6 (6/0)	15	43
13/06 K	09.11.2013	59	52 (19/33)	7 (7/0)	12	27
13/07 K	09.11.2013	68	58 (3/55)	10 (6/4)	15	67
13/08 K	09.11.2013	81	73 (4/69)	8 (6/2)	10	60
13/09 K	09.11.2013	83	76 (10/66)	7 (3/4)	8	23
13/10 K	09.11.2013	85	78 (22/56)	7 (6/1)	8	21
13/11 K	09.11.2013	93	81 (3/78)	12 (9/3)	13	75
13/12 K	09.11.2013	96	84 (31/53)	12 (11/1)	13	26
13/13 K	09.11.2013	97	90 (13/77)	7 (5/2)	7	28
13/14 K	09.11.2013	114	103 (12/91)	11 (6/5)	10	33
13/15 K	09.11.2013	129	123 (80/43)	6 (6/0)	5	7
13/16 K	09.11.2013	134	127 (9/118)	7 (4/3)	5	31
13/17 K	09.11.2013	156	131 (29/102)	25 (25/0)	16	46
13/18 K	09.11.2013	166	151 (13/138)	16 (10/6)	10	43
13/19 K	09.11.2013	216	202 (23/179)	14 (14/0)	6	38
13/20 K	09.11.2013	223	205 (56/149)	18 (15/3)	8	21
13/21 K	09.11.2013	236	195 (37/158)	41 (41/0)	17	53
13/22 K	09.11.2013	243	219 (11/208)	24 (15/9)	10	58
13/23 K	09.11.2013	250	241 (2/239)	9 (8/1)	4	80

Table 3. Colonies collected in 2013 in South Africa in Mokopane (M), Middlefontain (MF) and Krüger National Park (K) around Shingwedzi and Skukuza. Sex ratio at maturation (MSR) was measured as percentage of males among all spiders of the colony. Adult sex ratio (ASR) was measured as percentage of adult males among all adult spiders at time of dissection. All values are rounded.

Colony ID	*Collection date*	*Total number of spiders*	*Total number of females (adult/premature)*	*Total number of males (adult/ premature)*	*MSR (percent)*	*ASR (percent)*
14/01 KSH	12.11.2014	19	17 (0/17)	2 (1/1)	11	100
14/02 KSH	12.11.2014	23	19 (0/19)	4 (0/4)	17	-
14/03 KSH	12.11.2014	31	27 (0/ 27)	4 (2/2)	13	100
14/04 KSH	12.11.2014	41	38 (1/37)	3 (2/1)	7	67
14/05 KSH	12.11.2014	62	55 (3/52)	7 (5/2)	11	63
14/06 KSH	12.11.2014	77	74 (0/74)	3 (0/3)	4	-
14/07 KSH	12.11.2014	80	71 (0/71)	9 (0/9)	11	-
14/08 KSH	12.11.2014	93	67 (0/67)	26 (0/26)	28	-
14/09 KSH	12.11.2014	106	94 (0/94)	12 (0/12)	11	-
14/10 KSH	12.11.2014	151	130 (0/130)	21 (0/21)	14	-
14/11 KSH	12.11.2014	170	159 (0/159)	11 (0/11)	6	-
14/12 KSK	19.11.2014	44	35 (0/35)	9 (1/8)	20	100
14/13 KSK	19.11.2014	49	45 (0/45)	3 (0/3)	6	-
14/14 KSK	19.11.2014	49	44 (15/29)	5 (5/0)	10	25
14/15 KSK	19.11.2014	50	45 (45/0)	5 (5/0)	10	10
14/16 KSK	20.11.2014	61	50 (27/23)	11 (11/0)	18	29
14/17 KSK	20.11.2014	85	80 (2/78)	5 (4/1)	6	67
14/18 KSK	19.11.2014	172	156 (26/139)	16 (14/2)	9	35
14/19 KSK	19.11.2014	202	189 (0/186)	13 (0/13)	6	-
14/20 KSK	20.11.2014	219	183 (41/142)	36 (36/0)	16	47

Table 4. Colonies collected in 2014 in South Africa in Krüger National Park around Shingwedzi (KSH) and Skukuza (KSK). Sex ratio at maturation (MSR) was measured as percentage of males among all spiders of the colony. Adult sex ratio (ASR) was measured as percentage of adult males among all adult spiders at time of dissection. All values are rounded.

Colony ID	*Collection date*	*Total number of spiders*	*Total number of females (adult/premature)*	*Total number of males (adult/ premature)*	*MSR (percent)*	*ASR (percent)*
15/01 KZN	29.10.2015	30	28 (2/26)	2 (1/1)	7	33
15/02 KZN	29.10.2015	48	44 (7/37)	4 (3/1)	8	30
15/03 KZN	29.10.2015	71	59 (10/49)	12 (8/4)	17	44
15/04 KZN	28.10.2015	78	62 (0/62)	16 (9/7)	21	100
15/05 KZN	29.10.2015	99	85 (1/84)	14 (3/11)	14	75
15/06 KZN	28.10.2015	108	97 (4/93)	11 (4/7)	10	50
15/07 KZN	29.10.2015	109	107 (14/93)	2 (1/1)	2	7
15/08 KZN	29.10.2015	188	157 (9/148)	31 (24/7)	16	73
15/09 KZN	28.10.2015	217	197 (0/197)	20 (4/16)	9	100
15/10 KZN	28.10.2015	256	244 (0/244)	12 (0/12)	5	-
15/11 KZN	28.10.2015	259	234 (5/229)	25 (3/22)	10	38
15/12 KZN	28.10.2015	271	226 (0/226)	45 (19/26)	17	100
15/13 KZN	28.10.2015	533	514 (0/514)	8 (0/8)	2	-
15/14 KZN	28.10.2015	2743	2690 (0/2690)	53 (0/53)	2	-

Table 5. Colonies collected in 2015 in South Africa in Kwa-Zulu-Natal (KZN). Sex ratio at maturation (MSR) was measured as percentage of males among all spiders of the colony. Adult sex ratio (ASR) was measured as percentage of adult males among all adult spiders at time of dissection. All values are rounded.

3.3.2 Maturation experiments

From the total of 220 females that were investigated, 161 did not provide useful data, as 119 matured after their first recorded molt, 19 females died, and 22 females did not reach adulthood during the observation period. One juvenile spider turned out to be a male after the penultimate molt nine days after establishment of the experimental group and was replaced by a juvenile female. We were able to record the time between the penultimate and ultimate molt of 29 females in 9 experimental colonies with a male and of 30 females in 9 experimental colonies without a male present (see Table 6 for details). The time until the first female matured after the establishment of the experimental colony did not differ between the two treatments (19 days: min. 5 days, max. 33 days; U = 49.5; p = 0.4893). The shortest span between the penultimate and the ultimate molt (12 days) was observed in a female in an experimental colony in which a male was present. In comparison, the shortest span recorded in females of experimental colonies without a male was 25 days. The longest spans between penultimate an ultimate moult were 42 days in experimental colonies with male presence and 59 days in experimental colonies without a male. Overall, 66 percent (19 of 29 females) in experimental colonies with a mature male present matured within 30 days after the penultimate moult whereas only 30 percent (9 of 30 females) in experimental colonies without males matured in 30 days or less. Nevertheless, statistical analyses revealed no significant difference in timing between the penultimate and ultimate molt when means of individual experimental colonies were used (Weighted ANOVA: $F_{1,16}$ = 1.821, p = 0.2, Figure 10) in absence (weighted median: 31 days: min. 24; max .37) or presence of a male (weighted median: 27 days: min. 22; max. 37). Oviposition occurred in all groups that included a male (N = 11) and seven of eleven groups without a male (Table 6). The time between the first female maturing, and the first egg sac production varied between the two treatments (U = 65.5; p = 0.02, Figure 11). In groups with males the first egg sac was produced after a median of 39 days (min. 26; max. 50 days). In those groups without males, in which oviposition events were recorded, the first egg sac appeared after a median of 46 days (min. 37; max. 78 days) on average.

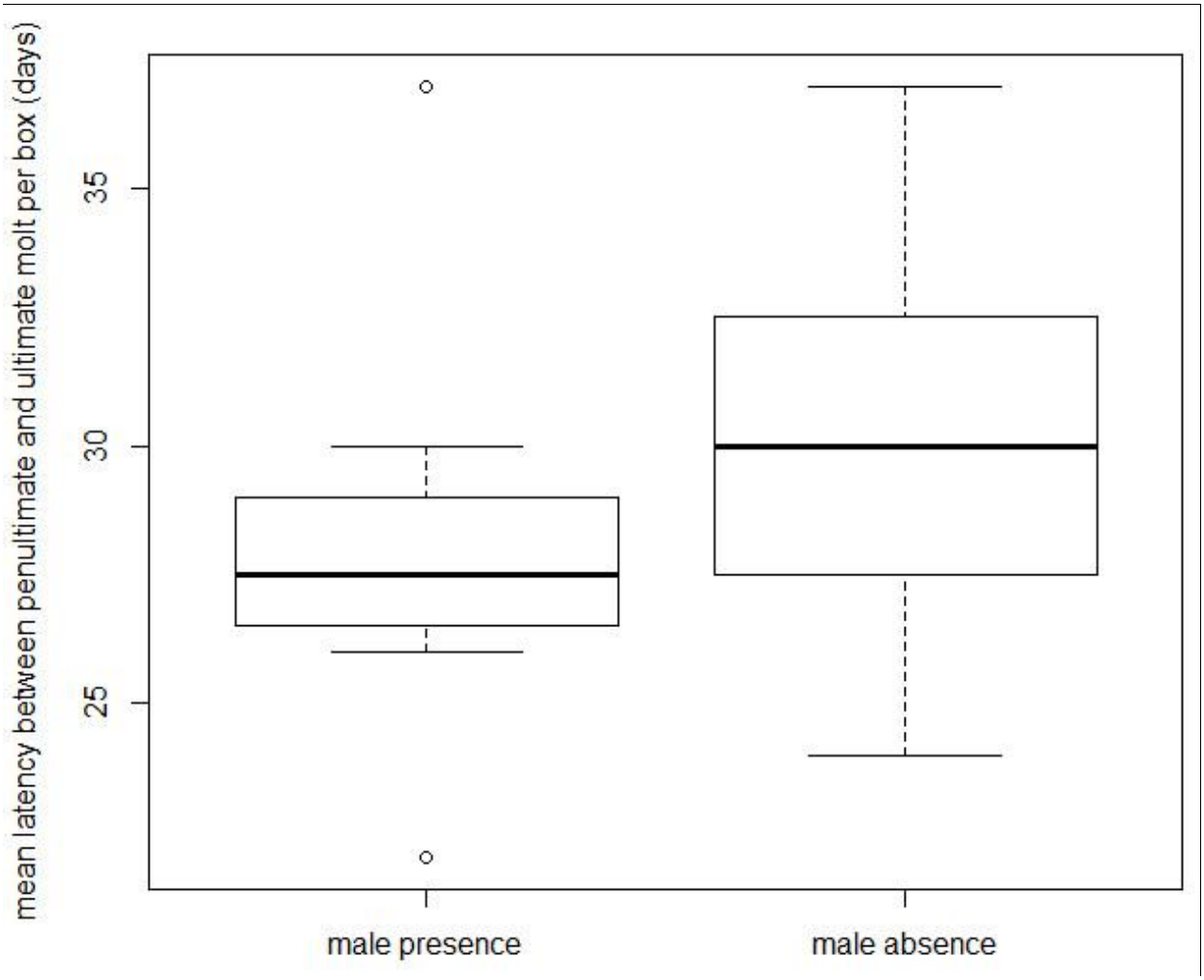

Figure 10: Mean latency between the penultimate and ultimate molt in experimental colonies of each ten juvenile and subadult females either in the absence (N = 9) or presence (N = 9) of an adult male.

	Experimental colony ID	*Source colony*	*Number of females for which maturation time is known*	*Median time until maturation in days (min / max)*	*Days until first oviposition after first mature female appeared*
With an adult male	M01	14/10 KSH	-	-	50
	M02	14/08 KSH	3	30 (22 / 44)	39
	M03	14/09 KSH	5	25,2 (12 / 38)	34
	M04	14/09 KSH	2	32 (28 / 36)	30
	M05	14/19 KSH	6	27,1666667 (26 / 30)	41
	M06	14/19 KSH	2	27,5 (26 / 29)	46
	M07	14/19 KSH	1	29	31
	M08	14/07 KSH	-	-	26
	M09	14/18 KSK	1	30	39
	M10	14/12 KSK	2	27 (24 / 30)	43
	M11	14/17 KSK	7	28,28571 (20 / 39)	28
median				**27 (min. 22; max. 37)**	**39**
Without any male	W01	14/10 KSH	1	24	78
	W02	14/08 KSH	5	31 (25 / 43)	46
	W03	14/09 KSH	5	41,4 (33 / 59)	37
	W04	14/09 KSH	3	35 (30 / 41)	-
	W05	14/19 KSH	2	27,5 (25 / 30)	63
	W06	14/19 KSH	4	32 (30 / 33)	44
	W07	14/19 KSH	-	-	45
	W08	14/07 KSH	-	-	-
	W09	14/18 KSK	2	27,5 (25 / 30)	-
	W10	14/12 KSK	1	29	-
	W11	14/17 KSK	7	31,28571 (25 / 46)	55
median				**31 (min. 24; max. 37)**	**46**

Table 6: Experimental colonies (EC) for maturation experiment, each composed of 10 females from natural colonies collected in 2014 and either with or without an adult male from the same natural colony. For each EC with a male, an EC without a male was created at the same time. Median maturation time (column 4) is based on a varying number of females per EC (column 3), as maturation time could not be detected for all females.

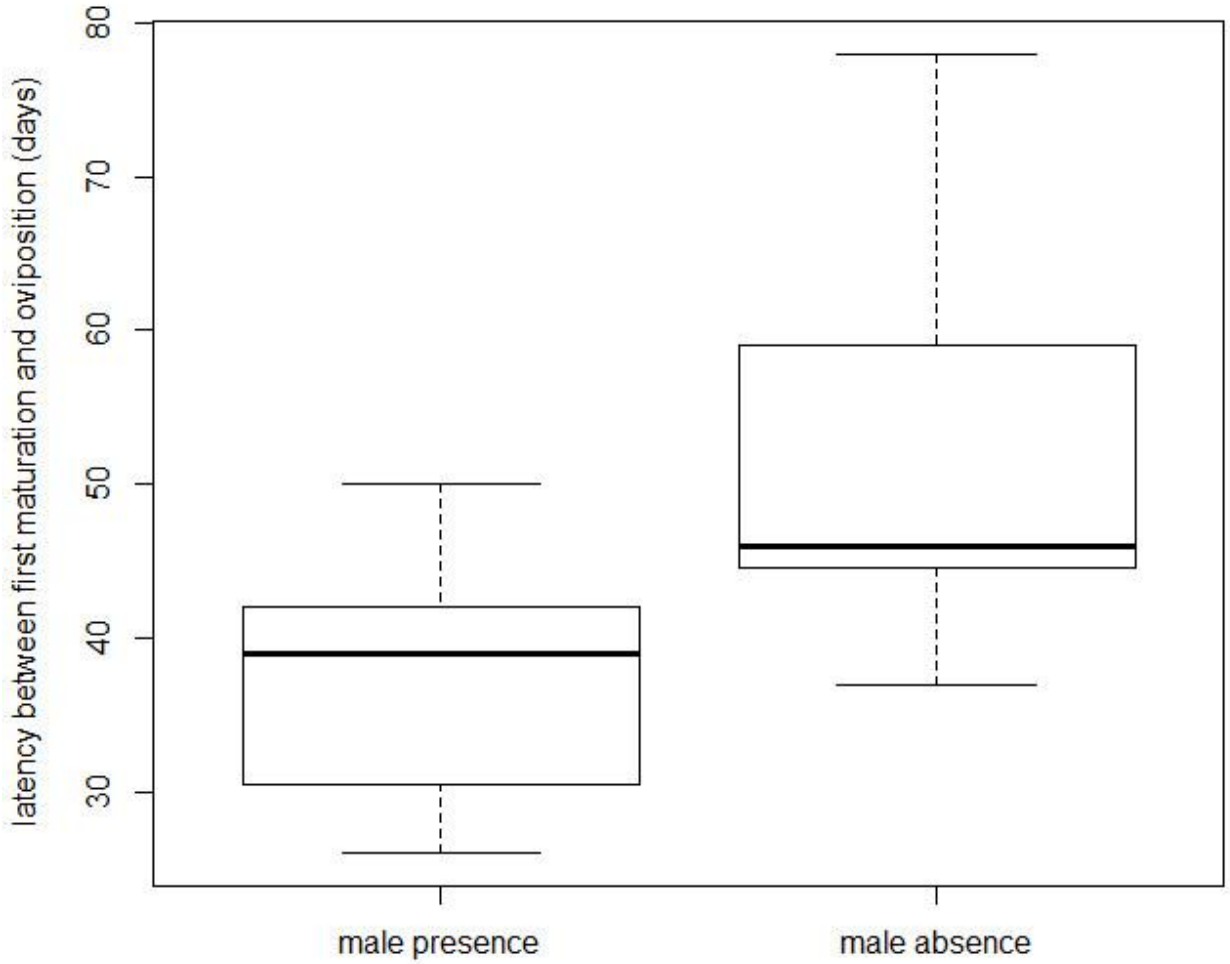

Figure 11: Time until an egg sac occurred after the first maturation in experimental groups of ten premature females without (n) or with (y) an adult male present in the group.

3.3.3 Mate choice trials

The mating trials revealed no mating preference by the male when presented with a large and a small virgin female simultaneously (binominal test p = 0.8, Figure 7; Table 12). Weight was correlated with size, with large females being heavier, thus only size was used for further analysis. Males copulated with the large female first in nine and small females first in seven of 16 trials. In 14 of 16 trials the male copulated with the first female he encountered. In two cases, the first encountered female was not receptive to the male. In both cases, the second female actively approached the male, which then copulated with the second female.

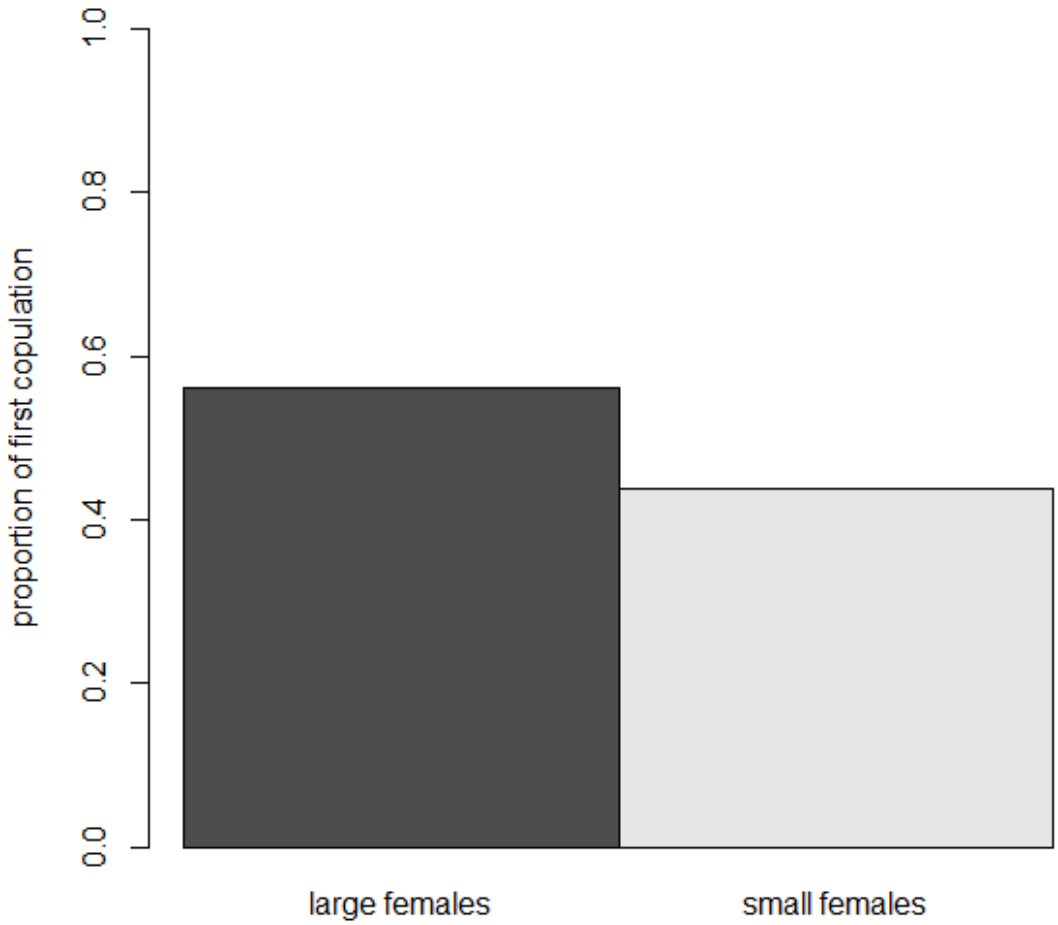

Figure 12: Mating trials conducted with virgin males. Males did not show a mating preference for either the large or the small female.

Trial/ Male ID	*Source colony*	*Large female size (Patella tibia length in µm)*	*Small females size (Patella tibia length in µm)*	*First female that was mated*	*Other female mated as well?*	*Remating of females occurred?*
M01	14/12 KSK	4185,74	3593,96	Large	Yes	No
M02	14/12 KSK	3878,86	3618,43	Small	Yes	Yes
M03	14/12 KSK	4157,88	3677,96	Small	Yes	No
M04	14/17 KSK	3832,35	3541,49	Large	Yes	Yes
M05	14/09 KSH	3755,97	3462,26	Small	Yes	No
M06	14/09 KSH	3848,71	3424,15	Large	Yes	Yes
M07	14/08 KSH	3639,57	3393,22	Small	Yes	No
M08	14/08 KSH	3678,28	3486,62	Large	No	No
M09	14/18 KSK	3679,46	3342,61	Large	No	Yes
M10	14/19 KSK	3662,26	3289,04	Large	No	Yes
M11	14/19 KSK	3682,48	3305,23	Large	No	Yes
M12	14/19 KSK	3608,10	3412,18	Large	No	No
(*) M13	14/10 KSH	3581,08	3314,96	Small	No	No
M14	14/10 KSH	3632,30	3259,70	Large	Yes	No
M15	14/10 KSH	3609,61	3255,45	Small	Yes	No
(*) M16	14/10 KSH	3597,14	3379,71	Small	Yes	Yes

Table 7: Mating trials conducted with 16 virgin males and females from 7 different colonies. Males were presented with a large and a small female at the same time and it was recorded which female was mated first. Within a timeframe of 3 hours it was also noted whether the second female was mated as well and if a remating of females occurred. Asterisks (*) mark trials that had to be repeated the following day due to no mating occurring on the first day.

In ten of 16 trials the male was observed copulating with the second female as well within two hours of mating with the first female (Table 7). During that time-frame, eight females re-mated with the male after they had copulated with the male before, leading to up to 4 copulations of a single male in one trial. No female re-mated more than once. Between copulations, males were regularly observed reloading their palps which usually took between five and ten minutes. While the male copulated with one female, the second female usually remained passive and motionless. However, in five cases the female that was currently not mating started to move its legs, shifted its position several times, walked around and touched the mating pair with its first pair of legs. One female assigned to the small category constantly interfered with the ongoing mating between the male and the large female by touching the male, causing the couple to stop mating several times before they resumed copulatory position.

3.3.4 Potential rate of reproduction in males

From 16 virgin males, eight copulated only twice (N = 2) or three times (N = 6), as it was no possible to present them with more females due to lack of adult virgin females over the course of the experiment. The remaining eight males copulated five times or more often (Table 8). The two males that were mated *ad libitum* (Male 7 and Male 8) copulated with 14 and 16 different females, respectively, over the course of 22 days before they died. Ten and eleven, respectively, of the females that had copulated with those males produced an egg sac of which nine and ten, respectively, hatched.

Male ID	*Source colony*	*Number of copulations*	*Number of ovipositing females*	*Number of hatching egg sacs*
Male1	15/05 KZN	5	4	3
Male2	15/03 KZN	6	3	1
Male3	15/08 KZN	6	3	2
Male4	15/12 KZN	7	3	0
Male5	15/05 KZN	8	4	2
Male6	15/11 KZN	10	4	0
Male7	15/04 KZN	14	10	9
Male8	15/04 KZN	16	11	10

Table 8: List of males that copulated with a virgin female five times or more often during an experiment estimating potential rate of reproduction in males, and the reproductive success of the females they copulated with.

Copulations took on average 31 ± 23 minutes with the shortest copulation lasting 4 minutes and the longest 124 minutes. The shortest copulation leading to oviposition was 5 minutes long and copulations as short as 7 minutes led to hatching success. The longest copulation leading to hatching success lasted for 91 minutes.

3.4 IV) LIFE HISTORY

3.4.1 Observations on reproductive traits in experimental colonies

After mating the first egg sac appeared, on average, after 35 ± 10.34 (mean ± SD) days (min = 9 days; max = 70 days, Table 9). There was a significant difference between the years ($t = 4.73$, $df = 58.53$, $p < 0.001$, Figure 13), with females in 2014 (N = 55) producing an egg sac sooner after the mating (mean ± SD = 31 ± 8,74 days) compared to females in 2015 (N = 32; mean ± SD = 41 ± 9,92 days). In ten groups a second egg sac was produced before the hatching of offspring. At the end of the brood care period 23 of 50 groups (46 %) contained more than one egg sac, with 17 groups containing two, five groups containing three and one group containing four egg sacs. All egg sacs were opened at the end of the brood care period. There were four unhatched egg sacs in three groups (two groups with one unhatched egg sac, one with two unhatched egg sacs) containing dead and dry spiderlings inside. In two of those experimental groups the survival rate of the egg sac that had hatched was very low, with only one spiderling surviving until the end of the brood care period. In one experimental group, 51 spiderlings survived until the end of brood care.

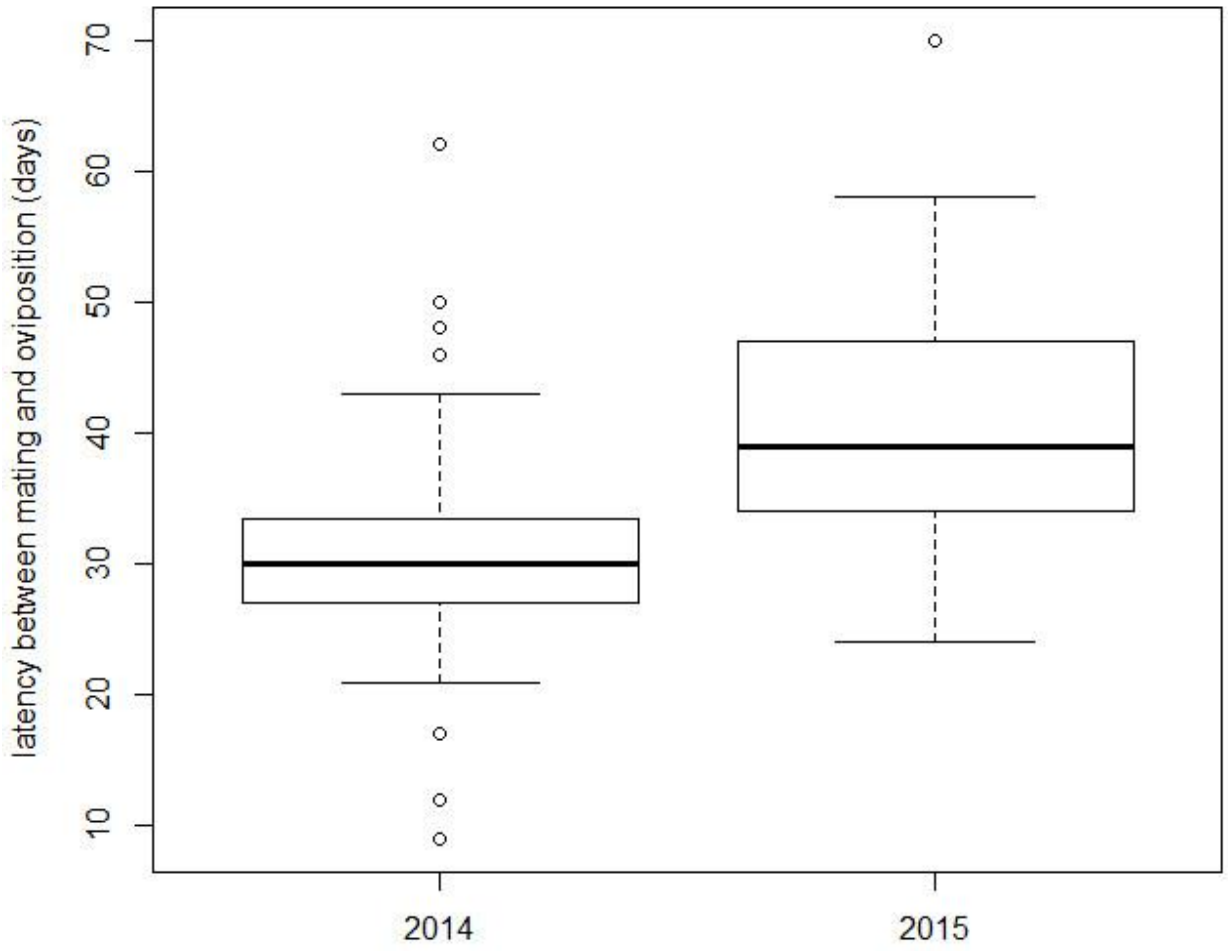

Figure 12: Latency between mating and oviposition in *S. dumicola*. In 2015 females took significantly longer to oviposit than in 2014.

Offspring hatched on average 37 ± 6.13 days after the first oviposition event (min = 29 days; max = 50 days, Table 9) and differed between the years as well (t = - 3.08, df = 79.00, p = 0.003, Figure 13). In 2014, the offspring hatched later (mean ± SD = 38 ± 6.4 days, min. = 31 days; max. = 67 days) than in 2015 (mean ± SD = 35 ± 4.86 days, min. 28 days; max. 50 days).

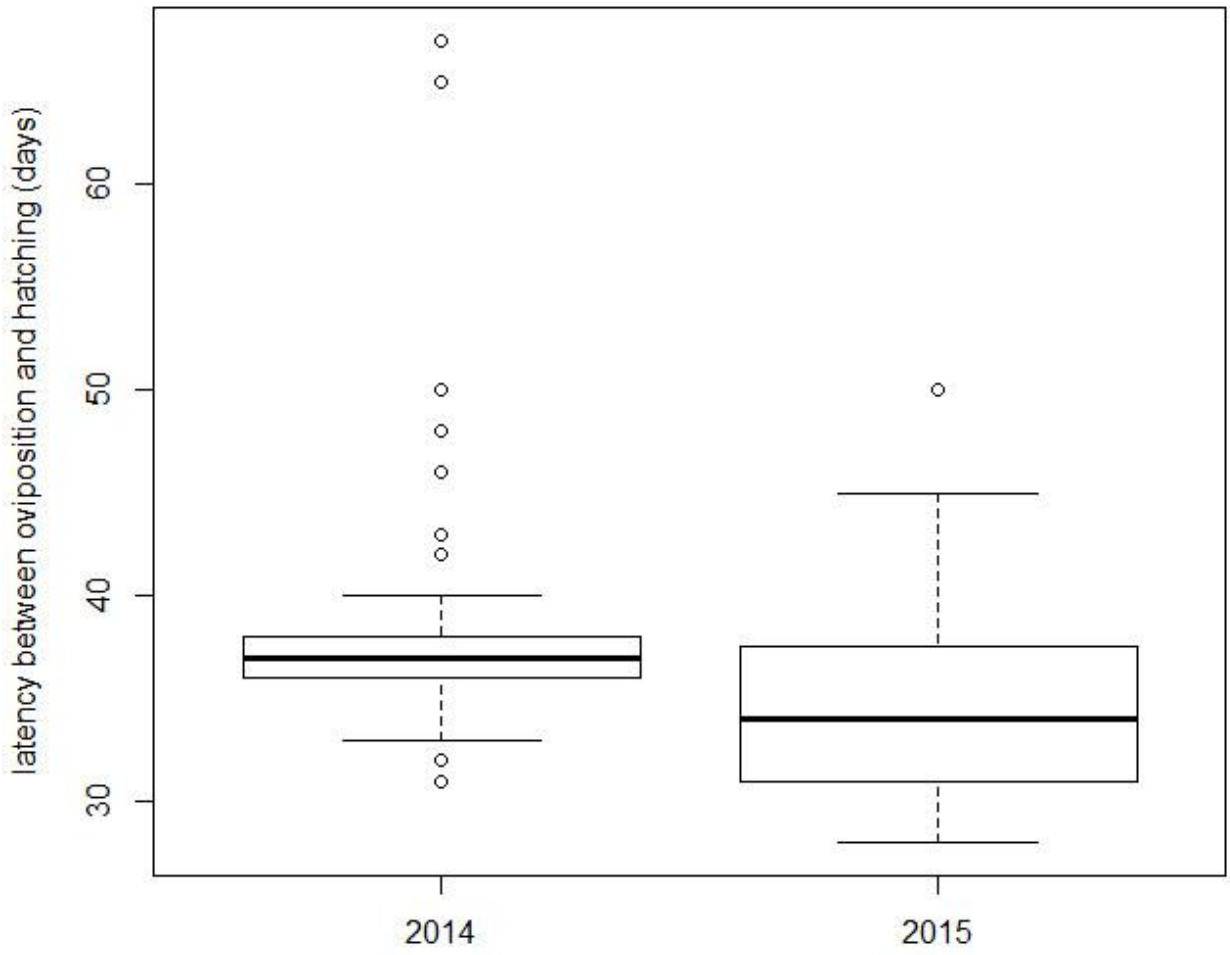

Figure 13: Time between oviposition and hatching of the offspring in *S. dumicola*. In 2015 offspring hatched significantly earlier than in 2014.

In three cases in 2014 and two cases in 2015 (5.7 % of all experimental groups) a second egg sac hatched while females were still caring for the first brood, indicating a successful second oviposition event by the mated female in the group. In all of these groups the second egg sac had been produced before the first hatching event and no further egg sacs occurred. Brood care after hatching of the offspring usually lasted between two and three months. However, in some experimental colonies all adult females were still alive after 4 months of regurgitation feeding. This coincides with my own observations on some experimental groups in 2013 in which matriphagy only took place after several months of brood care and when feeding of the groups ceased. The number of offspring that survived until the end of the brood care period was

recorded in 51 groups in 2014 and varied between 102 and none, with the 102 spiderlings being counted in a box with a second hatching event (Table 9). Due to the developmental stage of the spiderlings, it was possible to distinguish that 53 spiderlings had hatched from the first egg sac and 49 from the second. The highest number of surviving offspring from a single egg sac was 80. An average of 44 ± 21.65 spiderlings survived until the end of the brood care period if only groups in which at least one spiderling survived were considered (if all groups were included, a mean of 38 ± 25.18 spiderlings survived). In seven groups no offspring survived. The number of surviving offspring in the experimental groups did not differ between the regions where the natural nests had been collected in 2014 ($t = -0.82$, $df = 6.65$, $p = 0.44$).

Female ID	*Source colony*	*Days between mating and first oviposition*	*Total number of egg sacs*	*Days between first oviposition and hatching*	*Number of surviving spiderlings*
F01	14/05 KSH	17	1	33	80
F02	14/05 KSH	29	1	36	0
F03	14/05 KSH	30	2	46	1
F04	14/05 KSH	30	1	36	0
F05	14/05 KSH	30	1	38	11
F06	14/05 KSH	31	1	36	0
(*) F07	14/05 KSH	32	2	37	26
(*) F08	14/05 KSH	33	2	38	84
F09	14/08 KSH	24	1	36	0
F10	14/08 KSH	28	1	37	36
F11	14/08 KSH	28	1	40	0
F12	14/09 KSH	24	1	50	0
F13	14/09 KSH	27	1	35	3
F14	14/12 KSK	27	1	34	35
F15	14/17 KSK	12	1	48	17
F16	14/17 KSK	21	1	37	37
F17	14/17 KSK	24	-	38	-
F18	14/17 KSK	24	1	38	27
F19	14/17 KSK	26	1	37	26
F20	14/17 KSK	29	1	38	50
F21	14/17 KSK	27	1	39	0
F22	14/17 KSK	29	2	43	2
F23	14/17 KSK	31	4	31	66
F24	14/17 KSK	31	3	31	1
F25	14/18 KSK	9	2	37	46
F26	14/18 KSK	22	2	34	70
(*) F27	14/18 KSK	25	2	36	102

F28	14/18 KSK	27	1	38	55
F29	14/18 KSK	28	3	35	57
F30	14/18 KSK	28	1	35	55
F31	14/18 KSK	29	2	35	60
F32	14/18 KSK	30	2	32	34
F33	14/18 KSK	30	2	38	34
F34	14/18 KSK	30	1	33	41
F35	14/18 KSK	31	1	36	44
F36	14/18 KSK	32	3	33	24
F37	14/18 KSK	32	2	36	51
F38	14/18 KSK	33	2	36	41
F39	14/18 KSK	33	2	36	52
F40	14/18 KSK	34	1	35	55
F41	14/18 KSK	34	1	37	51
F42	14/18 KSK	35	3	37	45
F43	14/18 KSK	38	1	42	62
F44	14/18 KSK	39	1	38	64
F45	14/18 KSK	40	3	38	39
F46	14/18 KSK	40	2	37	45
F47	14/18 KSK	40	2	38	53
F48	14/18 KSK	43	2	39	40
F49	14/18 KSK	46	2	38	50
F50	14/18 KSK	48	1	35	51
F51	14/18 KSK	50	1	37	52
F52	14/18 KSK	62	1	36	50
F53	14/19 KSK	26	-	40	-
F54	14/19 KSK	31	-	38	-
F55	14/19 KSK	41	-	37	-
F56	15/01 KZN	36	-	30	-
F57	15/02 KZN	34	-	28	-
F58	15/04 KZN	37	-	42	-
F59	15/04 KZN	42	-	37	-
F60	15/04 KZN	43	-	38	-
F61	15/04 KZN	45	-	34	-
F62	15/04 KZN	45	-	37	-
F63	15/04 KZN	47	-	34	-
F64	15/04 KZN	49	-	34	-
F65	15/04 KZN	50	-	31	-
F66	15/04 KZN	52	-	34	-
F67	15/04 KZN	56	-	38	-
F68	15/04 KZN	58	-	38	-
F69	15/04 KZN	70	-	31	-
F70	15/05 KZN	35	-	50	-
F71	15/05 KZN	44	-	45	-
F72	15/06 KZN	34	-	32	-
F73	15/07 KZN	38	-	34	-
F74	15/07 KZN	42	-	30	-

F75	15/07 KZN	50	-	35	-
F76	15/09 KZN	24	-	29	-
(*) F77	15/09 KZN	26	-	33	-
F78	15/09 KZN	29	-	33	-
F79	15/09 KZN	33	-	29	-
F80	15/09 KZN	35	-	31	-
F81	15/09 KZN	38	-	31	-
F82	15/09 KZN	38	-	39	-
F83	15/09 KZN	40	-	29	-
F84	15/09 KZN	47	-	38	-
(*) F85	15/12 KZN	28	-	37	-
F86	15/12 KZN	34	-	33	-
F87	15/12 KZN	34	-	32	-

Table 9. Reproductive history of females from 15 source colonies in 2014 and 2015. A single mother was kept with 3 virgin females. Asterisks (*) indicate groups with a second hatching event. Number of egg sacs and spiderlings was evaluated for F01 to F52 only.

4 DISCUSSION

4.1 I) TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES

I investigated whether in communities of the social spider *Stegodyphus dumicola,* the investment in different tasks in the nest differs between virgin helpers and mothers, and whether virgin helpers perform brood care and show the same repertoire of brood care behaviors as mothers do. The data demonstrates that virgin helpers tended the egg sacs in virtually all groups, providing proof that allomaternal care by virgins is a common feature of social groups of *S. dumicola*. I also observed virgin helpers taking part in regurgitation feeding of the offspring and being consumed at the end of brood care via matriphagy. Even though virgin helpers engaged in the same tasks as mothers, they were more likely to engage in prey attack, irrespective of whether an egg sac was present in the group, while mothers were more likely to tend egg sacs. Although the degree of differential task participation was slight, it suggests that reproductive state determines some degree of behavioral specialization between virgin helpers and mothers.

In many cooperatively breeding societies with alloparental care, helpers are adult individuals that care for the offspring of other adults (Clutton-Brock, 2002; Cockburn, 1998). The same is true for social spiders, in which brood care involves caring for the egg sac as well as extreme behaviors such as regurgitation feeding and matriphagy. Providing food via regurgitation is cost intensive as it involves the liquefaction of body contents (Junghanns, 2013; Nawabi, 1974; Salomon et al., 2015), and thus the consumption of bodily resources, while (allo)matriphagy leads to the death of the female (Kullmann et al., 1971; Salomon et al., 2005). In contrast to many other animal societies in which alloparents help raise the offspring, allomothers in social spiders are thus deprived of the opportunity of a future reproductive event. Although allomaternal care has been demonstrated for social *Stegodyphus* and *Anelosimus* species (Christenson, 1984; Furey, 1998; Grinsted, Agnarsson & Bilde, 2012; Kraus, 1989; Kullmann et al., 1971; Samuk & Avilés, 2013), until this time it was unknown whether mating status determines the ability and propensity for individuals to perform extreme brood care relative to other tasks in the group. Observations in females of subsocial spiders suggested that the propensity to accept brood and perform care is linked to the reproductive status of the female (Schneider, 2002; Assi Bessékon & Horel, 1992;

Assi Bessékon et al., 1996). Even when confronted with an egg sac, it was not possible to stimulate virgin *S. lineatus* to provide care, suggesting that the egg sac is not a sufficient catalyst for triggering brood care. Instead, being in the right internal state probably plays an important role (Schneider, 2002). In contrast, our study shows that virgin helpers of the social *S. dumicola* provide extensive brood care and can thus rightfully be termed allomothers. The fact that virgin females are – as opposed to the subsocial species – able to provide all forms of brood care is highly intriguing. This ability represents an adaptation to cooperative breeding, and thus to a permanently social life style, and entails a shift in the trigger for brood care behaviors from mated to virgin females.

In *S. dumicola,* a large proportion of females remain unmated (Salomon et al., 2008), and their engagement in allomaternal care must enhance growth and survival of the offspring in the nest. In return, despite the costs of extreme allomaternal care, fitness for virgin helpers will be maximized due to inclusive fitness benefits by staying in the natal colony and helping their sisters (Lubin & Bilde, 2007; Settepani et al., 2017), instead of taking the risk of dispersal from the colony, which is associated with an increased risk of predation and low chances of successful reproduction (Crouch et al., 1998; Henschel, 1992). The physiological ability of virgins to provide extreme brood care may also provide insurance in the event of death of reproductive females (Schneider & Lubin, 1997). In the subsocial *S. lineatus,* more than 50 % of females with an egg sac die before their offspring hatch (Schneider, 1996b), and in *S. dumicola* up to 90 % of single-female nests go extinct (Bilde, et al., 2007; see also Henschel, Schneider & Meikle, 1996; Henschel, 1998). While in the subsocial species the offspring rely heavily on the care by the mother alone and will have low or no chance of survival if the mother dies before matriphagy (Schneider, 2002), in the socials species the dependence of offspring is distributed over several adult individuals. If one female dies, the remaining females can take over brood care of her offspring and ensure the survival of the brood. Indeed, in multi-female nests, allomaternal care by females of unknown mating status was demonstrated to secure growth and survival of offspring even in the case of the mother's death (Jones, et al., 2007; Kullmann, 1972; Lubin & Bilde, 2007). It remains to be investigated whether virgin helpers are flexible in altering their participation in various tasks in

response to the needs of the breeding group (Mooney et al., 2015).

High relatedness among group members is expected to reduce the cost of helping in individuals that are unlikely to reproduce, compared to individuals with a high chance of reproduction, which can ultimately lead to worker specialization (Boomsma, 2009). For example, nonbreeding and breeding banded mongoose appear to be totipotent. Nevertheless, subordinate males increase their contribution to costly babysitting of pups at the time of day presumed to be the most energetically demanding, and at oestrous when dominant males and females reduce their babysitting effort (Cant, 2003). We found that in *S. dumicola*, females are largely totipotent, but our point sampling trials revealed a propensity to engage in different tasks between virgin helpers and mothers. Virgin helpers were less likely to tend egg sacs than mothers, and more likely to engage in the risky initial prey attack that requires abandoning the safe retreat, confrontation with a potentially dangerous or defensive prey (Bradoo, 1980; Griswold & Meikle, 1990; Henschel, 1998; Junghanns & Holm, pers. obs. 2013; Settepani, pers. obs. 2010), and the costly use of venom to subdue the prey (Morgenstern & King, 2013). It is possible that the participation of virgin helpers in prey attack enhances foraging success by improving the nest's prey capture efficiency (Pasquet & Krafft, 1992). Furthermore, by engaging more in prey capture, virgin helpers may spare energy use by mated females while reducing their risk of injury during prey attack, and at the same time provide them with food before and during brood care. Mated females need to acquire energy to overcome the threshold for successful oviposition (Drent & Daan, 1980), from which virgin helpers profit through inclusive fitness. This behavioral differentiation, together with recent evidence for individual specialization (Settepani, et al., 2013; Pruitt & Riechert, 2011; Wright, et al., 2014), suggests a more complex social organization in social spiders than previously recognized. Because relatedness within the nest is extremely high (Lubin & Bilde, 2007; Settepani, et al., 2017), the nest represents the reproductive unit, and fitness is defined by nest-productivity and not solely by individual reproductive success (Boomsma, 2009; Grinsted & Bilde, 2013; Keller, 1999). High intra-nest relatedness should reduce conflict over reproduction because indirect fitness of helpers will be similar to direct fitness, thereby aligning the interests of individuals. If reproductive conflict is reduced, and task differentiation is beneficial (Dukas & Visscher, 1994;

Julian & Cahan, 1999; Wright, et al., 2014), this may further promote behavioral differentiation. Moreover, since social spiders are sedentary and dependent on the prey that arrives in their capture web, they are particularly limited by resources (Bilde, et al., 2007), which was proposed to favor a life history strategy of investing in fewer but larger offspring (Grinsted et al., 2014). Cooperative breeding and behavioral differentiation may be adaptations that increase reproductive success of the group under ecological constraints (Clutton-Brock, 2002; Emlen, 1995; Grinsted, et al., 2014).

The higher engagement by virgins in prey attack may also be explained by virgins trying to secure resources for a reproductive event later on. Our data demonstrate that even before egg sac production, virgin helpers engaged relatively more in foraging behavior, and it is known that the first attacking spider feeds longer (Willey, 1993) and gains more weight (Amir, Whitehouse & Lubin, 2000; Whitehouse & Lubin, 1999). It is assumed that those females in the colony remain virgin helpers that are not able to gain enough resources during growth to mature in time (Whitehouse & Lubin, 1999), thus, virgin prey attack could be mainly driven by hunger and the aim to secure resources. However, differential participation in foraging does not seem to be determined by hunger status (Ainsworth et al., 2002). Furthermore, I assume that mating opportunities for dispersing unmated females are low, when they were not able to mate within their own colony, especially the further the brood care season has progressed. Males in a nest are short lived and there is typically little male dispersal activity between nests (Lubin, et al., 2009). Furthermore, close by interconnected nests, which could serve as source for mating partners, are in a similar maturation status (Salomon et al., 2008; own observations) suggesting that males in those nests will die off around the same time as in the natal colony. On the other hand, females dispersing further distances by ballooning (Crouch, 1998; Schneider et al., 2001) and creating an isolated nest on a new location, face a low chance to encounter a male, as males typically only migrate short distances and preferably along silk threads among interconnected nests (Lubin et al., 2009). Hence, I assume that the resources acquired by virgin helpers will directly benefit the growth and survival of offspring in the nest by means of allomaternal care. As all females take part in the regurgitation feeding and serve as a final meal during matriphagy,

regardless of their reproductive state, food taken up by virgins during feeding bouts is not lost but will eventually benefit the offspring. Indeed, the process of cooperative regurgitation feeding enables the colony to maximize the profit of the periods that are rich in food and extend the feeding period into the periods when external food supply becomes scarce. When offspring hatch around March, the southern African summer is almost over, and prey diversity starts to decline. However, the females of the colony serve as a reservoir for resources accumulated during the hunting success of the summer. While external food supply diminishes, the offspring will still be able to grow and thrive on the resources distributed via regurgitation feeding by mothers and helpers. In this way the effects of the unfavourable winter period with low prey availability can be reduced and the chance of survival of the offspring until more prey is available is increased.

The mechanisms determining whether a female stays virgin and becomes an allomother or reproduces remain to be investigated (Grinsted & Bilde, 2013). It is possible that reproductive ability is determined by resource availability during early development that shapes a certain developmental trajectory of an individual (Beldade, Mateus & Keller, 2011; English, Browning & raihani, 2015; Mayntz, Raubenheimer, Salomon, Toft & Simpson, 2005; Miura 2005; Nijhout 2003; Salomon et al., 2008). Scramble competition may imply that some females do not develop beyond a specific threshold in time (Whitehouse & Lubin, 1999), and as pointed out above, late maturing females are unlikely to be able to reproduce and are therefore destined to become allomothers. On another note, the male scarcity within a colony might factor into the reproductive skew by mechanisms such as mate competition among females and/or mate choice by the comparably fewer males in a colony. However, that the helper role is not determined solely by mating status is indicated by observations that mated females can also act as allomothers (Kullmann et al., 1971), and even immature females are reported as helpers (Christenson, 1984; Gómez, Rojas-Buffet & Viera, 2015; Viera et al, 2005). In general, we have little understanding of how early life experience influences ‘social trajectories’ (English, et al., 2015; Zöttl, Thorley, Gaynor, Bennett & Clutton-Brock, 2016), and to what extent individuals have control over the flexibility to move among social trajectories or to specialize (Carter, et al., 2014; Huchard, Charmantier, English, Bateman, Nielsen & Clutton-Brock, 2014; Jeanson & Weidenmüller, 2013). The degree to which

individuals specialize or remain flexible may further depend on what type of information they have over the fitness outcomes of their decision (English, et al., 2015; Holman, 2014; Reale, Reader, Sol, McDougall & Dingemanse, 2007). When direct and indirect fitness outcomes are aligned, as in the social spiders, the ability of totipotent helpers to modify their effort and task strategically may evolve in response to ecological and social parameters.

4.2 II) MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE

I investigated the physiological adaptations to extreme maternal care in the solitarily breeding subsocial *S. lineatus* and the cooperatively breeding social spider *S. dumicola.* By applying an experimental approach of removing eggs or offspring at different stages, I investigated whether physiological responses to brood care are reversible. This study included both mothers and virgin helpers of the cooperative species, to examine whether virgin helpers show similar physiological changes as do mothers. This would additionally to the behavioral observation be a further indication of participation in brood provisioning and may represent an adaptation to cooperative breeding.

The re-evaluation of paraffin samples of the solitary *S. lineatus* confirmed that massive changes in the midgut tissue and accumulation of extracellular material occur only in mothers. This suggests that reproducing females undergo morphological changes to meet the demands of regurgitation feeding with liquefied body tissue (Junghanns, 2013; Kullmann, 1968; Nawabi, 1974; Salomon et al., 2015). After oviposition, females accumulated material for secretion in the form of blue stained granules, perhaps of alkaline content (Burck, 1988), in their cells in preparation for regurgitation feeding. By the time the offspring hatched, the midgut tissue of the mother contained cells brimming with blue stained granules. Additionally, the formation of large extracellular lacunae and signs of dissolving cell structures in the midgut region at that time indicate an ongoing disintegration of midgut tissue (Junghanns, 2013; Salomon et al., 2015). The fine-grained blue stained material that fills the lacunae at the time of hatching and during regurgitation feeding, most likely represents the material that females will regurgitate to the young. This regurgitant was already present in high amounts at the time when offspring hatch.

This suggests that the transformation of midgut tissue in preparation for provisioning of young is already initiated when an egg sac is present. During regurgitation, females lose weight (Salomon et al., 2005), which in *S. lineatus* is reflected in significantly reduced amounts of extracellular material in the late regurgitation stage and a smaller opisthosoma compared to earlier stages. Semelparous species are expected to invest maximally in their single reproductive bout and die once reproduction took place (Alonso-Alvarez & Velando, 2012; Collatz, 1987; Stearns, 1992). Our data indicate that mothers in *S. lineatus* are able to reverse physiological adaptations to brood care if their offspring were removed early in the maternal care period. Histological examination showed that after the removal of hatched offspring, mothers showed a reduced amount of blue stained secretion granules and extracellular material. Usually, only remnants of the extracellular material were found in the lacunae. This suggests that mothers were able to terminate the production of secretion for regurgitation and to reabsorb existing extracellular material using the remaining intact diverticula. The implication could be that mothers possess sufficient resources to produce a second clutch to care for, if the first brood is lost early in the provisioning period (Schneider & Lubin, 1997b). Indications of reversal processes were less pronounced when removal of offspring took place when regurgitation feeding had started in the early regurgitation stage. At this stage, mothers of *S. lineatus* appeared unable to reabsorb existing extracellular material.

The semithin sections of the midgut tissue, in which lipids are preserved, provided new insights to the processes involved in regurgitation feeding in *S. lineatus*. During periods of rapid growth, fat ingested by the spider during meals will mainly be metabolized to phospholipids, which serve as components to synthesize new membranes. However, when fat resources are abundant, lipids will be stored in the form of lipid droplets in the tissues (Jensen, Mayntz, Toft, Raubenheimer & Simpson, 2010). Virgins usually showed no lipid droplets in their midgut tissue and the lipid content of the midgut tissue in females across all stages in the control and removal group was highly variable. The lack of lipids in the interstitial tissue of many females was especially surprising, as this tissue had been found to be a storage facility for lipids in the related social *Stegdoyhus pacificus* (Nawabi, 1974) and other spiders (Alberti & Storch, 1983). In our

experiments, mothers of *S. lineatus* were not fed as soon as an egg sac was produced. Thus, the lack of lipids in all parts of the midgut in most virgins and the new occurrence of lipids in control and removal mothers indicates that those they originated from other sources in the body than from lipids stored in the interstitial tissue. Especially intriguing are mothers from the removal group, from which hatched offspring were removed before regurgitation had started, as they showed huge amounts of lipids in the cells of the diverticula as well as in the interstitial tissue. That mothers in this stage are able to reabsorb the extracellular material, which had accumulated in preparation for regurgitation feeding, was shown by the paraffin sections. The new observation adds that fat present in the regurgitant is reabsorbed by the cells of the diverticula and as lipid droplets transported to and stored in the interstitial tissue. I suggest that these observations are explained by the disintegration of tissues and thereby freeing of fat which had been confined in membranes. However, why *S. lineatus* females in the first place do not store lipids in the interstitial tissue remains unclear. It is possible that they are only rarely able to acquire a surplus of fat that can be stored.

The ability to halt and reverse processes related to brood care, despite *S. lineatus* being a semelparous species, can be explained by *Stegodyphus* females regularly losing their egg sac to infanticidal males, predation, or parasitism in the field (Bilde et al., 2007; Schneider & Lubin, 1997b, 1997a;). Females can compensate for brood loss by producing a second egg sac, as evidenced by the presence of late vitellogenic oocytes in the ovaries of some females in late stages of regurgitation feeding. This suggests that there is no predetermined point to cease egg production, and females retain the ability to produce a replacement clutch. However, as the season progresses, increased risk of predation decreases the chance of successful reproduction (Schneider & Lubin, 1997a). The life history of *Stegodyphus,* in which high mortality may have favored semelparity, appears to be aligned with physiological adaptations to extreme maternal care that are irreversible once regurgitation feeding has begun. At this point, females may have invested an amount of energy that reduces the likelihood that their energy budget would meet the threshold for yet another successful reproductive bout (Drent & Daan, 1980; Stearns, 1992). It is unlikely that a second clutch would survive and develop successfully without the intensive maternal investment (Salomon et al., 2005; Staerkle & Kolliger, 2008).

Similar morphological changes as in *S. lineatus* were observed in mothers of the cooperatively breeding *S. dumicola*, although the changes were not quite as comprehensive. In *S. dumicola,* extracellular blue stained material did not accumulate in high amounts in lacunae but were often limited to natural lumina of the diverticula in the anterior part of the midgut. Extracellular material accumulated when the offspring had been removed during regurgitation, indicating that similar to *S. lineatus*, mothers were incapable of reabsorbing existing extracellular material at this point, and unable to stop the process of producing additional regurgitant. This suggests that physiological plasticity in mothers of both species diminishes from the onset of regurgitation, marking the time of regurgitation feeding as a physiological 'point of no return.' How far into the regurgitation phase a mother can stop and reverse physiological brood care processes may depend on her physical condition. This could be reflected in the ability of some, but not all mothers, to mature oocytes in their ovaries even in late regurgitation stage. Different from *S. lineatus,* the lipid content of the midgut in *S. dumicola* females is higher. In virgin non-helpers the occurrence of lipids in the midgut is highly variable, but at the time of oviposition all mothers of the control group store huge amounts of lipids in their interstitial tissue. When offspring hatch and at all later stages in the brood care process, most lipids are present in the cells of the diverticula, presumably to release them into the lumina during regurgitation feeding. However, when offspring were removed at oviposition or hatching, in some cases lipids have been transported back to the interstitial tissue. The differences in lipid content of the midgut tissue between the two species are striking: Why are females of *S. dumicola* able to accumulate large amounts of storage lipids, while *S. lineatus* females are not? In later stages of brood care this could be explained by the ongoing foraging events in *S. dumicola*, which allow females to acquire additional resources. But if those new resources are ingested at all, they do not seem to be transported into the interstitial tissue, but remain in the cells of the diverticula, since the lipid content in the interstitial tissue remains low. However, the ongoing feeding during brood care does not explain why the difference is already apparent in virgin non-helpers and females at the stage of oviposition. Possibly, cooperative foraging females in the social species are generally able to acquire more surplus resources during development, allowing them to store lipids.

In cooperative breeders, helpers may develop morphological and physiological adaptations to brood care (Adkins-Regan, 2005; Cant, 2012; Roces & Núñez, 1995; Wilson, 1971;). In the solitary breeding *S. lineatus*, maternal care behavior occurs only in females that had mated and oviposited, whereas virgin females do not engage in maternal care (Schneider, 2002). In contrast, virgin female helpers of the social species perform all tasks associated with maternal care (Junghanns, Holm, Schou, Sorensen, Uhl & Bilde, 2017; Salomon & Lubin, 2007; Seibt & Wickler, 1988a). The physiological ability of virgin helpers to provision the offspring suggests that they have adapted to cooperative breeding. The histological examination showed that virgin helpers undergo similar changes in the midgut to those observed in mothers. As in the midgut tissue of mothers, in virgin helpers blue stained granules and small amounts of extracellular material accumulated in the midgut tissue (Figure 5D and F) and the dynamics of lipids followed the same pattern. Surprisingly, already at the stage of hatching, virgin helpers were unable to terminate and reverse the internal processes in preparation of regurgitation feeding. Instead, they accumulated more extracellular material (Figure 6D). Since the virgin helpers cannot invest their resources in own offspring, and the chance to mate with a male immigrating from another colony is especially low late in the reproductive season (Lubin et al., 2009) their reproductive fitness depends solely on the survival of their sisters´ brood (Lubin & Bilde, 2007). The physiological ability of unmated females to provide brood provisioning is therefore key for acquiring indirect benefits of helping, and likely represents an adaptation to cooperative breeding.

The less dramatic changes of the midgut tissue in the social *S. dumicola* likely reflect an adjustment of resource allocation to a longer maternal provisioning period, as egg sac production and hatching is not entirely synchronised within a nest. Indeed, the provisioning period in social *Stegodyphus* is much longer than that of solitary breeding congeners (Lubin & Bilde, 2007; Seibt & Wickler, 1988a). In the solitarily breeding *S. lineatus,* mothers often close their nest and stop hunting as soon as the offspring hatch (Schneider et al., 2003), as hunting or maintaining a capture web would entail abandoning offspring at times and leaving them vulnerable. Subsocial *Stegodyphus* mothers thus rely solely on the resources they have accumulated in their body up to this point, allowing for a limited regurgitation period. In *S. dumicola,* the participation of

helping females distributes the work-load of regurgitation feeding among them, each of them using a limited amount of her own resources at a time. Additionally, cooperative maintenance of the capture web and foraging provides all females with incoming resources that may prolong the care period. The ability to perform continuous allomaternal provisioning may also provide insurance against the loss of caring mothers (Jones & Riechert, 2008). Even if a mother dies, other females will provision offspring.

The physiological capacity to engage in regurgitation feeding may rely on an internal maturation process triggered by mating or oviposition that physiologically enables the female to provide offspring care (Krafft & Horel, 1980; Fénéron et al., 1996; Schal et al., 1997; Schneider, 2002; Mas & Kölliger, 2008; Pinilla et al., 2012). Interestingly, virgin helpers and virgin non-helping females of the cooperative *S. dumicola* showed early and late vitellogenic oocytes in their ovaries - a mating event thus does not seem required for egg maturation. Virgin helpers experienced morphological changes similar to those of mothers during cooperative brood care, and the presence of late stage oocytes in their ovaries might indicate the physiological maturation process that precedes and triggers regurgitation feeding and suicidal care. In ants, ovarian maturation of workers is linked with the performance of certain tasks in the nest, with nursing workers showing the most developed ovaries (Fénéron et al., 1996). The link between ovarian maturation and brood care seems to have played a role in the evolution of sociality within the genus *Stegodyphus* since in the solitary species *S. lineatus*, virgin females only contained pre-vitellogenic oocytes, and were shown not to oviposit and do not provide care when in contact with spiderlings. In *S. dumicola* and possibly in other social *Stegodyphus* species, the maturation of ovaries in virgin helpers may thereby represent an adaptation to cooperative brood care.

To summarize, in both *Stegodyphus* species, the onset of reproduction triggers massive changes in the opisthosomal midgut tissue, indicating physiological responses to regurgitation feeding with liquefied body tissue. Females were able to terminate and partially reverse these internal morphological changes until the start of regurgitation feeding, which marks a physiological ‘point of no return.’ Virgin helpers showed similar or even stronger physiological response to brood care

than mothers. This could be an adaptation to continued allomaternal care over prolonged periods in social nests, or it could facilitate the production of a second brood by mothers, as mothers retain the ability to mature oocytes upon the loss of offspring. In contrast to virgin females of the subsocial *S. lineatus*, virgin helpers in *S. dumicola* often contained early and late vitellogenic oocytes in their ovaries. This suggests that oocyte maturation is a prerequisite for the onset of extreme brood care in virgin helpers, and that oocyte maturation has shifted to an earlier stage in ontogeny during the evolution of cooperative breeding.

4.3 IV) MALE SCARCITY AND ITS IMPLICATION ON REPRODUCTIVE SKEW

That the sex ratio at maturation (MSR) is biased in favor of females was documented in *S. dumicola* before (Griswold & Meikle, 1990; Henschel et al., 1995; Seibt & Wickler, 1988a) and is confirmed by our examinations of natural nests in three consecutive years. In accordance to the above-mentioned authors, the average MSR was between 11 and 13 percent in all three years, suggesting a more or less steady female skew within the species. However, the MSR between the nests varied between 2 % and 28 %, showing that MSR in natural colonies is plastic (Salomon et al., 2008). We collected the colonies at the beginning of the reproductive season when males started to mature, suggesting the variation in MSR was probably not a result of sex-biased dispersal. Premating dispersal, especially over large distances, is assumed to be uncommon in undisturbed colonies (Bilde & Lubin, 2011). Instead, mated females leave the colony by the end of the mating season (Schneider et al., 2001), possibly resulting in changing ASR (Lubin et al., 2009). As the dissected colonies were in an early phase of their maturation the latter is unlikely to have affected our results. Indeed, the plasticity in sex ratio was proven to be already present at the early zygotic stage (Avilés et al., 1999) and earlier even in sex determining sperm among males from the same colony (Vanthournout et al., 2018). Hamilton (1967a) discussed that inbreeding societies favor a female-biased sex ratio with only as many males being produced as are needed to fertilize all receptive females. Additionally, in societies with cooperative breeding, the sex ratio should be skewed towards the most helpful sex (Emlen, 1986), which is the allomaternal females in *S. dumicola*. However, optimal sex ratio might depend on variable environmental factors, which can cause variation in the parent's condition and thus determine

their investment in a certain sex (Trivers & Willard, 1973). This could explain why the MSR is not stable but oscillates around the low value repeatedly found in *S. dumicola*.

The consequences of male scarcity in colonies on reproductive skew among females had not been evaluated yet. Reproductive skew is often assumed to be a result of the skewed maturation between the sexes and also among females, thus resulting in male biased ASR especially in the beginning of the mating season when males are most active (Henschel et al., 1995). It is assumed that males start maturing before females and all males within a colony will mature within a week (Henschel, 1995). As females mature asynchronously over the course of several weeks and months and early maturing males die after 6 to 8 weeks (Henschel, 1995), late maturing females are expected to remain virgin due to lack of mating opportunities. The dissection of natural nests in this study provides evidence for the skewed maturation of the sexes, with males maturing earlier than females. Several colonies contained no adult females although males had already matured. The opposing case, with adult females but no adult males in the colony, has never been observed this early in the reproductive season. This fact reaffirms the observation that males are the first to start maturation (Henschel, 1995). In most colonies, females had started maturing as well when only half or even fewer males were adult, showing an overlap of maturation of the sexes. In one colony (15/11 KZN, Table 5) only 12 percent of males (3 of 25) had matured when first females matured as well (5 of 234; 2 %). In half of the colonies this concurrent maturation of the sexes led to a situation with an ASR considerably biased towards the females, sometimes with a ratio as high as 1 male facing 14 adult females. In one nest, all of the males and females (5 males and 45 females) were already adult. These observations challenge the idea that a male-biased ASR in the beginning of the reproductive season persist for long. Instead, the ASR quickly changes to a female-biased ratio even before all males have matured in colonies, in which a large proportion or even all of the females mature while males are still around, female maturation skew cannot be the only determining factor for reproductive skew. The female-biased ASR might play another key role in determining reproductive roles of females, as reproduction in females might be limited by the number of females a male can inseminate.

If reproductive skew is indeed linked to the skewed maturation in females and diminished chances of mating in late maturing females (Bilder & Lubin, 2011; Henschel et al. 1995; Schneider, 2002), a female's chances of being fertilized could be increased by expediting maturation as soon as first males start to mature. This ability would allow females to outcompete other females that do not meet the threshold for maturation yet, even if it is at the expense of a larger body size that could increase fecundity and chances of mating with choosy males. Although subadult females showed a tendency to mature faster when an adult male was present, statistical analysis did not detect significant differences between the two treatments. This could indicate that timing of maturation is less aligned with adult male presence and depends more on competition among females for resources. This could be due to the fact that adult male presence is not a limiting factor to the degree previously thought. Male maturation was more skewed than expected in seven colonies collected in the end of October in 2015. Instead of all males maturing within a week (Henschel et al., 1995), those colonies contained adult males when they were dissected at the beginning of November and subadult males were still observed several weeks later in mid-December (own observations). Additionally, in the maturation experiments with ten mated females in each experimental group one individual turned out to be a subadult male more than a week after the experiments, which included adult males from the same colony, had started. This observation also indicates a skewed maturation in males. Furthermore, longevity of adult males might vary according to mating possibilities in the colony. Indeed, adult males of unknown age survived in groups of maturing females for 11 to 15 weeks, thus clearly exceeding the 4 to 6-week life expectancy suggested before (Henschel et al., 1995). Under limited mating conditions, males seem to able to persevere for several weeks until more mating opportunities emerge. Similar observations had been conducted in nematodes, in which reduced sperm production increases life span (Van Voorhies, 1992). With maturation of males being skewed and a life expectancy of up to 3 months and longer, the time window for mating opportunities is less restricted for females than has been assumed, as even later maturing females might commonly encounter males. Adult male presence might thus not be an essential cue for the decision to mature. Instead, presence of mature females or a combination of both might be decisive. In this

setup we were not able to distinguish between both effects and generally the low sample sizes might pose a limitation to the interpretation of the data. Thus, future examination of maturation patterns with increased sample sizes could allow for more conclusive results.

The female-biased ASR in half of the dissected colonies raises the question of whether males are choosy, with mating decisions influenced by differences in females' traits. Male choosiness can arise if males have a limited mating ability, resulting in mate preferences which could lead to a skewed reproduction in females. A suitable factor for mate selection in spiders would be female size, which correlates with female fecundity (Marshall & Gittlemen, 1994). However, the results from the mating trials suggest that sexual selection does not act on female size, as males readily mated with the first female they encountered, no matter if it was comparably large or small. An alternative potential target for male selection might be female mating status. Males might prefer to mate with virgin females over already mated ones. An evolutionary advantage could arise from mating with virgin females, which could explain the skewed maturation between the sexes. This is especially true if there is a first male priority in fertilization success, as is indicated by the conduit spermatheca females possess (Austad, 1984; Schendel, Junghanns, Bilde & Uhl, 2017). In the mating trials males and females were willing to re-mate within a short time and in the multiple mating experiment, males proved to be ready to mate even after many matings and several days. In contrast, in females the willingness to re-mate seems to diminish once some hours have passed, even if they only mated once before (Tuni, Mester, Berger-tal, Lubin & Bilde, in prep.). Males are thus more likely to mate with virgin females than with already mated females, which restricts reproductive skew compared to a scenario in which males are generally attracted to certain females, for example because of size differences among them. The observation that males can mate up to 16 times with up to 11 successful broods might explain why male choosiness is not supported. Even taking the possible intensity of the female-biased ASR into account, the high mating frequency should allow males to fertilize most adult virgin females they encounter. Aiming to mate as often as possible seems thus to be the more promising option to

increase male fitness than restricting their mating possibilities by only fertilizing best suited females.

The above discussed results beg the question: is reproductive skew a flexible trait in spider colonies? Reproductive skew is viewed as a main characteristic of social spider communities and I was able to show that when a cohort of females remains unmated and act as helpers, these virgin females will contribute to colony fitness by providing allomaternal care. However, the observations on the extended period of male maturation and male longevity, as well as on strongly overlapping maturation periods between males and females, suggest that under favorable conditions all females might get the chance to reproduce. Our data thus do not support the idea that the low number of males is directly involved in the formation of reproductive skew. With a surprisingly high remating ability and an average MSR of 12 % within colonies, males should be able to fertilize all females, provided they become adult in time. This finding supports Hamilton's (1967b) theory on sexual skew, which claims that in inbreeding systems males are just abundant enough to fertilize all females. However, if reproductive skew in females is an obligatory trait of spider communities, the above discussed results shift the suspect back to a mainly female driven cause of reproductive skew. Especially when resources are restricted, competition among females before maturation could prevent a subset of females to mature in time for reproduction. If conditions are especially favorable and allow all females to mature in a timely manner, intrinsic or social factors could be responsible for maintaining a basic reproductive skew. Further investigation of the flexibility of reproductive skew in colonies of adult females might provide fundamental insights to identify what determines who becomes a reproducing female and who remains a virgin helper. The low male presence however seems to contribute little to female reproductive role.

4.4 III) LIFE HISTORY

The observations of reproductive traits of mated *S. dumicola* females provide new insights into the reproductive biology of this social spider, which could help to understand the consequences of the cooperative lifestyle. The latency between mating and oviposition showed a high variability similar to that found in *S. lineatus* (Schneider, 1999) while the latency between oviposition and hatching of the offspring is less variable. Interestingly, both latencies differed significantly between the years with females in 2015 ovipositing about ten days later but offspring hatching a few days earlier than in 2014. As animals were kept in similar conditions in the lab in both years, there are two potential explanations for this difference: a) different climatic conditions between the years (e.g. due to the persistent drought in 2015 and decreased insect abundances, as they are linked to rainfall patterns; Wolda, 1978) or b) differences in the location of the collected nests, that might entail climatic and/or genetic differences, as natural nests were collected at different locations between the years. However, in this experiment it was not possible to determine reasons for the observed differences.

Intriguingly, about half of the experimental groups comprising a single mother and virgin female helpers, contained more than one and up to four egg sacs by the end of the reproductive season. This result contradicts the assumption that the number of egg sacs within a group can serve as an indication of the number of reproducing females within colonies. In fact, knowing that groups might contain more egg sacs than reproductive females, the reproductive skew in natural colonies might have been even underestimated in the past. Previously I provided both indirect and direct evidence of virgin egg sac production in *S. dumicola.* This included histological evidence of oocyte maturation in virgins (see results and discussion of topic II) and direct observations of virgin egg sac production in maturing groups of unmated females (see results and discussion of III). However, the present observations did not allow us to distinguish whether the egg sacs had been produced by virgin helpers or by the reproductive female. In the subsocial *S. lineatus*, mothers produce a replacement clutch only when the first one is lost (Schneider, 1999). By opening the unhatched egg sacs in experimental groups of *S. dumicola*, it was possible to determine in 5 cases that the egg sac clearly had been produced by the mated female, as they contained brood that failed only after the spiderlings had developed. This finding indicates that

S. dumicola mothers can produce a replacement clutch in case of a failed brood, just as mothers in *S. lineatus* do. In one case, two unhatched egg sacs containing dead spiderlings occurred in the group in addition to the hatched egg sac, showing that the reproductive female had produced 3 fertilized egg sacs in total. However, it was not possible to clarify whether these unhatched fertile egg sacs were first or second clutches. Surprising reason to doubt that further fertilized egg says in a group were replacement clutches was provided by five experimental groups in which a second egg sac hatched, while the first brood was still being cared for, leading to a second successful reproductive event by the reproducing female. In all those cases, the second clutch had been produced before the first egg sac was due to hatch, indicating that cues alluding to a possible reproductive failure are unlikely to have triggered the additional oviposition event. The latency between the first and the second oviposition event of a reproductive female was as short as 4 days in some experimental groups, including groups with two successful broods as well as groups in which the unhatched egg sac contained dead spiderlings. It is thus reasonable to assume that the groups in which a second fertile egg sac was produced represent part of a reproductive strategy rather than a replacement clutch. Life history strategies deviating from semelparity and including multiple reproductive events might thus be not uncommon in *S. dumicola*. This is especially interesting, as in the subsocial *S. lineatus* semelparity is not plastic (Schneider et al., 2003).

Reports in the literature of how many egg sacs are produced by single mated females in social spiders are mixed. While it has been observed that social spider females never producing more than one egg sac (Rypstra, 1993) others report at least occasional multiple egg sac production (Bradoo, 1973; Vollrath, 1986), although in some cases it was not clear, how multiple reproduction by an individual was investigated (Bradoo, 1973). That helpers can influence a parent's future reproductive success positively is known from fish (Taborsky, 1984). Now, *Stegodyphus dumicola* provides another case, in which females with helpers at the nest are capable of successfully reproducing multiple times, thus employing an iteroparous lifestyle. Multiple egg sac production seems to be a facultative trait, as more than half of the groups contained only a single egg sac at the end of the care period. The factors which determine if a female can reproduce more than once remain unclear. Possibly, resource accumulation before

and during the reproductive season plays an important role. It is assumed that resource allocation during growth determines whether a female becomes a reproducer or a virgin helper (Bilde & Lubin, 2011). The recent findings suggest that the amount of resources a female can accumulate might not only determine if she reproduces at all, but also how often she is able to oviposit. In any case, the option for *S. dumicola* mothers to invest in more than one brood, provides an unexpected additional fitness advantage of cooperative brood care. The reproductive strategy of *S. dumicola* might represent a (facultative) case of within-nest iteroparity as postulated by Trumbo (2013), in which an extended foraging period and allomaternal care reduces the costs of brood care for the mother such that the threshold for reproduction can be met a second time. Social *Stegodyphus* mothers might thus be able to take advantage of especially favorable conditions by increasing reproductive output with a second clutch.

Several authors have found that social spiders produce fewer eggs per egg sac than subsocial species (Avilés et al., 1999; Seibt & Wickler, 1988b; Grinsted et al., 2014). In the present study a mean of 44 spiderlings survived until the end of brood care. Although reproductive success in some experimental groups with only one hatching event was much higher, the two highest numbers of surviving offspring were counted in experimental groups with a second hatching event. Possibly, reproducing females employing an iteroparous strategy can reach a similar total clutch size as the subsocial congener, which is averaged at 80 (Schneider, 1999). However, observations on second hatching clutches were comparably rare in our experiments (in 5 of 87 experimental groups, 5.7 %), posing the question of how frequently this advantage comes into effect in nature. The possibility to increase the number of offspring with a second clutch might only be a secondary advantage of cooperative brood care for reproductive females, in cases when the external conditions are especially favorable. A more frequent advantage of social living becomes apparent when comparing the duration of the regurgitation phase between subsocial and social species. Cooperation in groups of social *S. dumicola* results in a considerably longer care period compared to subsocial species, with a prolonged regurgitation period of 8 to 12

weeks or even longer, compared to 3 weeks in *S. lineatus* (Salomon et al., 2005). In the subsocial species, females experience massive morphological changes during brood care which exhaust the resources of the female rather quickly. Parts of the midgut tissue disintegrate (Junghanns, 2013; Salomon et al., 2015; Nawabi, 1974) and in the midgut region large amounts of extracellular material are accumulated, which the females feed to their young during regurgitation (Kullmann, 1971). In comparison, as has been shown in the present work (results and discussion of topic II), brood care-related morphological changes in mothers of the cooperatively breeding *S. dumicola* are less intense and the midgut tissue of allomaternal virgin females is in a similar condition as in mothers. By distributing the work load of brood care amongst females (Junghanns et al., 2017), individual females can economize on their own resources, allowing for a prolonged care period. At the same time, the cooperation not only in brood care but also in foraging allows females to restock their resources during the regurgitation phase. This might result in a more flexible duration of the regurgitation phase depending on food availability. Only when prey starts to get scarce by the end of summer, females increasingly use up their bodily resources until finally matriphagy takes place. In this way, brood care can be considerably prolonged, increasing the chance of successful survival of the period of food scarcity during winter that the juveniles face after matriphagy.

When benefits of cooperation outweigh the costs of competition over resources and reproduction, group living evolves. Compared to the solitary breeding *S. lineatus*, cooperation in brood care in *S. dumicola* facilitates a prolonged provisioning period that might improve the chances of survival for the fewer offspring (Salomon & Lubin, 2007). The new results add an unsuspected benefit to cooperative brood care: allomaternal help enables mothers to change their reproductive strategy from semelparous to iteroparous reproduction. With multiple clutches and an enhanced care for offspring, reproducing individuals of the cooperative breeding *S. dumicola* might indeed be able to increase their fitness compared to solitary breeders, despite the lower egg number per egg sac (Avilés et al., 1999). The potential for elevated reproductive success in group breeding females and at the same time low chances of successful solitary reproduction (Henschel, 1995; Bilde et al., 2007) favor cooperative breeding in S. dumicola. While mothers increase their reproductive success in groups, virgin females benefit from increased

inclusive fitness, overcoming the low chances of mating and reproducing outside the natal group. Although facultative iteroparity is not a cause, but rather a consequence of cooperative breeding, it leads to increased benefits of helping behavior, thus representing a mechanism that helps to maintain and strengthen the social lifestyle in *S. dumicola*.

5 Summary

Most animals live solitarily, but for some species the benefits of group living outweigh the costs and social communities have evolved. Truly social societies are characterized by cooperation in tasks like foraging, predator defense and brood care. In the most extreme cases, non-reproducing individuals act as helpers and provision offspring of reproducing individuals at the cost of their own reproductive success. This alloparental care is attributed to kin selection that provides the helpers with inclusive fitness benefits. However, how reproductive role is determined and in which ways virgin helpers in a group benefit the community is not always well understood.

Spiders are known to be generalist hunters, which in many cases do not shy away from cannibalism. Thus, most spiders live solitarily. However, in a few species a permanently social lifestyle has evolved in which individuals live together throughout their life, providing an intriguing case of social evolution. These spider communities are characterized by lack of premating dispersal leading to extreme inbreeding, by reproductive skew, in which only a proportion of females reproduce and by cooperative breeding of the reproducing females. It has been assumed that the large proportion of virgin females act as helpers not only in foraging and web maintenance but also during brood care. In the social spider *Stegodyphus dumicola* brood care involves the intensive task of regurgitation feeding, at which mothers regurgitate their own liquefied body tissue. At the end of brood care, the offspring sucks the mothers dry during matriphagy, leading to the death of brood caring females and a semelparous lifestyle. In the closely related solitarily breeding *Stegodyphus lineatus* virgin females do not provide brood care. The ability of virgin females in *S. dumicola* to care for offspring would thus depict an adaptation to sociality and cooperative breeding. I therefore aimed to clarify the role and significance of virgin females in colonies of social spiders and furthermore investigated a possible mechanism of how reproductive role within a colony is determined.

I investigated whether there is differential task participation in a non-reproductive task and the task of brood care among reproducing mothers and virgin females (helpers) in *Stegodyphus dumicola.* The study provides explicit evidence that brood care – including egg sac care,

regurgitation feeding and matriphagy – is performed by mothers as well as by virgin helpers. Virgin females in a colony can thus rightfully be termed allomothers. However, the task participation differed between the reproductive states. While mothers engaged more often in brood care, virgin females were more active in foraging. However, the active provisioning of offspring by the virgin females decreases the motherly workload as is suggested by the extended brood care period in comparison to solitary breeders. The observations on virgin allomaternal care are supported by histological studies on the midgut tissue of brood caring females, which revealed that mothers and virgin helpers undergo comparable morphological changes in preparation of regurgitation feeding. The changes in virgin females correlate to ovarian development that might depict an internal maturation process which sets virgin females in the right state to provide care. The morphological changes in mothers and virgin helpers of *S. dumicola* are less comprehensive than in the solitarily breeding *S. lineatus* mothers. This indicates that cooperatively caring females are able to save on their resources, provision offspring for longer and thus are probably able to increase survival of the brood by an extended care period. A surprising consequence of cooperative brood care is the ability of mothers to produce a second viable egg sac, even when the first brood is successful. Mothers of the cooperative breeding *S. dumicola* can thus depart from the strictly semelparous lifestyle and instead invest part of their resources in a second clutch. This finding identified a new way of how cooperative breeding enhances breeding success of reproducers and thus inclusive fitness for helpers as well, thus adding to the benefits of allomaternal care.

Virgin females did not store significantly lower amounts of lipids in their midgut tissue than mothers, raising the question of how much reproductive role of females is determined by competition for resources during growth, as often assumed. Another possible determinant of female reproductive skew is the characteristic male scarcity in spider colonies, with only about 12 percent of spiders being male. Males are assumed to mature early within a few days and die early, thus leaving late maturing females unmated due to lack of mating partners. However, my studies provided evidence that male maturation is more skewed than expected and males might survive several months. Subadult females did not accelerate molting when an adult male was present, which could further indicate, that male presence is not a limiting factor on reproduction

in males. Furthermore, males are able copulate with up to 16 females and did not show e preference for large females during mating trials. Males are thus able to fertilize all females, provided all females mature in time. I therefore suggest, that male scarcity is not major determinant of reproductive skew in females, especially in small and middle-sized colonies in which female maturation might only be moderately skewed.

My studies were able to demonstrate the meaning of the large proportion of unmated females in a colony of the social spider *S. dumicola*. Virgin helpers support mothers during brood care and thus do not only enhance the brood care period but facilitate mothers to produce multiple clutches. Virgin females are able to care as they undergo similar morphological changes as mothers' do. This seems to be facilitated by an internal maturation process,

indicated by ovarian development and oviposition by virgin females, both of which has never been observed in virgins of the subsocial species. How reproductive role is determined remains unclear, but I was able to exclude male scarcity as a major factor influencing reproductive skew.

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7 List of definitions

Colony:

entirety of spiders that inhabit a single nest

Nest:

dense and roundish web structure used as a retreat by the colony

Capture web:

two-dimensional sheet web for prey capture

Subsocial spiders /subsociality in spiders:

Solitarily breeding spiders; refers to solitary spiders that in which the mother and offspring cooperate during a period of extended brood care

Social spiders /sociality in spiders:

Cooperatively breeding spiders; refers to permanently social spiders that live in groups throughout their life and cooperate in different tasks

Mother / reproducer:

mated female in experimental colonies

Allomother / virgin helper:

Virgin females cohoused with mated females and offspring in experimental colonies

Virgin / Virgin non-helper:

Virgin females reared separate from males, mothers or offspring in the lab

Regurgitation feeding:

food provisioning by regurgitation of a nourishing fluid

Regurgitant:

extracellular material that is used for regurgitation feeding, presumably consisting of liquefied body parts

(allo)Matriphagy:

“mother-eating”; process at the end of brood car, at which offspring eat the mother (or virgin helper)

Midgut gland:

midgut branches and interconnecting interstitial tissue in the opisthosoma

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.
Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Own Contributions to Thesis

The present thesis includes data that is part of two manuscripts of which I am the first author.

The parts of this thesis on TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES are based on the first manuscript “Extreme allomaternal care and unequal task participation by unmated females in a cooperatively breeding spider”, which has been published in Animal Behaviour.

The parts of this thesis on MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE are based on the second manuscript “Physiological adaptations to extreme maternal and allomaternal care in spiders”, which is submitted to Functional Ecology.

The following list provide estimates of the proportions of my own contributions to the chapters of the present thesis considering the contributions of the co-authors to the manuscripts.

I TASK PARTICIPATION BY VIRGIN AND REPRODUCTIVE FEMALES
Conception: 50 %
Execution: 50 %
Writing: 85 %

II MORPHOLOGICAL ADAPTATIONS TO COOPERATIVE BROOD CARE
Conception: 20 %
Execution: 90 %
Writing: 90 %

III LOW MALE PRESENCE AND ITS IMPLICATIONS ON REPRODUCTIVE SKEW
Conception: 100 %
Execution: 95 %
Writing: 100 %

IV LIFE HISTORY
Conception: 100 %
Execution: 95 %
Writing: 100 %

The above stated information is confirmed:

Candidate:_____ Academic Supervisor:_____

Erklärung zur Abgabe einer elektronischen Kopie der Dissertation

Mathematisch-Naturwissenschaftliche Fakultät
Einverständniserklärung nach § 4 Abs. 1 Nr. c Promotionsordnung

Hiermit erkläre ich, dass von der Arbeit eine elektronische Kopie gefertigt und gespeichert werden darf, um unter Beachtung der datenschutzrechtlichen Vorschriften eine elektronische Überprüfung der Einhaltung der wissenschaftlichen Standards zu ermöglichen.

Datum:

Unterschrift:

Curriculum Vitae

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academic career

Since 04/2014	PhD studies: Evolution of Sociality: mechanisms and dynamics of social b behavior in spiders" at Greifswald University
01/2014	diploma thesis: "Histologische Untersuchungen der Matriphagie bei der Röhrenspinne *Stegodyphus lineatus* (Latreille, 1817)" ("Histological investigations of matriphagy in the velvet spider *Stegodyphus lineatus* (Latreille, 1817)"
10/2008 - 01/2014	Diploma studies "landscape ecology and nature conservation" at Greifswald University
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05/2018	Kinder- und JugendUNI at the University Greifswald/ Germany "Einblicke in die faszinierende Welt der Spinnen"
09/2017	110th Annual Meeting of the German Zoological Society (DZG), Bielefeld /Germany. "Extreme allomaternal care by unmated females in a cooperatively breeding spider"
06/2016	Tag der Wissenschaft (Science Day) at the University of Greifswald /Germany "Von Gruppenkuscheln bis Muttermord – aus dem faszinierenden Leben einer sozialen Spinne"
08/2015	European Congress of Arachnology in Brno /Czech Republik "Reproductive role shapes task differentiation in a social spider"

07/2015	International Course “Advanced Behavioural Ecology” of the universities of Hamburg, Aarhus and Greifswald in Peevesdorf /Germany “Histological investigation of extreme maternal brood care in the subsocial spider *Stegodyphus lineatus*”
08/2014	European Congress of Arachnology in Turin /Italy “Histological examination of the mechanisms and dynamics of maternal care in the subsocial spider *Stegodyphus lineatus* (Araneae, Eresidae)”
02/2014	University Aarhus /Denmark “Histological dynamics of the midgut during maternal care in the subsocial spider *S. lineatus*”
12/2013	University Pretoria /South Africa “The evolution of cooperation and allo-maternal care”
05/2013	University Aarhus /Denmark “Histological investigation of matriphagy in a subsocial spider”

publications

submitted	**Junghanns, A**., C. Holm, Schou, M. F., Overgaard, J., Malte, H., Uhl, G. and Bilde, T. Physiological adaptations to extreme maternal andallomaternal care in spiders. The American Naturalist.
2018	Schendel V., **Junghanns A**., Bilde T. and Uhl G. (2018). Comparative female genital morphology in *Stegodyphus* spiders (Araneae: Eresidae). Zoologischer Anzeiger - A Journal of Comparative Zoology. DOI: 10.1016/j.jcz.2018.01.011
2017	**Junghanns A**., Holm C. *****, Schou M. F., Sørensen A. B., Uhl G. and Bilde T. (2017). Extreme allomaternal care and unequal task participation by unmated females in a cooperatively breeding spider. Animal Behaviour 132: 101-107. *shared first authorship/°shared last authorship
2014	Schmidt V., Mock R., Burgkhardt E., **Junghanns, A**., Ortlieb F., Szabo I., Marschang R., Blindow I. and Krautwald-Junghanns M.-E. (2014): Cloacal aerobic bacterial flora and absence of viruses in free-living Slow Worms (*Anguis fragilis*), Grass Snakes (*Natrix natrix*) and European Adders (*Vipera berus*) from Germany. EcoHealth. DOI: 10.1007/s10393-014-0947-6.
2013	Jährling F., **Junghanns, A.** and Ortlieb F. (2013). *Zootoca vivipara* (Common Lizard). Record Weight. Herpetological Review 44(3): 517.

Grands, scholarships, stipends

12/2017	German Academic Exchange Service (DAAD) for Aarhus
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09/2016	German Academic Exchange Service (DAAD) for Aarhus
12/2015	German Academic Exchange Service (DAAD) for Aarhus
2015	Student Grant of the Organising Committee of the 29th European Congress of Arachnology
2014	Student Grant of the Organising Committee of the 28th European Congress of Arachnology
2014 to 2016	State Mecklenburg-Vorpommern, Germany State Scholarship for PhD studends
2013	University Aarhus, Denmark employment as field assistent in South Africa
2013	Forschungsnetzwerk Ostseeraum, University Greifswald, Germany financial support of the project work on the subsocial spider *Stegodyphus lineatus* at the University of Aarhus

languages

German	mother tongue
English	business fluent
French	basic knowledge
Spanish	basic knowledge

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