

## Dramatic histological changes preceding suicidal maternal care in the subsocial spider *Stegodyphus lineatus* (Araneae: Eresidae)

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**Abstract.** Parental care entails physiological costs to the mother. These costs, even if dramatic, are usually reversible and do not result in mortality of the mother. In the spider *Stegodyphus lineatus* Latreille 1817 (Eresidae), maternal care is extreme and irreversible: mothers regurgitate food for the young and then die when consumed by them (matriphagy). We examined whether the mother's midgut tissues undergo structural changes in preparation for regurgitation and matriphagy. Our histological data show that the midgut diverticula (MD) tissues start to degrade during the egg sac incubation period. When the young emerge from the egg sac, the midgut tissues are partly liquefied and are retained within the MD. The degradation process intensifies when the female feeds her young by regurgitation and liquid tissue is observed within and among the diverticula lobes. The presence of the lumen of a diverticulum during the regurgitation process suggests that degenerated tissues enter the lumen and form the regurgitated fluid. At matriphagy, the abdomen is filled with liquid containing nutritional vacuoles, which the young imbibe after piercing the female's abdomen. We conclude that the MD undergoes a gradual degradation process that maximizes the nutritional potential of the female's body and finally enables complete consumption of her soma. These changes are consistent with the extreme semelparous reproductive system of *S. lineatus*, where a female invests all of her resources into a single reproductive event. This is the first demonstration of the mechanism underlying suicidal maternal care in an arthropod.

**Keywords:** Matriphagy, midgut diverticula, nutritional vacuoles, regurgitation, tissue degradation

Raising offspring is costly, and investment in parental care may be traded off against future reproduction (Fox & Csezak 2000). A major cost of parental care is the physiological burden on the mother. Maternal care in spiders may be transient or extended (Yip & Rayor 2013). Transient maternal care is widespread in spiders and is mainly restricted to protection of egg sacs or of the young until dispersal shortly after emergence (Lubin & Bilde 2007). Extended maternal care of offspring after emergence from the egg sac (subsocial behavior) has evolved multiple times, and occurs in at least 65 species in 16 families (Yip & Rayor 2013). The behaviors include feeding the young with captured prey (Gundermann et al. 1988; Schneider 1996), producing trophic oocytes (Gundermann et al. 1991; Evans et al. 1995), regurgitating food for the young (Kullmann & Zimmerman 1975) and matriphagy, i.e. the consumption of the mother by her young (Seibt & Wickler 1987; Kim & Horel 1998; Kim et al. 2000). While transient maternal care does not restrict the production of additional broods, extended maternal care, such as providing prey for the young, regurgitation feeding, and production of trophic eggs involve prolonged association between the mother and her brood and may constrain the female to a single clutch (Yip & Rayor 2014). Matriphagy is an extreme form of maternal investment and is an irreversible dead-end for the mother that precludes the possibility of future reproduction. Do such constraints on future reproduction

involve changes in physiology and internal anatomy that at some stage become irreversible?

Female *Stegodyphus lineatus* Latreille 1817 (Eresidae) exhibits intensive maternal care for newly emerged young, first by regurgitating liquid food and later by allowing matriphagy. Females lay a single clutch of ~80 eggs that constitutes less than 3% of the body mass of the mother at oviposition (Schneider 1996), and will lay a second clutch of eggs only if the first is lost due to predation or male infanticide (Schneider & Lubin 1997a). After emergence of the young, the female provides the young with regurgitated fluid (Kullmann & Zimmermann 1975). Young of females fed with radioactive flies became radioactive a few days after emergence, indicating a transfer of digested prey from the mother to her young (Kullmann 1972). After two weeks of regurgitation feeding, the young climb on the mother's body and consume her (matriphagy; Seibt & Wickler 1987); within 2–3 hours they extract her body fluids leaving behind only a dry exoskeleton (Salomon et al. 2005). In this species, the mother ceases to eat after the young emerge. She does not provide prey to the young and the young are incapable of catching and ingesting prey independently, and thus they will die without her regurgitated fluid (Salomon & Lubin unpubl. data). Therefore, the body mass of the female at the emergence of the young constitutes all the food available for the young during maternal care. During regurgitation, the female loses 41% of her body mass, while in matriphagy, the young consume an additional 54% of her body mass measured at emergence of the young. Thus a total of 95% of her body mass is provided to the young as food (Salomon et al. 2005). These maternal care behaviors lead to a threefold increase in the body mass of

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the young from emergence until dispersal (Salomon et al. 2005). After matrophagy, the young remain in the maternal nest for approximately two weeks, undergo another molt, and only then start to disperse gradually (Aviram 2000).

Provisioning of food by regurgitation and matrophagy are linked to the reproductive stage of the female. Schneider (2002) showed that unmated *S. lineatus* females and females after egg-laying will not regurgitate food to foster young, but once females have young of their own, they will feed foster young of other females. One possible interpretation of this finding is that females are physiologically incapable of regurgitating to the young if they are not in the appropriate reproductive stage, namely with young of their own. Matrophagy, like regurgitation, occurs only when the female is in the right reproductive stage. In the spider *Amaurobius ferox* Walckenaer 1820 (Amaurobiidae), matrophagy is initiated only after reciprocal communication between mother and young through web vibrations (Kim & Horel 1998). Females after egg-laying and females with young, but not virgin females, were all consumed when experimentally introduced to foster young ready for matrophagy. However, only females before matrophagy exhibited the web vibration behaviors that activated the young and directed them towards her abdomen (Kim & Horel 1998). The evidence that both regurgitation feeding and matrophagy are closely linked to the female's reproductive stage suggests that internal changes are necessary well in advance to prepare the female for these functions. In the present study we asked: What are these changes and when do they occur in the reproductive sequence from maturation to matrophagy?

We used histological sections to examine the changes occurring in the opisthosoma (abdomen) of *S. lineatus* females at different reproductive stages. The digestive system of arachnids is divided into foregut (pharynx and esophagus), midgut, and hindgut (Bertkau 1885, cited in Ludwig & Alberti 1988; Collatz 1987; Klann & Alberti 2010). The midgut of spiders consists of the midgut tube and numerous blind branches called 'midgut diverticula' (henceforth, MD), located mostly in the opisthosoma (Ludwig & Alberti 1988, 1990; Laino et al. 2009). These diverticula function as a digestive and storage organ (Collatz 1987). The MD consists of a dimorphic epithelium containing secretory and digestive cells (also called 'resorption cells') connected by intermediate tissue (Bertkau 1881, 1885, cited in Ludwig & Alberti 1988; Nawabi 1974; Ludwig & Alberti 1990). Spiders use extra-oral digestion to obtain nutrients from prey, regurgitating an enzymatic digestive fluid into the body of the prey, and then sucking out the liquefied tissues (Collatz 1987; Cohen 1995). The secretory cells containing the digestive fluid empty their content into the lumen of the MD during feeding (Nawabi 1974 for *Stegodyphus pacificus* (Pocock 1900)). One hour after feeding, the digestive cells are filled with nutritional vacuoles containing freshly ingested nutrients (Nawabi 1974; Ludwig & Alberti 1988 for *Coelotes terrestris* (Wider 1834) (Agelenidae)). Segregation of nutrients occurs within these vacuoles, forming droplets of lipids and glycogen that are then extruded from the nutritional vacuoles. Most of the lipid and glycogen droplets are transported to the intermediate tissue for storage (Nawabi 1974; Ludwig & Alberti 1988). Thus the MD is responsible for absorption, synthesis and storage of lipids, and the transfer of

energy (Laino et al. 2009). Nawabi (1974) studied the histology of the digestive system of *S. pacificus* females. In her unpublished M.Sc. thesis, Nawabi detailed changes in specific MD tissues (secretion and resorption cells, intermediate tissue and lumen) over periods of time post-feeding. She noted that at the stage of regurgitation-feeding of the young, the midgut tissue underwent a process of degradation, and suggested that these tissues were irreversibly transformed into liquid regurgitate for the young. In the present study, we recorded the sequence of changes occurring during different stages prior to and during maternal care. In particular, we focused on the process of breakdown of opisthosomal MD tissue during early stages of maternal care, in order to gain insight into the timing of changes in relation to regurgitation feeding and matrophagy.

## METHODS

**Experimental set-up.**—Juveniles, adult females and females with egg sacs of *S. lineatus* were collected from field populations near Lehavim (31° 22' 04.08"N, 34° 49' 41.9"E) and in Shagririm forest (31° 19' 28.45"N, 34° 46' 44.36"E), Israel. Spiders were maintained at 25–26° C, 14L:10D cycle, and were fed lab-reared adult crickets (*Acheta domestica* Linnaeus 1758) once a week. To examine the MD of females before and during maternal care, we classified females into six reproductive stages (Salomon et al. 2005): 1) sexually mature virgin, 2) post-mating (1 week after mating), 3) mid-egg incubation period (15 days after egg laying), 4) emergence day of the young, 5) mid-regurgitation period (6 days after emergence of the young), and 6) pre-matrophagy (12 days after emergence of the young). Juveniles were raised separately to adulthood in the lab in order to obtain virgin females.

**Histological sections.**—Three to four females of each stage were sacrificed in order to prepare histological sections. Females of stages 1–4 were sacrificed  $3 \pm 2$  days after feeding. Females in stages 5 and 6 were not fed as they do not capture prey at these stages. Sections from different females of a given stage were checked for consistency. We anesthetized the females with CO<sub>2</sub>, made longitudinal incisions in the cuticle of the opisthosoma, and fixed the spiders in 10% formalin. They were then dehydrated gradually in a series of increasing ethanol concentrations and the opisthosoma was cleared and embedded in paraplast (Paraplast Plus: Kendall, Tyco Healthcare, Mansfield, MA). Cross sections (thickness, 5 µm) were cut onto silane-coated slides (Superfrost plus; Menzel-Gläser, Braunschweig, Germany). With the use of a razor blade, we dissected the opisthosoma of each female into three parts; anterior, median and posterior regions. Five-micron sections were obtained from the anterior and posterior parts while the median parts were sectioned at least 750 microns before collection of sections for staining. All the sections were prepared from anterior, median, and posterior regions of the opisthosoma of each female and stained with Hematoxylin-Eosin. Hematoxylin has a blue-purple color and stains acidic structures such as nucleic acids, while eosin is pink and stains proteins nonspecifically, thus staining a variety of structures in the cell (Fischer et al. 2008). Structures in the histological sections were distinguished based on the literature (Nawabi 1974; Ludwig & Alberti 1990, 1992; Ludwig et al. 1994). Lipid droplets remain unstained due to their extraction by the

ethanol dehydration. Variation in the details of the cross sections among the 3–4 females examined within each stage was minimal, thus allowing us to generalize from the observations. The sections were examined using a Nikon eclipse E600 light microscope and photographed with a Nikon digital camera DXM1200.

## RESULTS

The histological sections showed that changes occurred in the midgut diverticula (MD) tissue as female reproduction progressed from mating to caring for the young. Figures 1–3 show respectively, the MD tissues as seen under different magnifications, the polarity of changes in the MD with respect to the longitudinal axis of the opisthosoma, and changes in the lumen and other tissues.

**Prior to maternal care.**—In sexually mature females, the MD were intact and filled up the entire opisthosoma (Fig. 1A). The midgut lumen was surrounded by well-defined, organized lobes (Ludwig et al. 1994) filled with nutritional vacuoles (after Nawabi 1974) and lipid droplets (distinguished by their round, empty form) occur within and among the diverticula lobes (Fig. 1B, C). After mating, the MD remained clearly visible and contained many nutritional vacuoles surrounding the distinct diverticula lobes (Fig. 1D–F). Within each lobe, some islets of cells stained dark blue while others stained pink (Fig. 1E, F). Tissue degeneration began in the middle of the egg incubation period. Although there was still a clear border between the diverticula and the intermediate tissue, the cell boundaries within both tissues became blurred as the cells and vacuoles started to dissolve (Fig. 1G–I). There was a visible reduction in the number of lipid droplets at this stage.

**During maternal care.**—At emergence of the young, the borders of the MD became blurred as it continued to degenerate (Fig. 1J–L). The degenerating diverticula and intermediate tissue with their cells and vacuoles were all condensed into an undifferentiated, liquid tissue that was accumulating among the lobes. The pattern of tissue degeneration was unevenly distributed within the opisthosoma. The tissues began to dissolve primarily at the perimeter of the opisthosoma (Fig. 1J, 2B), near the cuticle, but not interiorly (Fig. 1K). Lipid droplets were apparent among the dissolved MD lobes (Fig. 1L). Vital organs within the body such as the heart and the intestine were unchanged at this stage.

In the middle of the regurgitation period, more of the MD tissue degenerated and fluid containing nutritional vacuoles was apparent (Fig. 1M–O). Tissue degeneration at this stage resulted in a distinct separation between liquefied tissue and the remaining cellular tissue of each diverticulum within the opisthosoma (Fig. 1N). Contrary to the previous stage where the dissolved tissue was apparent only at the perimeter of the opisthosoma (Fig. 2B), during the regurgitation process, the interior lobes of the opisthosoma were also dissolved, creating patches of liquefied tissue among the dissolving lobes (Fig. 1M). Interestingly, the diverticula lobes surrounding the heart remained intact at this stage (Fig. 1M). Similarly, the muscles, ovaries, and the midgut tube remained intact (Table 1; not shown). The extra-cellular nutritional vacuoles within each degenerating lobe appeared much larger (Fig. 1O)

than those at the previous stage (Fig. 1K). The MD lobes that had not dissolved had few lipid droplets and nutritional vacuoles (Fig. 1O).

A few days before matrophagy, the remaining MD tissues appeared granular and condensed, surrounding the still functional heart while the perimeter of the opisthosoma was filled with liquid that contained large nutritional vacuoles (Fig. 1P–R). Few lipid droplets were still observed within the remaining MD tissue.

In addition to the differential degeneration of the MD seen in the median cross-sections of the opisthosoma, there was also an anterior-posterior polarity in the degradation pattern. At emergence of the young, the MD tissues started to dissolve at the median area of the opisthosoma followed by the tissue at the posterior end, while most of the MD in the anterior part of the opisthosoma remained intact (Fig. 2A–C). During regurgitation, the main body of liquid was concentrated at the posterior rather than the median part of the opisthosoma, while almost no liquid accumulation was observed in the anterior part (Fig. 2D–F). A few days before matrophagy, the anterior, median, and posterior parts of the opisthosoma appeared similar in the level of degradation of the MD lobes (Fig. 2G–I). In all sections, liquid containing nutritional vacuoles was apparent in the perimeter of the section, while the interior contained the remaining MD tissue.

The presence of the lumen of the diverticulum may indicate whether the MD is still functional in the transfer of nutrients through the different stages of maternal care. In sexually mature females, the lumen appeared full of liquid at the median section and empty in the posterior section (Fig. 3A, B). This difference can stem from different ingestion stages of nutrients between the median and posterior sections, but it is clear that the lumen is functional in both areas. The lumen was clearly visible as tissue degeneration began during egg incubation and emergence of the young (Fig. 3C–F). The apparent absence of the lumen in the median section during the egg incubation period may result from the orientation of the section (Fig. 3C), but it was observed after the emergence of the young (Fig. 3E). During the regurgitation stage the lumen was clearly visible in the posterior but not the median section (Fig. 3H), but was not observed before matrophagy in either the median or posterior sections (Fig. 3I, J).

## DISCUSSION

Distinct histological changes occurred in the MD of *S. lineatus* females before and during maternal care. The degradation of the MD was gradual, starting after oviposition and ending at matrophagy. However, this degradation process was highly selective; organs, including the ovaries, did not degenerate until after the middle of the regurgitation period. Table 1 summarizes the changes that occurred in different tissues and organs during maternal care.

**Prior to maternal care.**—In sexually mature females, the MD filled the opisthosoma and surrounded organs such as the heart and ovaries. This finding is in accord with previous work indicating that the MD functions as a nutrient storage site (Nawabi 1974; Ludwig & Alberti 1988, 1990). Indeed our histological sections of sexually mature females show many lipid droplets occurring among and within the MD lobes.



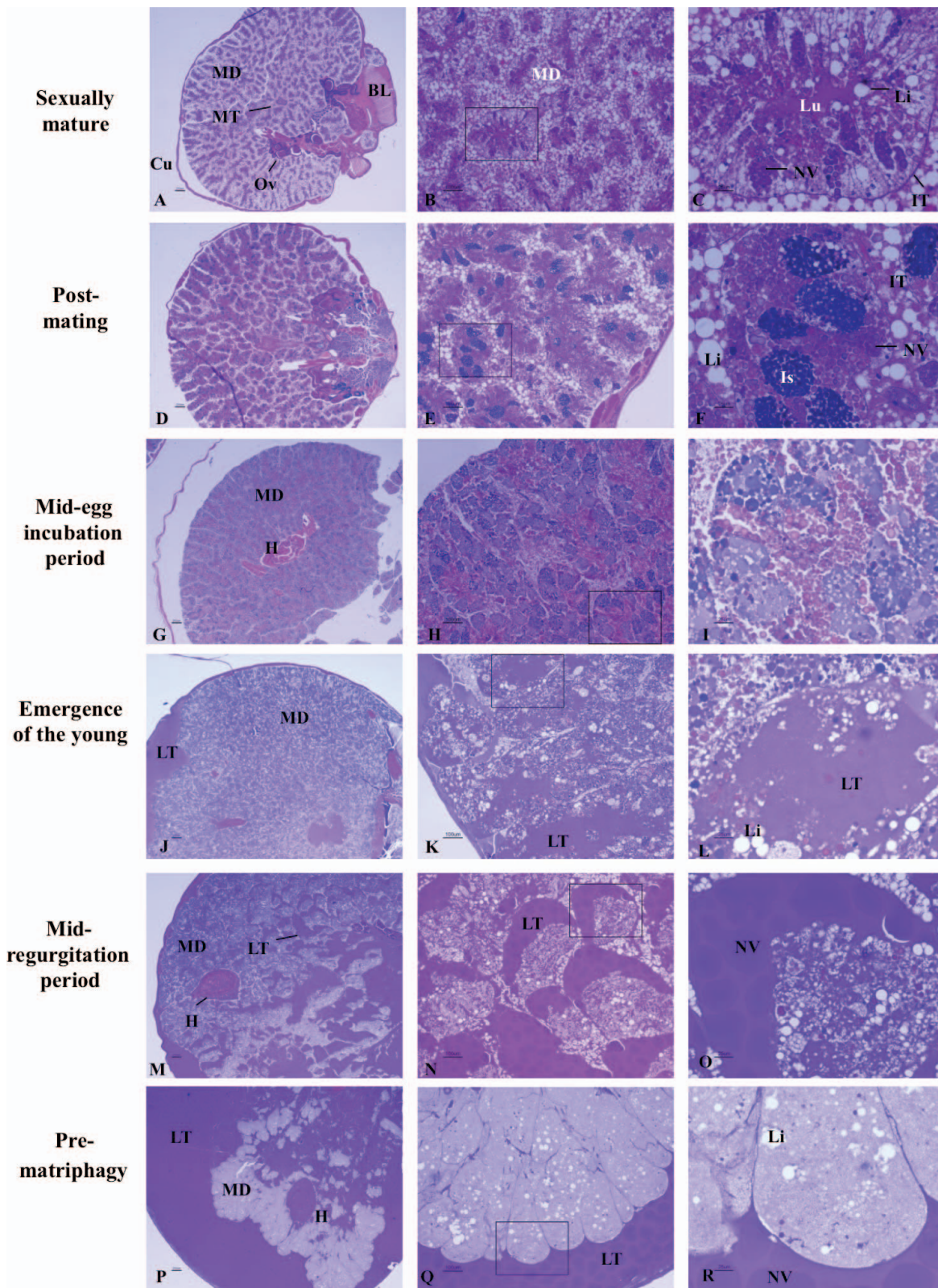


Figure 1.—Light microscopy of median cross-sections in the opisthosoma of *Stegodyphus lineatus* females during reproduction and maternal care. A–C: Sexually mature, virgin female; D–F: Female after mating. Note the dark-blue islets. See text for explanation. G–I: Female during egg incubation; J–L: Female at emergence of the young; M–O: Female halfway through the regurgitation phase; P–R: Female before matriphagy. The magnification of the first image of each stage (left column) is  $\times 2$  while the following image (middle column) is  $\times 10$ . Boxed areas (right column) are enlarged at  $\times 40$  magnification. The bar represents  $100\ \mu\text{m}$ . Dorsal side facing left. *Cu*: Cuticle; *BL*: Book Lung; *H*: Heart; *Is*: Dark blue islets; *IT*: Intermediate tissue; *Li*: Lipid; *LT*: Liquefied Tissue; *Lu*: Lumen; *MD*: Midgut Diverticula; *MT*: Midgut Tube; *NV*: Nutritional vacuoles; *Ov*: Ovaries.



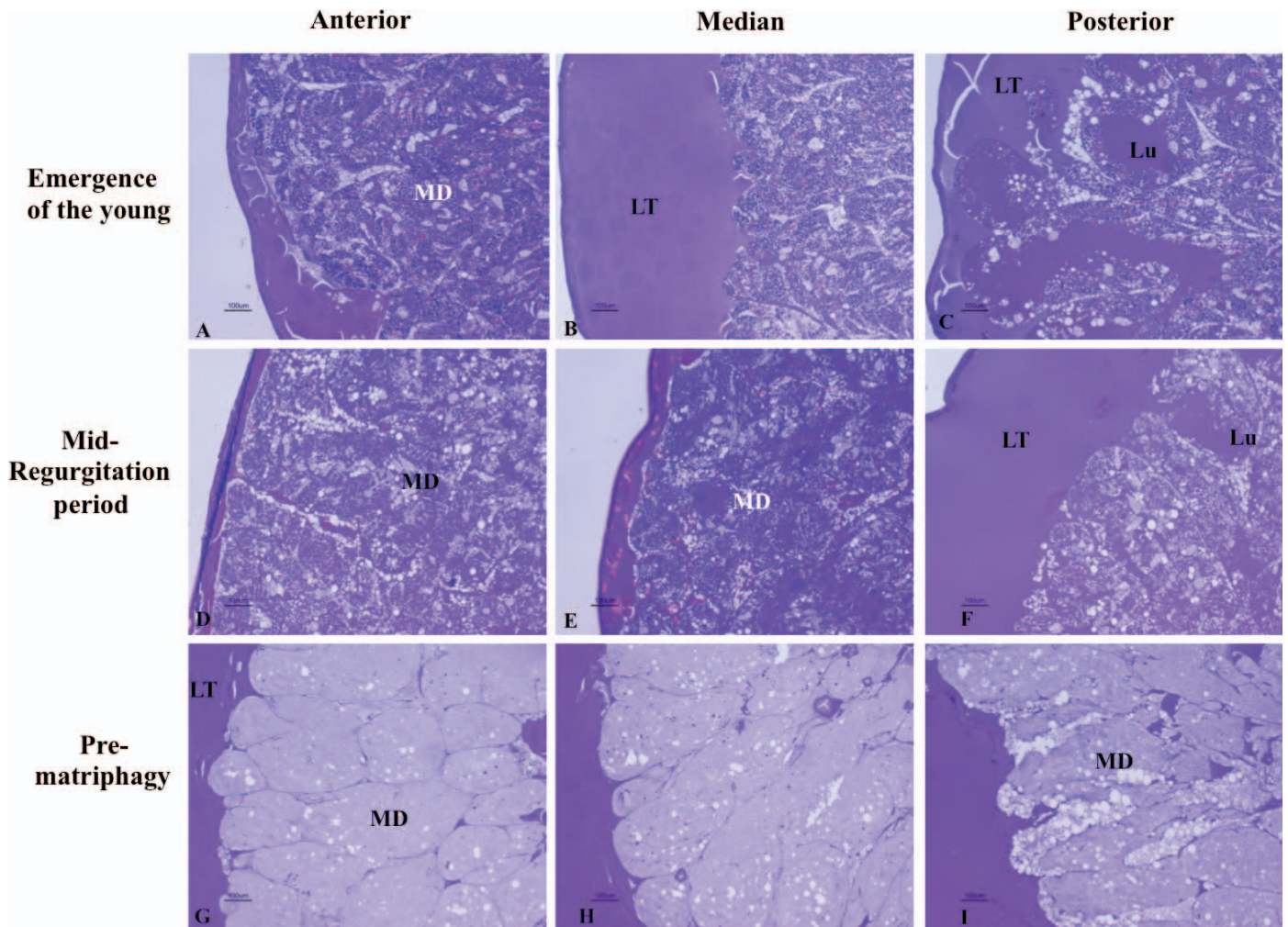


Figure 2.—Light microscopy of cross-sections showing the dorsal side of the opisthosoma of females during maternal care. A–C: Female at emergence of the young; D–F: Female half way through the regurgitation phase; G–I: Female before matiphagy. Images in the left, middle and right columns show anterior, median, and posterior cross-sections, respectively (magnification  $\times 10$ ). The bar represents 100  $\mu\text{m}$ . Dorsal side facing left. MD: Midgut Diverticula; LT: Liquefied Tissue.

After mating occurs, the structure of the MD remains intact, but the increased dark-blue (basophilic) staining (dark blue islets) observed may indicate increased presence of ribosomal RNA (di Fiore & Eroschenko 2005). The secretory cells of the MD contain a voluminous, rough endoplasmic reticulum and are characterized by dense granules containing digestive enzymes (Ludwig & Alberti 1990). Thus the dark-blue staining observed in females after mating may indicate increased gene transcription for the production of digestive enzymes (Collatz 1987). We suggest that mating triggers an increased production of digestive enzymes, allowing the female to ingest more nutrients before the young emerge. The timing of these processes corresponds to the period of greatest availability of prey in the spring (Salomon & Lubin unpubl. data). Finally, increased enzymatic activity may be linked as well to the start of the process of MD degradation.

While guarding the egg sac, the female continues to renew her capture web and catch and consume prey (Salomon & Lubin unpubl. data). At this stage the cells and vacuoles are blurred as they start to dissolve within the MD. According to

Nawabi (1974), the secretion cells dissolve first, merging their content into the lumen, followed by the digestive cells which deposit their nutritional vacuoles in the intermediate tissue and are then dissolved into the lumen. Consequently, we suggest that nutrients from prey items consumed during the egg incubation period are stored in the intermediate tissue and at a later stage dissolved to constitute the regurgitation fluid accumulating in the lumen together with the degraded secretory and digestive cells in the lumen. The reduced amount of lipid droplets that we observed at this stage may be explained by their having been deposited in the eggs (Laino et al. 2013).

**During maternal care.**—After the young emerge, the female does not renew her capture web and does not consume prey even when offered under laboratory conditions (Salomon & Lubin unpubl. data). It is not clear from the work of Nawabi (1974) when the digestive cells degenerate, but it seems likely that they absorb nutrients from ingested prey until the emergence of the young. At this stage, we show that the MD lobes in the perimeter of the opisthosoma are

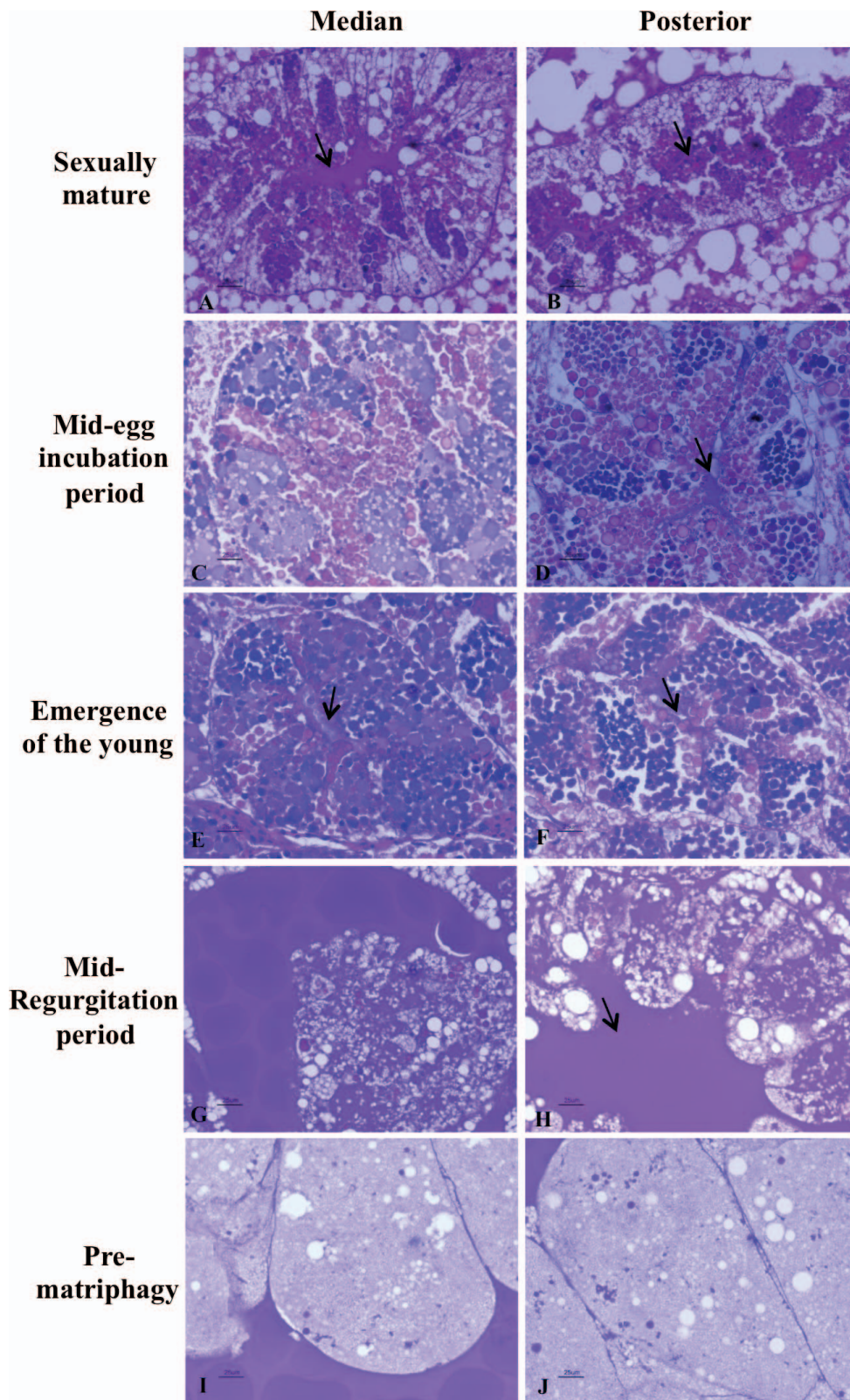


Figure 3.—Light microscopy of cross-sections showing the dorsal side of the opisthosoma of females during maternal care. A, B: Sexually mature female; C, D: Female during egg incubation; E, F: Female at emergence of the young; G, H: Female halfway through the regurgitation phase; I, J: Female before matriphagy. Images on the left are median cross-sections, while those on the right are posterior cross-sections (magnification  $\times 40$ ). The lumen is indicated by a black arrow. The bar represents  $100\ \mu\text{m}$ .



Table 1.—A summary of the change of key players in the opisthosoma during maternal care. ✓ stands for 'present.' Observations of the heart, midgut tube, ovary and MD lumen are not always seen in the representative Figures 1–3.

	Heart	Midgut tube	Ovary	MD- lobe degradation	MD lumen	MD nutritional vacuoles	Lipid droplets	Accumulation of liquefied tissue
Sexually mature	✓	✓	✓	Intact & organized lobes	✓	Within the lobe	Within & among the lobes	Not observed
Post-mating	✓	✓	✓	As above	As above	Within the lobe	✓	Not observed
Mid-egg incubation	✓	✓	✓	Blurred boundaries	Barely seen	Within the lobe	Few observed	Not observed
Period	✓	✓	✓	Degraded lobes	Not observed	Observed in the liquefied lobe	Observed among the lobes	Observed near the cuticle
Emergence of the young	✓	✓	Degraded	Degradation intensifies	Not observed	Large vacuoles appear in each lobe	Few observed	Observed near the cuticle & among the lobes
Mid-regurgitation	✓	✓	Not observed	As above	Not observed	Occur in the liquefied tissue	Few observed	Occupies most of the perimeter of the abdomen
Pre-matriphagy	✓	Not observed	Not observed					

dissolved and liquid containing nutritional vacuoles starts to accumulate.

The ovaries are still intact at emergence of the young and even during the regurgitation period (Table 1; not shown), but are no longer visible just before matriphagy. These results are in agreement with a study showing that when all but five spiderlings were removed two days after they emerged, *S. lineatus* females were still able to produce a new clutch of eggs, but females did not produce a new clutch after a similar reduction five or ten days after emergence (Schneider et al. 2003). We suggest that once the female begins regurgitation feeding and tissue degradation intensifies, the window of opportunity for future reproduction closes. Therefore, there is likely a trade-off between current maternal investment, determined by the number of offspring to be fed and future reproductive potential. This trade-off appears strongly skewed towards investment in current reproduction. The reasons for this may be first, regurgitation and matriphagy demand large and possibly irreversible internal changes as shown in this study, and second, the probability of surviving to produce a successful second clutch decreases with time in the season due to ecological pressures such as increased parasite and predator pressure and lower food availability for emerging young (Schneider 1996; Schneider & Lubin 1997b).

By the middle of the period of regurgitation feeding (day 6 after emergence), the young have grown and bodily demand for food increases. This corresponds to increased degeneration of the MD and the appearance of large patches of liquid and nutritional vacuoles. By the 9<sup>th</sup> day of regurgitation, each young has increased its mass by a factor of 3.8 since emergence (from  $0.78 \pm 0.11$  mg to  $2.98 \pm 0.28$  mg (mean  $\pm$  SE); Salomon & Lubin unpubl. data) and massive tissue degradation occurs at this time. The large nutritional vacuoles observed may be the result of merging of nutritional vacuoles into large ones or absorption of additional nutrients resulting from degeneration of the tissues. The lipid droplets are not dissolved in this degeneration process and small droplets occur in the MD tissues after emergence of the young, during regurgitation, and even before matriphagy.

Tissue degeneration is unequally distributed within the opisthosoma. At emergence of the young, the tissues are dissolving at the perimeter of the opisthosoma and after 9 days of regurgitation, the interior MD lobes also start to dissolve. Additionally, tissue degeneration starts from the median area followed by the posterior and finally the anterior parts of the opisthosoma. We observed liquid accumulation at the perimeter after emergence of the young, but it was not observed during regurgitation. This remains unexplained. The ovaries, located at the posterior end of the opisthosoma, thus likely remain functional until late in the regurgitation stage.

The presence of the lumen during the egg incubation period indicates that nutrients from ingested prey pass through the lumen. After emergence of the young, the female ceases to consume prey (Salomon & Lubin unpubl. data). The presence of a lumen filled with liquefied tissue at the emergence of the young and during the regurgitation phase confirms that nutrients continue to pass through the lumen. This suggests that nutrients flow from the lumen into the gut and towards the female's mouthparts, constituting the regurgitation fluid that feeds the young. Our data, together with that of Nawabi

(1974), show a gradual process of tissue degradation according to the function of the tissue. First to degenerate are the secretory and digestive cells that are no longer needed to produce digestive fluid or absorb nutrients after the emergence of the young (Nawabi 1974). Next is the intermediate tissue between the MD lobes, leaving a functional MD lumen during the stage of regurgitation. Lastly, the MD lobes degenerate together with the lumen, precluding further regurgitation and setting the stage for matrophagy.

The histological sections show that matrophagy occurs when all the MD tissues are degenerated into a granular non-functional tissue and the perimeter of the opisthosoma is filled with liquid containing numerous large nutritional vacuoles. In *S. lineatus*, matrophagy does not occur earlier than 9 days after emergence of the young (Salomon & Lubin unpubl. data), suggesting that time may be needed to prepare the female's body for matrophagy. Furthermore, after two weeks of regurgitation feeding, the young have grown and molted at least twice (Kullmann 1972). At this stage, their chelicerae are functional and the young have been observed feeding on the opisthosoma and leg joints of the mother (M.S., pers. obs.). The concentration of the nutritional liquid in the perimeter of the opisthosoma allows the young easy access to the liquid food, and as the female's digestive enzymes have already liquefied most of the tissues for the young, they only need to pierce the female's opisthosoma and imbibe the liquid. It remains unknown whether young of *S. lineatus* are capable at this stage of regurgitating digestive enzymes and consuming the remaining MD tissue.

These dramatic changes in the body of female *S. lineatus* are consistent with an extreme semelparous reproductive system in which females invest all of their resources, including their soma, in a single small clutch of offspring (Schneider & Lubin 1997b). This is the first demonstration of the mechanism at the cellular level that underlies suicidal maternal care in an arthropod. In spiders, matrophagy has evolved independently in Amaurobiidae, Eresidae, Theridiidae, and Thomisidae (Pekar 2000; Lubin & Bilde 2007; Yip & Rayer 2013). Similar processes may occur in other taxa exhibiting suicidal maternal care such as the hump earwig *Anechura harmandi* Burr 1904 (Suzuki et al. 2005).

The family Eresidae is an evolutionarily basal family (Johannesen et al. 2007). Maternal care, including regurgitation feeding and matrophagy, occurs in all eresids studied to date (Lubin & Bilde 2007). In the genus *Stegodyphus* Simon 1873, cooperative breeding species have evolved independently three times from subsocial ancestors similar to *S. lineatus* (Johannesen et al. 2007). In colonies of the social spider, *Stegodyphus dumicola* Pocock 1898, non-reproducing females help the mother feed the young through regurgitation and are even consumed by the young (Salomon & Lubin 2007). It is likely therefore that tissue degeneration occurs also in these non-reproductive female helpers. This evolutionary pathway towards cooperative breeding may involve further specialization of females for maternal and allomaternal care of offspring, and at the same time increased dependence of the young on caring adults. Finally, the combination of regurgitation feeding and matrophagy occurs sporadically in other spider lineages (Lubin & Bilde 2007), but whether a similar physiological mechanism underlies this behavior remains unknown.

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