Reconstruction of the female genitalia at molting in the isopod crustacean, *Armadillidium vulgare* (Latreille, 1804)

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**Abstract.**—In *Armadillidium vulgare*, the female external genitalia (cuticular copulatory apparatus) consist of a copulatory opening and a spermathecal duct. Adult females change the structure of the genitalia at molting during a reproductive cycle. This study was done to investigate the relationship between the structural change of the genitalia and their reconstruction at the non-parturial (normal) and parturial (reproductive) molts. When females have immature ovaries at molting, they completely cast off the genitalia, together with the old exoskeleton, and reconstruct new genitalia of a non-reproductive type. On the other hand, females with mature ovaries cast off the copulatory openings together with the old exoskeleton at parturial molting. However, the old spermathecal ducts are retained in the oviducts through and after molting. In females with mature ovaries, new genitalia of a reproductive type are not reconstructed at the parturial molt. The present observations demonstrate that adult females change the structure of the genitalia at molting in accordance with the degree of ovarian maturation. The non-reproductive type genitalia are reconstructed at the non-parturial molt but the reproductive type genitalia are not reconstructed at the time of the parturial molt in *A. vulgare*.

**Introduction**

Studies of the female genitalia of isopod crustaceans have revealed considerable variation in their morphological structure (Calman, 1909; Vandel, 1925; Veuille, 1978; Lincoln, 1985; Wilson, 1986, 1991; Wägele, 1990, 1992; Suzuki, 2001). Adult females of the terrestrial isopod, *Armadillidium vulgare*, possess a pair of genitalia which are situated on the ventral surface of the fifth pereionite and are attached to the fifth sternite (Suzuki, 2001; Figs. 1A–C). The genitalia of this species are morphologically classified into two types: a non-reproductive type with the copulatory opening of a short and narrow tube (Fig. 1B) and a reproductive type with a funnel-shaped copulatory opening (Fig 1C). The two types are not present simultaneously but at the different phases of the female reproductive cycle. Females without a marsupium (five pairs of oostegites) have non-reproductive type genitalia but females with a marsupium have reproductive type genitalia. Suzuki (2001) has described that structural changes of the genitalia from the non-reproductive type to the reproductive type occur at the parturial molt in *A. vulgare*. The female molt preceding oviposition is termed the parturial molt in this species (Suzuki, 2001).

Under natural conditions females of *A. vulgare* produce 3–4 broods either in rapid succession or discontinuously during a reproductive season (Suzuki, 1991; Warburg, 1993). Around Yokohama all the hibernating females do not bear a marsupium (Suzuki, 1986) and possess non-reproductive type genitalia, suggesting that they have made a non-parturial...
molt at the end of the previous reproductive season. Therefore, the genitalic structure is probably changed and reconstructed several times each year in the field population of females. The present study was carried out in order to elucidate the relationship between the structural changes of the two genitalic types and the degree of ovarian maturation at the time of molting.

Materials and Methods

Animals

Adult females and males of Armadillidium vulgar were reared in petri dishes, as described by Suzuki (2001). Females were divided into two groups: (a) females without a marsupium and (b) those with an empty marsupium. When females of each group formed the sternal calcium carbonate (CaCO₃) deposits (termed sternoliths) before molting, they were divided further into two groups: (a) females with immature ovaries and (b) those with mature ovaries. The degree of ovarian maturation at molting was determined by the shape of the CaCO₃ deposits (Moreau et al., 2001), as mentioned below. Specimens were observed at four stages of the reproductive cycle: (1) females without a marsupium and with immature ovaries, (2) females with a marsupium and immature ovaries, (3) females without a marsupium and with mature ovaries, and (4) females with a marsupium and mature ovaries.

Relationship between the shape of CaCO₃ deposits and the stage of ovarian maturation

The shape of the CaCO₃ deposits is an external marker of female reproductive status (Moreau et al., 2001). When females possess immature ovaries, they form CaCO₃ deposits with a complete shape (having no opening) and a non-parturial molt occurs. When females possess mature ovaries, they form CaCO₃ deposits with incomplete shape (having a diamond-shaped opening at the level of the central furrow) and a parturial molt occurs. The shape of the CaCO₃ deposits was used to estimate the degree of ovarian maturation before molting.

Observation of morphological changes of genitalia

Over 50 females, 10–12 mm in body length, were used in the present study. The females with CaCO₃ deposits were kept separate for daily observation and sacrificed just before or after molting. The times before and after molting were determined from precise observations on each individually reared female. All isopods first molt the posterior half of the body and then the anterior half of the body (Wägeler, 1992; Ziegler, 1997). In this study the start of exuviation of the posterior half of the body initiates molting. Completion of exuviation of the anterior half of the body indicates that molting is complete. The female reproductive system was dissected out, together with the fifth sternite, to facilitate morphological observations of the genitalia, as described by Suzuki (2001). The exuvial exoskeleton was examined under a dissecting microscope to confirm the presence of the genitalia attached to the fifth sternite, which lies in the posterior half of the body. The presence of genitalia attached to the exuvial exoskeleton is clear evidence that they are cast off together with the exoskeleton at the posterior molt.

Results

Detailed examination of genitalia was made of females just before and after molting of the posterior and anterior halves of the body. Structural changes of genitalia are described at the four stages of the female reproductive cycle.
**Fig. 1.** Ventral view of the female reproductive system, showing the position and morphology of the genitalia. (A) Overview of the female reproductive system. An arrow indicates the right genitalium of the non-reproductive type in the oviduct. (B) Left genitalium of the non-reproductive type, attached to the fifth sternite. The genitalium is positioned opposite to the fifth sternite after dissection for observation. (C) Right genitalium of the reproductive type, attached to the fifth sternite. The oostegite in (C) is removed. as, abdomen (abdominal segment); co, copulatory opening; h, head; od, oviduct; ov, ovary; pe, pereopod; po-5, fifth pereonite; sd, spermathecal duct; st-5, fifth sternite; ts, thorax (thoracic segment). Scale bars represent 500 μm.

**Females without a marsupium and with immature ovaries**

The genitalia are of the non-reproductive type in females without a marsupium (Figs. 1A, 1B, 3A). When ovarian maturation was incomplete at the time of molting, females underwent a non-parturial molt in the posterior half of the body. The old genitalia were cast off together with the exuvial exoskeleton (Figs. 2A, 3B). New genitalia of the non-reproductive type were reconstructed and the copulatory openings of them were continuous to the fifth sternite of the new exoskeleton (Fig. 3C). Internally the copulatory openings were connected to the spermathecal ducts. Two hours after molting, females consumed the exuvial exoskeleton of the posterior body region. Molting of the anterior half of the body occurred about 22 hours after the posterior molt and the exuvial exoskeleton was consumed about 2 hours after molting.

**Females with a marsupium and immature ovaries**

The genitalia of females with a marsupium are the reproductive type (Figs. 1C,
Fig. 2. Ventral views of the fifth exuvial sternite, showing the genitalia cast off together with the fifth sternite at molting. (A and C) are the non-reproductive type genitalia. (B and D) are the reproductive type genitalia. The oostegites in (B and D) are removed for genitalic observation. (A) Right copulatory opening and spermathecal duct at the non-parturial molt. (B) Left copulatory opening and spermathecal duct at the non-parturial molt. (C) Right copulatory opening at the parturial molt. (D) Right copulatory opening at the parturial molt. co, copulatory opening; sd, spermathecal duct; st-5, fifth sternite. Scale bars represent 200 μm.
Fig. 3. Structural change of the non-reproductive type genitalia at the posterior body molting. Ventral views of the fifth sternite. (A) Non-reproductive type genitalia attached to the sternite before molting. (B) Exuvial sternite with the copulatory openings and spermathecal ducts after non-parturial molting. (C) New sternite with the reconstructed genitalia after non-parturial molting. (D) Exuvial sternite with the copulatory openings after parturial molting. (E) New sternite with the spermathecal ducts which are retained in the oviduct after parturial molting. The oostegites are omitted in the drawing (E). The half circle in (E) shows the cotyledon (Warburg, 1993). co, copulatory opening; sd, spermathecal duct; st-5, fifth sternite.

4a). When ovarian maturation was incomplete before molting, females cast off the reproductive type genitalia together with the exuvial exoskeleton of the posterior body region at the non-parturial molt (Figs. 2B, 4b). New genitalia of the non-reproductive type were reconstructed (Fig. 4c). That is, the old reproductive genitalia were changed into the new non-reproductive genitalia at the non-parturial molt. After the exuvial exoskeleton of the posterior end was eaten, the anterior body molted 22 hours after the posterior molt.

Females without a marsupium and with mature ovaries

Females without a marsupium possess non-reproductive type genitalia (Fig. 3A). When the ovaries were mature, the connection between the spermathecal ducts and the copulatory opening was broken before molting (Figs. 5A–D). Fig. 5D shows that the spermathecal duct is easily separated from the copulatory opening. Females carried out the parturial molt of the posterior body region and cast off only the copulatory openings of the non-reproductive genita-
lia together with the exuvial exoskeleton (Figs. 2C, 3D). The spermathecal ducts remained in the oviducts at the posterior molt (Fig. 3E). After the anterior molt the spermathecal ducts were retained in the oviducts (Figs. 6A–C, 7A, 7B). That is to say, females do not cast off but retain the old spermathecal ducts at the parturial molt. A pair of oostegites developed on the fifth sternite at the posterior molt. At the anterior molt four pairs of oostegites developed on sternites 1–4. Five pairs of oostegites form a marsupium on the ventral side of the body. However, new genitalia of the reproductive type were not reconstructed at the parturial molt (Figs. 6A–C, 7B). After parturial molting the spermathecal ducts of the old genitalia are easily freed and come out from the oviducts together with sperm during dissection (see Fig. 6C). The exuvial exoskeletons of the posterior and anterior molts were consumed soon after each molting.

**Females with a marsupium and mature ovaries**

After females had completed the process of ovarian maturation, the copulatory openings of the reproductive genitalia were broken away from the spermathe-
Fig. 5. Female reproductive system before the parturial molt. It is dissected out together with the fifth sternite from the right hand side and positioned opposite the sternite for observation. Ventral views. (A) Overview of the female reproductive system. (B) Enlargement of (A). (C) The sternite in (B) is pulled apart from the oviduct. (D) Enlargement of (C), showing the copulatory opening is attached to the sternite, and the connection between the spermathecal duct and the copulatory opening is severed (arrow). co, copulatory opening; mo, mature oocyte; od, oviduct; ov, ovary; s, sperm; sd, spermathecal duct; st-5, fifth sternite. Scale bars represent 500 μm.
Fig. 6. Female reproductive system after the parturial molt. It is dissected out together with the fifth sternite from the right hand side and positioned opposite the sternite after dissection. Ventral views. (A) Ovary and oviduct with the sternite. The oostegite is removed in (A). (B) Dissected oviduct, showing that the spermathecal duct is retained in the oviduct. (C) Dissected oviduct and the spermathecal duct, showing that the spermathecal duct is easily freed out of the oviduct together with sperm. mo, mature oocyte; od, oviduct; ov, ovary; s, sperm; sd, spermathecal duct; st-5, fifth sternite. Scale bars represent 500 μm.
cal ducts and cast off with the exuvial exoskeleton at the parturial molt of the posterior body region (Figs. 2D, 4a, 4d). The spermathecal ducts remained in the oviducts (Fig. 4e). At the posterior and anterior molts, five pairs of oostegites develop and form the marsupium. After parturial molting, the spermathecal ducts of the old genitalia are still retained, but new genitalia are not present in the oviducts (Fig. 4e).

Discussion

Structural change and reconstruction of the genitalia at the non-parturial molt

When females, with or without a marsupium, possess immature ovaries before molting, old genitalia of the non-reproductive or reproductive type are cast off with the exuvial exoskeleton (Figs. 3B, 4b). At the same time new genitalia are reconstructed (Figs. 3C, 4c). The newly reconstructed genitalia are the non-reproductive type.

Structural change and reconstruction of the genitalia at the parturial molt

When females, regardless of whether they have a marsupium, possess mature ovaries before molting, they cast off only the old copulatory openings of the non-reproductive or reproductive genitalia with the exuvial exoskeleton at the parturial molt (Figs. 3D, 4d). The old spermathecal ducts of both types are retained in the oviducts (Figs. 3E, 4e, 6B). After molting a marsupium is formed on the ventral surface of the body. As shown in Fig. 7, females partially change the structure of the old genitalia but do not reconstruct new genitalia at the time of the parturial molt. However, reconstruction of the reproductive type genitalian has been suggested to occur 1–2 days after molting and oviposition in A. vulgare (Suzuki, 2001).

As mentioned above, in females with immature ovaries the structural change from the old to new genitalia corresponds to the genital reconstruction. Both occur simultaneously at the time of the non-
parturial molt. By contrast, in females with mature ovaries the structure of old genitalia is partially changed at the parturial molt. The reconstruction of new genitalia does not take place at molting. Therefore, the relationship between the structural change and the reconstruction of the female genitalia is dependent on the status of ovarian maturation at molting in *A. vulgare*.

**Persistence of the spermathecal duct**

In female *A. vulgare*, the copulatory openings and the spermathecal ducts are connected to one another in the oviducts. The other end of the copulatory opening is continuous to the sternite. When a female completes ovarian maturation, the connection between the copulatory opening and the spermathecal duct is severed (Fig. 5D). Then, when females undergo the parturial molt of the posterior body region, the copulatory openings are cast off together with the fifth exuvial sternite, but the spermathecal ducts are left behind in the oviducts. Why do only the spermathecal ducts remain at the parturial molt? The function of the retained spermathecal ducts is unknown but appears to be associated with fertilization and oviposition (Fig. 7B).

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**Literature Cited**


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