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# CHRONOLOGY OF THE FEMALE MOLT CYCLE IN *SIRIELLA ARMATA* M. EDW. (CRUSTACEA: MYSIDACEA) BASED ON MARSUPIAL DEVELOPMENT

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## ABSTRACT

An alternative method for staging molt cycles is proposed for adult females of the mysid *Siriella armata*. Cultured females have a 12-day molt and breeding cycle. At the end of each cycle, liberation of the young, ecdysis of the female, copulation, and egg laying occur successively in less than 8 h. A synchronization between marsupial development, secondary ovarian vitellogenesis, and the integumental cycle is observed. Marsupial development is described and its chronology is related to molt cycle staging. Molt stages are defined following Drach's method complemented by an ultrastructural study of the evolution of the integument. The corresponding 12-day cycle of oocyte development in the ovary is described. Embryonic development corresponds to the female postmolt period. The nauplioid phase matches the intermolt period and the postnauplioid phase is simultaneous with the premolt period. *Siriella armata* is suggested as a suitable experimental organism for studies of the control of molt and reproductive cycle in mysids.

## RÉSUMÉ

Une méthode différente de la méthode classique est proposée pour définir les stades du cycle de mue chez les femelles adultes du mysidacé *Siriella armata*. Dans les conditions du laboratoire, la durée du cycle des femelles peut être fixée à 12 jours. A la fin de chaque cycle, la libération des jeunes, l'exuviation de la femelle, la copulation et la ponte ont lieu successivement en moins de 8 h. Une synchronisation du développement marsupial avec la vitellogenèse secondaire et le cycle tégumentaire est observée. Le développement marsupial est décrit et sa chronologie est établie en parallèle avec les stades du cycle tégumentaire. Les stades de mue sont définis selon la méthode de Drach complétée par une étude ultrastructurale de l'évolution du tégument. Le développement des ovocytes dans l'ovaire pendant le cycle de 12 jours est décrit. Le développement embryonnaire correspond à la période de postmue de la femelle; la phase naupliöide à l'intermue et la phase postnauplioïde à la prémue. *Siriella armata* est proposée comme support approprié pour des études du contrôle du cycle de mue et de reproduction.

In crustaceans most physiological and metabolic processes are cyclical and it is thus important to determine the various stages of the molt cycle prior to experimental studies. Since Drach (1939), the recognition of molt cycle stages in crustaceans has been based on in vivo observations of the integument and on developmental changes in the setae (Drach and Tchernigovtzeff, 1967). The method has been adapted to a wide variety of crustaceans (Lyle and MacDonald, 1983). Electron microscopical studies have been useful for describing the details of the deposition of cuticular layers by epidermal cells (literature cited in Green and Neff, 1972) and to follow the evolution of the integument during a molt cycle (Christiansen and Costlow, 1982). Using this technique, Tchernigovtzeff (1976) has described the organization of setal matrices.

The aim of the present study is to define stages of the molt cycle in the mysid *Siriella armata* and to establish a relationship between molt stages and development of eggs and of young in the marsupium in breeding females. Furthermore, we suggest that the stages of the cycle of the female could be determined by the state of development of the embryos in the marsupium rather than on observation of the structure of the integument.

*Siriella armata*, a neritic species distributed from the North Sea to the Mediterranean, has been successfully reared from the egg to the reproductive adult at the Station Zoologique (Villefranche-sur-mer). The juvenile development, and the differentiation and development of the gonads have been previously described (Cuzin-Roudy *et al.*, 1981).

Embryonic development of other mysid species has been described by Nusbaum (1887) in *Mysis chameleo*, Manton (1928) in *Hemimysis lamornae*, and Nair (1939) in *Mesopodopsis orientalis*. Marsupial development has been studied in *Gastrosaccus vulgaris* by Matsudaira *et al.* (1952), *Neomysis vulgaris* (Kinne, 1955), *Boreomysis arctica* (Jepsen, 1965), *Mysidium columbiae* (Davis, 1966), *Mysis relicta* (Berrill, 1969), *Mysis stenolepis* (Modlin, 1977), and *Leptomysis lingvura* (Wittmann, 1981). Wittmann observed that the major stages of marsupial development described in *L. lingvura* can be identified in *Siriella jaltensis*. No such description is available for *Siriella armata*.

## Materials and Methods

Swarms of *Siriella armata* were reared as described by Cuzin-Roudy *et al.* (1981). The photoperiod was 16 h day/8 h night, the temperature 20°C (± 1°), and the diet nauplii of *Artemia*. Under these conditions the molt cycle lasts 12 days. The females used in the present study were adults with total lengths of 20–24 mm. This is the maximum size observed at Villefranche in natural populations. The timing of the cycle was established for animals taken from the laboratory populations.

Since *Siriella armata* is completely transparent when the chromatophores are retracted, observations of the brood in the marsupium were done directly on live females. These were observed while in a drop of sea water, using a stereomicroscope. More precise examinations were made by washing the eggs or young from the marsupium with a gentle water current issuing from a pipette. This did not harm the female. Micrographs were also made from live animals maintained in a drop of sea water and held between slide and coverslip.

The criteria used for determining molt stages are those proposed by Drach and Tchernigovtzeff (1967) for the Natantia. Molt staging was done by observing flat appendages which bear rows of setae or spines. In *Siriella armata*, it was most convenient to use antennal scales and the external ramus of uropods since they are flat and thin. The antennal scale displays one wide indentation and a row of long setae, while the uropod has a row of setae and large spines. Preparations were made from freshly cut antennal scales and the external ramus of pleopods mounted anterior side up in sea water. Drawings made with a camera lucida were used to measure the degree of retraction of soft tissues following apolysis. It was estimated as the ratio of the distance of epidermis from the cuticle to the thickness of the cuticle. This ratio was measured both for the antennal scale (level of the indentation shown in Fig. 2D) and the external uropod (level of the first external seta). These 2 variates (a.r. = antennal retraction; u.r. = uropodal retraction) were measured on 18 females taken at day 7, 8, or 9 of their cycle.

The ovaries of live females were examined to estimate ovarian expansion, which we have defined as the ratio of thorax height occupied by the ovary in lateral view (Fig. 1, A' to E').

In vivo observations were made on several hundreds of females taken from cultures or from Villefranche Bay and the Bay of Cannes during two years (1980–1982).

Electron microscopy was used to follow thickening of the new cuticle in pre- and postmolt periods. Pieces of integument were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffered at pH 7.4, then postfixed in 1% osmic acid and finally embedded in Epon. Females were dissected in the fixative for better penetration. A Philips 201 electron microscope was used. In order to study cuticle secretion during the postmolt period 25 females were fixed at different times during this period. Since cuticular deposition rates are not the same throughout the organism, pieces of the integument were taken from the carapace, antennal scale, telson, and uropod. Cuticular layers were counted from electron micrographs. To allow for variability, the ratio of the number of postexuvial cuticular layers to that of preexuvial layers, rather than absolute thickness, was taken as an index of cuticular postmolt secretion. Electron microscopic studies were based on 40 females, fixed at various stages of their molt cycle.

A *G* test (Sokal and Rohlf, 1969) was used to determine whether marsupial development has the same timing in culture and in natural populations. Assuming that the breeding cycle is asynchronous, the relative frequencies of marsupial stages among broods in natural populations may be used to reflect the relative durations of stages (Mauchline, 1973). For this purpose, 472 breeding females were collected at Villefranche Bay in late spring when temperature and day length are similar to laboratory conditions.

Observed stage frequencies were compared to estimated frequencies from an equivalent number of females taken from the cultures.

Kendall's coefficient of concordance (Siegel, 1956), $W$, was used to estimate the correlation between timing of the female cuticular cycle with that of marsupial development. Eighteen breeding females, taken at day 7, 8, or 9 of their cycle, were ranked using three variates: the two variates a.r. and u.r. defined above and the age of the brood in the marsupium.

## RESULTS

In our laboratory, *S. armata* is cultured year-round in conditions resulting in a succession of twelve-day molt and reproductive cycles in adult females. As in other mysids, eggs are laid in a brood pouch and development from the egg to juvenile takes place during one molt cycle of the female. During this time, the female ovary matures for the subsequent oviposition. Liberation of young occurs just before the molt of the mother and deposition of a new batch of eggs just after molting. Thus, during every molt cycle of a female, three separate events appear to be synchronized: evolution of the integument between two ecdyses, cyclic secondary vitellogenesis of the ovary, and development of the young in the marsupium.

### Events Related to the Female Molt

Liberation of the young, molting, mating, and egg laying occur at night in *Siriella armata*. The whole process lasts less than 8 h. A female ready to molt first releases the young from the brood pouch a few hours after dark when the fully developed brood exceeds the capacity of the marsupium. This is indicated by the protrusion of the abdomen of the young anteriorly between the oostegites.

More active movements of the oostegites cause the release of the young, while females are swimming. Females were observed while liberating their young in dim light. The young molted immediately on being released and started swimming actively.

Ecdysis of the female occurs in the middle of the night, while she swims. Shortly after this molt, the female copulates. Copulation was never observed during the day nor in illuminated tanks.

Occasionally females molted early in the morning, much later than normal. These animals failed to copulate but were observed laying eggs. It was possible to see the oocytes passing one after the other from the ovary through the oviducts to the brood pouch. Prior to oviposition, each oviduct aperture, situated near the bases of the sixth thoracopods, extrudes a smooth, very elastic, mucous sac which will receive the first egg from each oviduct. With each additional egg, the sac swells. When full, the paired sacs are aligned in an anteroposterior position in the marsupium. The posterior sac belongs to the oviduct that oviposited first. The two halves of the brood remain packed in their sacs for about 12 h. Egg laying is normally completed by daybreak. Nonmated females lay eggs, but the process is slow and some oocytes may remain in the ovary where they are resorbed in a few days.

### Marsupial Development

Three phases of development occurred in the marsupium: (1) an *embryonic phase* with development inside the egg membrane, (2) a *nauplioid phase* after egg hatching, and (3) a *postnauplioid phase* after the nauplioid molt. These phases follow the description of Wittmann (1981). Each phase can be subdivided into

stages on the basis of morphological changes. Two morphological criteria were used to divide the second and third phases: the first appearence of eye pigmentation in the forming eye of the nauplius and the complete enclosure of yolk within the thorax of the postnauplius. Consequently 5 stages could be described in marsupial development.

*Stage 1: Embryonic Development.* Duration: 3 days (Fig. 1A).—Adult females, in the size range considered here, lay 30–50 eggs per brood, in two batches. Eggs taken from the egg sacs for observation, have a very thin, fragile vitelline membrane tightly applied to the uncolored yolk. They appear homogeneous in structure and are full of refringent yolk globules (Fig. 1A). They are nearly spherical in shape. However, when packed in the egg sac they assume a polygonal shape. About 12 h after egg laying, the egg sacs burst. They immediately shrink and are carried toward the anterior end of the marsupium by water currents created by the female and are expelled. The eggs are now free in the marsupium. They acquire a more spherical shape and their vitelline membrane becomes more resistant. Unfertilized eggs disintegrate within the first 12 h and are expelled when the sacs burst.

Twenty-four h after egg laying, the germinal disk forms in a depression of yolk at the animal pole of the egg which will become the ventral region of the animal. At 48 h, an invagination is visible on the surface of the germinal disk which will form the abdominal rudiment. Within 72 h the abdominal rudiment and the three pairs of cephalic appendages (antennules, antennae, mandibles) are completed. Hatching occurs when straightening of the abdominal rudiment bursts the egg membrane. The timing of embryonic development is given in Table 1.

*Stage 2: Early Nauplioid Phase.* Duration: 2.5 days (Fig. 1B).—Just after hatching, the nauplius is comma-shaped. The spherical body contains yolk and the small abdominal rudiment is bent ventrally. Within a short time, the abdomen flexes in the opposite direction, elongates, and becomes dorsally bent (Fig. 1B). This dorsal curvature of the abdomen is a constant feature of the larva prior to molting into first juvenile stage. During this abdominal flexing, part of the yolk flows into the abdomen. The embryonic tissues are located ventrally and the yolk mass anterodorsally. The cephalic appendages are tubelike, with short but conspicuous mandibles (Fig. 1B). The tapered end of the abdomen bears lateral rows of cuticular spines. Subsequent segmentation of the ventral part of the thorax can be observed through the thin transparent naupliar cuticle. The appearance of the thoracopods, uropods, and telson, and the delimitation of the ocular peduncles in the anteroventral region are observed easily. The antennules (a1) and antennae (a2) become biramous and segmented. At this stage the larva is colorless. The first appearance of black pigment in the eyes marks the beginning of the next stage.

*Stage 3: Late Nauplioid Phase.* Duration: 2 days (Fig. 1C).—Segmentation of the thorax and the abdomen is completed (Fig. 1C). Yolk continues to condense anterodorsally, clearing the abdomen and the dorsal region where the heart forms. At the end of this stage, the heart is functional, the eyestalks are fully formed, and the eyes are pigmented. Part of the yolk is enclosed in the posterior sacs of the digestive gland. The naupliar cuticle is completely free, indicating that molting is imminent.

*Stage 4: Early Post-nauplioid Phase.* Duration: 2 days (Fig. 1D).—The nauplius molts. This molt is slow and asynchronous, since it is possible to find molted, partially molted, and unmolted larvae together in the marsupium. Complete exuviae were never found in the marsupium, since they are extremely thin and

Table 1. Correspondence between marsupial development, female molt cycle, and ovarian cycle in breeding females of *Striella armata*. Temperature = 20°C. Photoperiod = D, 16 h day, N, 8 h night.

| Time | | Marsupial development | | Female molt cycle | | Ovary cycle |
|---|---|---|---|---|---|---|
| | | Characteristic steps | Stage | Characteristic steps | Stage | (ovary height/ thorax height) |
| Day 12 | D | | | ← Ecdysis | E | 3/4 |
| | N | ← Oviposition + fertilization | Stage 1 | | $A_2$ | ← Oviposition |
| Day 1 | D | ← Egg sacs disappearence | | Thickening of postexuvial cuticle | | Flat ovary |
| | N | | | | | |
| Day 2 | D | ← Germinal disc formation | | | | |
| | N | | | | | |
| Day 3 | D | ← Gastrulation | | | | |
| | N | | | | | |
| Day 4 | D | ← Hatching | Stage 2 | ← Cuticle completion | $C_3$ | 1/5 |
| | N | | | | $C_4$ | |
| Day 5 | D | | | | | |
| | N | | | | | |
| Day 6 | D | ← Eye pigment appearance | Stage 3 | | | 1/4 |
| | N | | | | | |
| Day 7 | D | | | | | |
| | N | | | | | |
| Day 8 | D | ← Naupliar molt | Stage 4 | ← Apolysis | $D_0$ | 1/3 |
| | N | | | | | |
| Day 9 | D | | | | | |
| | N | | | | | |
| Day 10 | D | | | ← "Invaginations," progressive splitting of matrices | $D_1$ | |
| | N | ← Yolk enclosure | Stage 5 | | | 2/3 |
| Day 11 | D | | | Pre-exuvial cuticle deposition and resorption of old cuticle | $D_2$ | |
| | N | | | | | |
| Day 12 | D | | | | | |
| | N | ← Young liberation | | | | |
| | | | | ← Ecdysis | E | 3/4 |

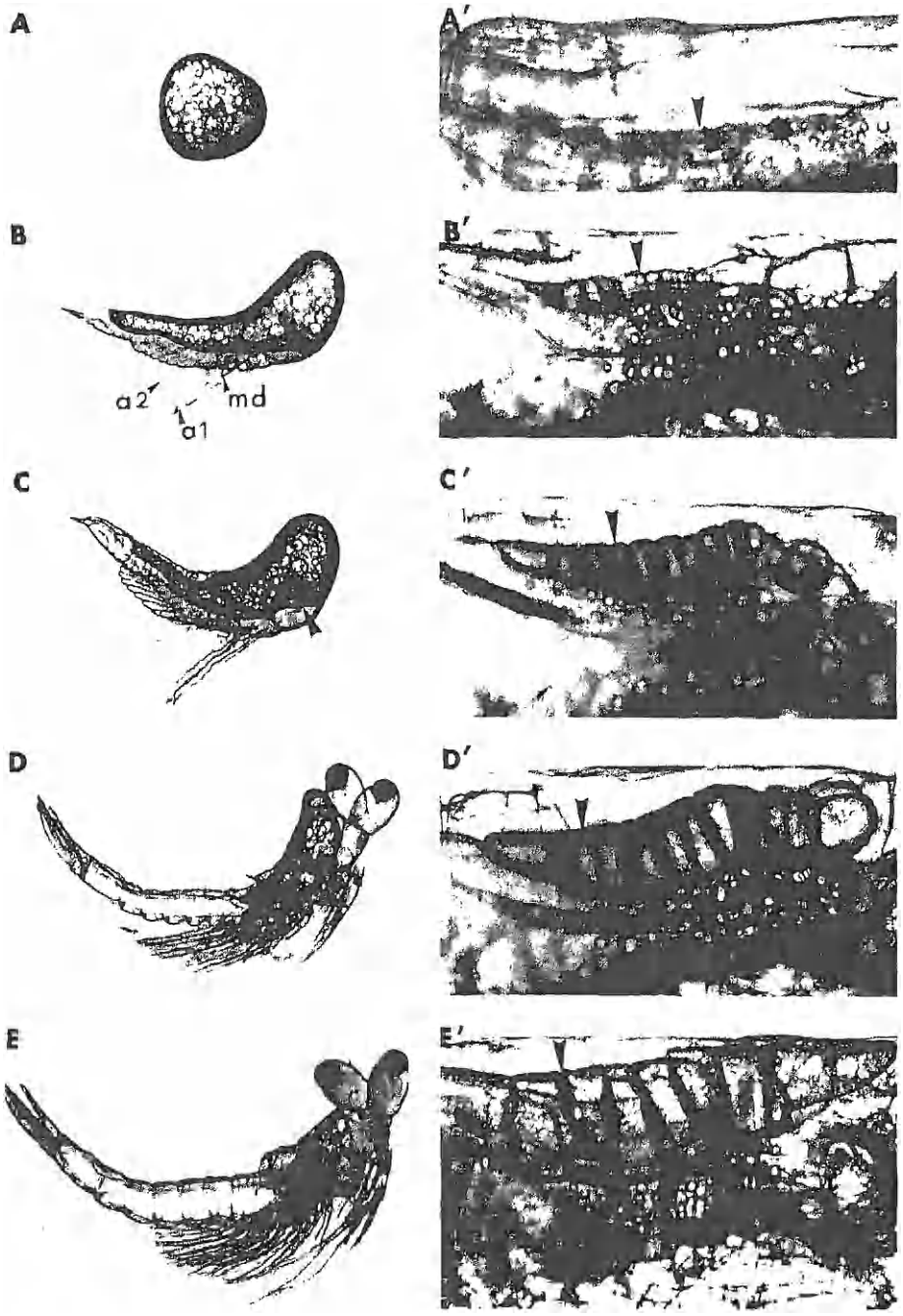


Fig. 1. Marsupial development and corresponding ovarian development in breeding females of *Siriella armata*. A–E: major stages of marsupial development. A, stage 1, developing egg. B, stage 2, early nauplioid form with cephalic appendages (a1 = antennules, a2 = antennae, md = mandibules). C, stage 3, late nauplioid form with eye pigmentation (arrow). D, stage 4, early postnauplioid form with free thoracic appendages and uropods and with a vitelline anterodorsal protuberance. E, stage 5,

expelled by water currents as small pieces. The eyestalks are free, as well as thoracic appendages, uropods, and telson. The yolk forms an anterodorsal protuberance.

During this stage, the most obvious event is the resorption of the yolk mass. At the beginning of the stage the yolk mass protrudes anteriorly as far as the eyestalks and at the end it is completely enclosed in the thorax. The carapace is first visible at the posterior end of the thorax, and as it develops it encloses the yolk mass. Melanophores appear on a1, a2, the thoracic and abdominal sternites, the uropods, and the telson.

*Stage 5: Late Post-nauplioid Phase.* Duration: 2 days (Fig. 1E).—The yolk becomes completely enclosed in the digestive glands and the digestive tract (Fig. 1E). The ventral chromatophores become functional and the pigment can expand or retract. The larvae are able to move jerkily inside the marsupium when stimulated. However, they are unable to bend ventrally even when removed from the marsupium. Liberation from the marsupium can be predicted by observing the uropods of the larva. When the new cuticle is visible inside the old, the young is ready to molt. Liberation from the marsupium and the subsequent molt occurs normally at night. Removal of the young from the marsupium during daytime was never followed by the juvenile molt.

## Duration of the Marsupial Stages

In order to determine if the timing of marsupial development observed in cultures differs from that in a natural population, a comparison was made using breeding females collected at sea. The observed frequencies for stages 1–5 were 22%, 18%, 20%, 17%, or 16%, respectively, and 7% of females had an empty brood pouch. This is not significantly different from relative durations in the laboratory: 25%, 21%, 17%, 17%, 17%, and 4%, respectively ($G = 4.578$ is less than the chi-square value at $P = 0.05$ with 5 d.f.). We can therefore assume that the relative duration of the marsupial stages is the same at sea and in the laboratory.

## The Ovarian Cycle

At the beginning of stage 1, after the molt of the female and egg laying, the ovary is extremely flat and thin. In lateral view melanophores situated in the epidermis at the level of the ovary indicate its position (Fig. 1A′). At the end of stage 1, a row of small oocytes is distinguishable inside each ovary. The oocytes grow continuously during the following stages without conspicuous steps. Ovarian expansion was estimated by the proportion of cephalothorax height occupied by the ovary in lateral view. The ovary occupied about one-fifth of the thorax height at stage 2 (Fig. 1B′), one-fourth at stage 3 (Fig. 1C′), one-third to two-thirds at stage 4 (Fig. 1D′), and two-thirds to three-fourths at stage 5 (Fig. 1E′).

It was not useful to measure ovarian expansion more precisely at the end of the cycle because of the wide variability in the size and number of oocytes. This variability may be linked to differences in the nutritional states of females. In a female ready to molt and lay eggs, the ovary may completely fill the thorax, leaving free only the dorsal heart space. In other cases the ovary occupied only

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late postnauplioid form with complete carapace and yolk enclosed in the digestive tract. — A′–E′: lateral views of thorax of female showing correlative states of development of ovary (arrow) at marsupial stage 1 (A′), stage 2 (B′), stage 3 (C′), stage 4 (D′), and stage 5 (E′). Scale, 1 cm — 0.35 mm.

two-thirds of the total height of the thorax. In both situations egg laying occurred normally. Normal development of the ovary is shown in Fig. 1 (A' to E') alongside the marsupial stages (A to E).

## The Cuticular Cycle

*Setae and Spines Formation.*—Just after the molt, the cuticle appears thin and lacks rigidity (Fig. 2A) with the tissues of the appendages having a loose structure. Lacunae and haemocytes invade setae and spines and epidermal cells are vacuolized. Later the lacunae condense to form a main blood lacuna which occupies a central position in the appendage with a conspicuous border at the bases of setae and spines. This condensation occurs during the end of marsupial stage 1 and marsupial stage 2. At stage 3, the tissues appear close-textured with slightly fibrillous setal matrices and a characteristic striped pattern. At the beginning of marsupial stage 4 apolysis has occurred and separation of the epidermis from the cuticle can be first seen in regions such as the large indentation of the antennal scale (Fig. 2D) or at the bases of the uropodal spines. During stage $D_0$ retraction of the epidermis becomes obvious between the bases of all setae and spines. At the end of marsupial stage 4, retraction of the epidermis is at maximum and invaginations appear at each side of the bases of the setal matrices. This marks the beginning of stage $D_1$. These ring-shaped invaginations actually correspond to the structure described by Tchernigovtzeff (1976) for the matrices of setae in *Palaemon serratus,* which involves a splitting of the matrices rather than an invagination. For each seta or spine, splitting of the matrix proceeds in a centripetal way, from the base of the seta toward the inner part of the appendage. When the splits have attained their full development (one-third of the size of the old seta), stage $D_1$ is terminated (Fig. 2E) and stage $D_2$ starts with the secretion of the new cuticle on the surface of the matrices. This secretion occurs on the direct as well as on the recurrent lamina of the splits. New cuticle secretion is better observed in the space between adjacent setae, where it appears as a thin but refringent deposit on the epidermis. During marsupial stage 5, this deposit becomes obvious, as well as the barbules formed on the new setae inside the old setae and along the splits inside the appendage (Fig. 2F). No further changes could be seen by in vivo observation before ecdysis.

*Ultrastructure of Cuticle Formation.*—The structure of the integument 24 h after ecdysis is shown in Fig. 2B. Newly secreted material is visible at the surface of the epidermal cell on which microvilli are present. Fully organized endocuticular

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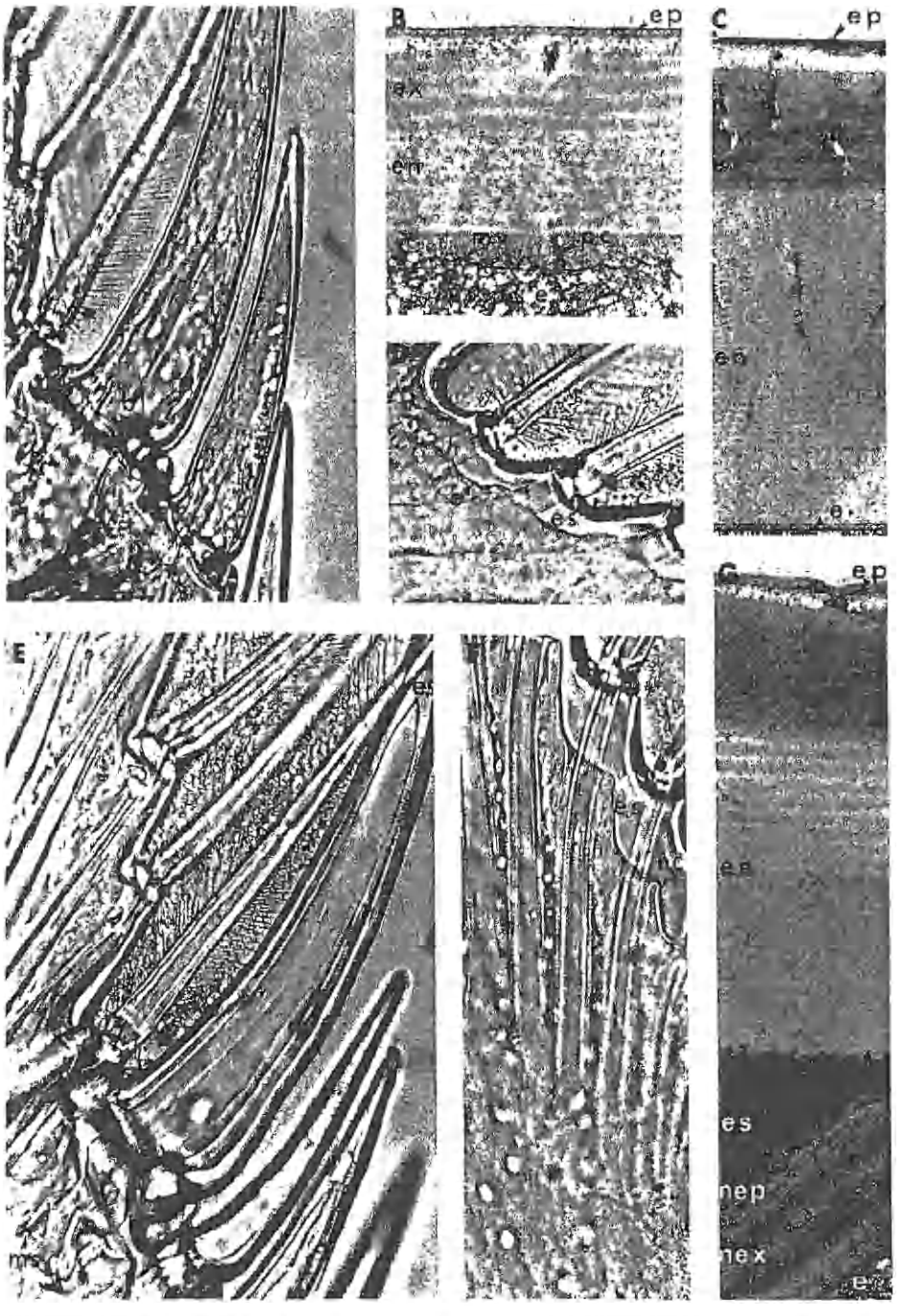
Fig. 2. Molt cycle in breeding females of *Siriella armata.* A, D, E, F: in vivo preparations of appendages. Scale, 1 cm = 20 μm. B, C, G: transverse sections of integument. A, external ramus of uropod, less than 1 h after ecdysis (the cuticle is thin, compare to E; tissues are vacuolated, blood lacunae are visible in spines and setae). B, telson integument about 24 h after exuviation (marsupial stage 1) (the epi- and exocuticle, separated by a "junction zone," are complete; endocuticle secretion is in progress; microvilli and pore canals from epidermal secreting cells are visible.) ×22,500. C, carapace integument at marsupial stage 2 and cuticular stage C (endocuticle secretion and organization is terminated; microvilli have disappeared.) ×10,000. D, antennal scale at $D_0$ stage (early marsupial stage 4) (epidermis is retracted from cuticle). E, external ramus of uropod at the end of stage $D_1$ (marsupial stage 5) (splitting of matrices is complete; secretion of new cuticle and formation of barbules has begun). F, antennal scale at stage $D_2$ (marsupial stage 5) (new cuticle is visible between setae and inside the splits, with arrows showing their inner folds). G, telson integument at marsupial stage 5 and cuticular stage $D_2$ (exuvial space and resorption zone at contact of endocuticle with exuvial fluid




are visible; rippled epicuticle of new integument is formed and exocuticle is in process of secretion by epidermis.) ×20,000. bl = blood lacuna; e = epidermis; en = endocuticle; ep = epicuticle; es = exuvial space; ex = exocuticle; ms = matrix split; mv = microvilli; nc = new cuticle; nep = new epicuticle; nex = new exocuticle; pc = pore canal; rz = resorption zone.

layers are already as thick as the epicuticle and exocuticle deposited before molt. The maximum development of the postexuvial cuticle, which marks the end of stage $C_3$, is shown in Fig. 2C. This is a transverse section from the carapace of a female fixed the fourth morning of her molt cycle. At this time microvilli have disappeared, secretion is terminated, and the postexuvial layers are completely organized. They are 2–3 times thicker than pre-exuvial layers in the different parts of the body. Two main layers are apparent in the endocuticle in some micrographs, as shown in Fig. 2G of the carapace. This could not be interpreted. Signs of resorption of the old cuticle were visible as early as stage $D_0$, on transverse sections of the integument made from females fixed from the ninth to the twelfth day of their cycle. They are shown in Fig. 2G, along with premolt secretion of the new cuticle.

*Synchronization between the Marsupial Development of the Young and the Cuticular Cycle of the Female.*—Under laboratory conditions, the cycle of adult females of *Siriella armata* could be stablized to a duration of 12 days. Timing of marsupial development is matched by cuticular evolution in breeding females (Fig. 3). Fertilization of the eggs occurs a few hours after ecdysis of the female and both cycles start the same night. It was established from the ultrastructural study of 25 females fixed at the beginning of their cycle that cuticle secretion was completed before the fourth day. The relative number of postexuvial layers was 35% twelve h after ecdysis, 50% at 24 h, and 70% before 84 h for all females studied. Thus, cuticular completion corresponds to hatching of the eggs in the marsupium and embryonic development occurs simultaneously with the postmolt period of the female.

Another series of micrographs from females fixed during the eighth day of their molt cycle indicated that apolysis occurs when the larvae undergo naupliar molt. The simultaneity of these events was ascertained by in vivo light microscope preparations of 18 breeding females taken at marsupial stages 3 and 4. The agreement between the age of the young in the marsupium and the cuticular stage attained by the females was tested by a $W$ Kendall coefficient of concordance. From the results ($W = 0.778$, highly significant) we can conclude that the timing of marsupial development is coincident with the timing of female integumental evolution and that the nauplioid phase corresponds to the intermolt period of the female.

Liberation of young occurs just before female ecdysis with the postnaupliar phase corresponding to the female premolt period. The main phases last 3, 4.5, and 4 days, respectively, with the events related to female ecdysis occurring during the twelfth night of the cycle.

Growth of the ovary, which performs cyclic secondary vitellogenesis during the 12-day cycle is shown, along with marsupial development and the molt cycle of the female, in Table 1.

## Discussion

From the descriptions of marsupial development of brood in mysids (reviewed by Mauchline, 1980), development is fairly uniform and occurs in three main stages: the "egg," "eyeless larva," and "eyed larva." However, there is some confusion about these marsupial stages as some authors use the term "embryos" to describe these three stages (Nair, 1939; Jepsen, 1965; Davis, 1966; Berrill, 1969; Amaratunga and Corey, 1975), while others use the term "embryos" only for the developmental stages occurring inside the egg membrane and "larvae" after hatching (Nusbaum, 1887; Matsudaira *et al.*, 1952; Green, 1970; Wittmann,

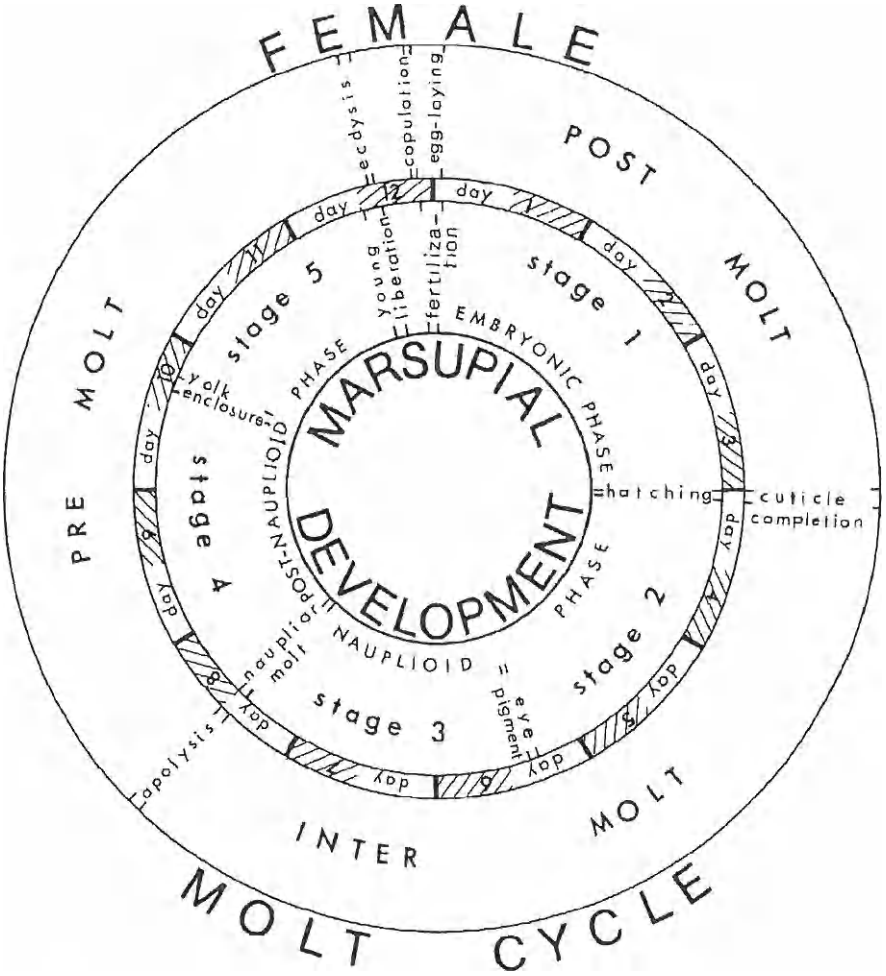



Fig. 3. Time schedule of the molt cycle of breeding females of *Siriella armata* based on marsupial development of the young (12-day cycle). Outer circle = major events of the female molt cycle; inner circle = major events and stages of marsupium development.

1981). We have chosen to use the nomenclature of Wittmann (1981). The embryo of *S. armata* develops inside the egg membrane, and the form liberated on hatching is not a true larva, since it has the typical appendages of the nauplius but lacks the structures suitable for living free such as the naupliar eye or the swimming setae. Like most nauplii of decapods and euphausiids it lives on vitelline reserves but is unable to move in the marsupium. A molt then occurs in the marsupium giving a postnauplioid form which is still immobile and relies on yolk. This form then undergoes another ecdysis, the juvenile molt, just after its liberation from the marsupium, to become a free juvenile.

By observing live females, we were able to establish the presence of egg sacs receiving the eggs. Similar egg sacs have been described only in *Neomysis vulgaris* by Kinne (1955) and in *Leptomysis lingvura* by Wittmann (1981). In *Mesopodopsis orientalis*, Nair (1939) described a thin membrane, secreted from the genital pore,

which surrounds each egg as it is extruded. This membrane was loose and sticky and was produced by the epithelial cells of the lower portion of the oviducts. It has been interpreted as a chorion. In *Mysidium columbiae,* Davis (1966) described a thin membrane around each egg that was distinct from the vitelline membrane. In *Hemimysis lamornae,* Manton (1928) found no chorion. The occurrence of a membrane secreted by the oviduct could be overlooked by authors working on fixed animals or on species where the egg sacs are very short-lived. Whether this membrane encloses each egg or a batch of eggs is probably a variable characteristic among the Mysidae.

The method of molt staging elaborated by Drach and Tchernigovtzeff (1967) for the Natantia has been adapted in the present study for a mysid. On account of the small size of the animal and the thinness of the integument macroscopic characteristics of the exoskeleton could not be used. Formation of the setae is not different from what has been observed by Tchernigovtzeff and Ragage-Willigens (1968) in the isopod *Sphaeroma serratum,* and is very similar to setal development in euphausiids (Buchholz, 1982). For example, the distal parts of the new setae develop inside the old setae. Though classical cuticular stages are easily recognized during the period between apolysis and ecdysis (i.e., premolt period), it was impossible to determine obvious stages during post- and intermolt periods (stages A, B, and C). The cuticle of *Siriella armata* is thin, poorly calcified, and weakly pigmented. Any changes in the condition of the exoskeleton cannot be taken as criteria for staging. Thickening of the cuticle could only be roughly estimated by direct observation (in Fig. 2, compare A and E). No special structures, such as the cones described by Freeman and Bartell (1975), exist in *Palaemonetes pugio,* nor is there any occlusion of the setal bases during stages A–B. Stage C could not be subdivided. In order to follow the endocuticular secretion from stage $A_2$ to the end of $C_3$, and to know when the intermolt periods starts, an electron microscopic study of the integument was necessary.

In consequence, an alternative staging method is proposed here. It is valid only for female mysids and is based on the synchrony between marsupial development of the young and molt cycle of the female, as summarized in Fig. 3. Parallel observations of the ovary in the live female do not provide a timing for secondary vitellogenesis. However, they do give information about the physiological status of the female, since the growth of oocytes is very sensitive to any limiting conditions in the cultures.

The succession of events related to female ecdysis shows precise synchronization, implying a linkage between the different physiological processes involved. Development of the young has to be completed to the point of the juvenile molt before the young are expelled from the marsupium. This expulsion always occurs before female ecdysis. Secondary vitellogenesis has to be terminated at the time of ecdysis of the female. A close relationship between exuviation and oocyte maturation has been demonstrated by Mathieu-Capderou (1980) in *Orchestia gammarellus,* even when exuviation is experimentally advanced or delayed. In *Siriella armata* it was observed that exuviation is occasionally postponed for 24 h. In this case, copulation and oviposition are also delayed and always occur shortly after exuviation. Thus, liberation of young may occur without being followed by female ecdysis during the same night. Such females stay at cuticular stage $D_3$ for 24 h until the subsequent night, when the cycle will resume. Day-night passage seems to be the starting signal for liberation of the young and for ecdysis.

During the molt cycle events which are important for the female and for the young are also synchronized. For example, hatching of the young coincides with

the end of the female cuticle postmolt secretion and naupliar molt in the marsupium with female apolysis. Juvenile molt of the expelled young usually occurs on the same night as female ecdysis, which implies direct or indirect communication between female and brood. In addition, there must be an integrated control of the ovarian and integumental cycles.

The general scheme given in Fig. 3 can be used for rapid dating of an adult breeding female by looking at marsupial development. It is accurate within 12 h. Coincidental observation of the state of development of the ovary aids staging and indicates whether the female cycle is normal. Direct observation of marsupial development is not only more rapid than other methods, but is also much less injurious for the female than the mutilations necessary in Drach's method. The same female can be followed during several successive cycles. This means that females of *S. armata* can be used to study the major physiological processes occurring during the molt cycle. In addition, it should be possible to investigate the control mechanisms involved in their synchronization.

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