COURTSHIP, MATING, AND EGG-LAYING BEHAVIOR IN THE LIMACIDAE (MOLLUSCA)

EDWARD J. KARLIN AND CHARLOTTE BACON
Bowling Green State University, Bowling Green, Ohio

The Limacidae include those slugs characterized by an oval mantle situated anteriorly and extending forward in a free lobe. All have a smooth jaw, a pneumostome opening behind the mantle cleft and an internal shell with concentric growth lines. Caudal mucous glands are absent.

The family is found throughout the world with comparatively few species occurring in North America. Considered here are those six limacids which have been reported from the eastern United States. Actually, the same six species are those which are most numerous throughout all of North America. Five of them have been introduced into this country and it is of interest to compare their observed behavior here with that reported by European malacologists (Taylor, 1907; Gerhardt, 1933–1937).

Gerhardt (1936) suggests that the copulatory position is similar in all species of slugs and that it orginates with the shelled ancestors. Since Pilsbry (1948) claims that slugs of the Limacidae are not even closely related to those of the Arionidae, apparent similarities between the copulatory positions of species of these two families probably are related to the fact that only a limited number of positions in which two hermaphroditic organisms can achieve cross-fertilization are possible. One even can find similarities in the mating postures of such distantly related organisms as annelids and snails. Obviously, such similarities are only matters of chance. As this paper indicates, there are actually significant variations in courtship and mating behaviors among the species of a single family of mollusks.

Deroceras reticulatum (Müller)

Mating pairs of *D. reticulatum* have been observed in the field on many occasions. No matings between captive specimens were ever observed but fertile eggs have been obtained from captive slugs. Insemination may have preceded their capture, however, since fertile eggs can be laid for many weeks after a single insemination. It is possible that confinement in small containers prevented mating since an elaborate courtship ritual precedes coition, a ritual which usually takes place in unobstructed circular areas several inches in diameter. Taylor (*l.c.*), Hawley (1922) and Pilsbry (*l.c.*) have discussed this ritual although not in detail.

All courtships observed by this author have been at night. Mating pairs are usually found on open areas of soil or vegetation and no attempts at concealment are made by this species. Three distinct types of actions constitute the courtship behavior. These may be designated as positional movements, excitatory movements, and so-called pugnacious activities.

Positional movements usually cover a circular area some two to three inches in diameter. The slugs normally lie along the periphery of the circle, facing in opposite directions. Crawling in a clockwise pattern

maintains the same relative position between the two slugs, *i.e.*, the right side of each is opposite the right side of the other. These crawling movements are most apparent early in the courtship ritual. Occasionally, one slug partially may overtake the other. The circular crawling pattern is always maintained, however, and areas in which matings have occurred may be recognized by the circular patches of slime which remain after mating has been completed. After courtship has proceeded for some time, one diameter of the circle may be decreased so that an oval is described by the slugs whose right sides may even come into contact with each other.

Excitatory movements are initiated during the later stages of crawling. *Deroceras reticulatum* exserts a pear-shaped excitatory organ or sarcobelum from an orifice just behind the right tentacle. This organ is used by each slug to stroke the other, these movements increasing in frequency and the size of the sarcobelum somewhat expanding until the actual transfer of sperm is accomplished.

Interspersed in all of the foregoing are certain apparently pugnacious movements of *D. reticulatum*. Periodically, each individual suddenly thrusts forward its head and strikes the other slug. This apparently pugnacious action appears to represent one slug feeding upon the slime of the other or "working" it in the manner that one "kneads" dough. It has been suggested that preliminary slime feeding is necessary for the production of the modified slime thread upon which *Limax maximus* hangs while mating. *Deroceras reticulatum* obviously does not eat slime for such a purpose. Slime-feeding or pugnacious actions have been noted in other genera of slugs and in some snails (Webb, 1950), thus suggesting that such behavior is an integral part of molluscan mating patterns.

Courtship may continue from one-half hour to almost two hours. Many researchers have associated the length of courtship with temperature of the environment. While it is true that external temperatures affect the activity of slugs, the authors have observed that simultaneous matings within a few feet of each other and under presumably similar conditions have varied three-fold in duration.

Sperm transfer requires less than one minute in *D. reticulatum*. The excitatory organs of both slugs previously have become swollen and interlaced. Finally, the organs are violently rotated back and forth while intertwined. Large quantities of clear slime are produced and the sarcobela reach their greatest tumescence. In some instances only the bases of the sarcobela are appressed but the increase in turgidity, the production of slime and the violent rotation all occur. Sperm transfer is accomplished in small slime balls which are, according to Taylor (1907), . . . "the forerunners of true spermatophores." Shortly after sperm transfer the slugs separate and crawl off.

Five matings of this species have been observed in which three slugs took part in the courtship. Invariably, all three slugs extended their sarcobela and stroked each other with them. Only two slugs, however, actually completed sperm transfer in each instance.

Lovett and Black (1920) indicated that eggs may be expected within 12 to 40 days following mating. After the first eggs are produced other clutches may be laid without refertilization over a period of from three to four weeks. Lovett and Black also claimed that *D. reticulatum* buries itself and becomes quiescent one to three days before laying eggs. Miles

et al. (1931) reported this quiescent period to be much longer, often up to three weeks in duration. These observations are in direct contrast with those of this study where *D. reticulatum* was noted to feed and move actively until the actual deposition of eggs commenced.

Several pairs of *D. reticulatum* were collected immediately after having mated in the field. These slugs were held at fluctuating temperatures averaging 70 degrees F. Fertile eggs were laid over a period of time ranging from three to eight days after coition. Several of the mated slugs were held as long as 35 days without any eggs being laid. One slug laid separate clutches of eggs on the fourth and the eight days after it had mated.

Deroceras reticulatum was observed engaged in egg-laying beneath an inverted clay pot in a greenhouse bench. More than 60 eggs had already been laid in a cluster when the pot was raised. Eggs became visible and were extruded from the genital orifice in from two to 30 seconds. Intervals of three to five minutes separated the depositions of the eggs which were laid singly with one exception when two eggs were laid in rapid sequence. The slug was observed to lay eggs when in a semi-contracted position with its tentacles withdrawn and while crawling with its tentacles fully extended. Crawling apparently was initiated by the disturbance of lifting the clay pot since the eggs laid prior to that time were neatly piled in a single cluster. A total of 73 eggs were deposited. The eggs were almost round and varied from 1.3 to four mm. in diameter. They were watery and translucent with some sculpturing of an irregular pattern on their surface. Eggs of this species were found to be extremely variable in shape. Over 500 eggs were obtained at various times. These ranged from round to oval and many had pointed ends. Karlin and Naegele (1958) illustrated eggs of this and other species. Those with attenuated ends were often attached by gelatinous threads at the points, thus forming sausage-like strings of eggs. The eggs also varied in opacity. They were usually clear, watery and translucent in appearance but occasionally almost white, opaque eggs were found.

Temperature influences the amount of time required by eggs for development. Frömming (1954) reported that eggs of this species hatched in 16 to 28 days at "laboratory temperatures". Carrick (1942) showed that oviposition and egg-development will not occur below 41 degrees F. This temperature was thus established as the threshold of development. At this temperature development took 105 days, but at 68 degrees F. only 18 days were required.

In this study, less than five percent of the eggs laid by *D. reticulatum* actually hatched. Those eggs which did develop hatched in 15 to 24 days at average temperatures of from 70 to 72 degrees F.

Deroceras laeve (Müller)

Deroceras laeve has a courtship behavior similar to that described for *D. reticulatum*. Frequently, however, the duration of courtship is shorter and the slugs do not come into as intimate bodily contact. *Deroceras laeve* normally is a more seclusive slug than *D. reticulatum* and mating occurs beneath dead leaves, boards and other objects instead of in exposed areas.

Eggs may be laid as shortly as three days after mating (Frömming,

l.c.). Young slugs often produce fewer eggs in the first clutch than in subsequent clutches. In this study, 17 different clutches of eggs were obtained ranging from one to 33 eggs per clutch. The eggs varied in size from one to three mm. and in shape from oval to round. In general, they resembled the eggs of *D. reticulatum* although none were found connected in gelatinous strings as in that species. All of the eggs obtained from *D. laeve* were found on the soil surface, beneath flower pots, boards, and other objects.

Frömming (*l.c.*) listed data from 31 egg clutches of *D. laeve*. He recorded the length of time to hatching and the temperature ranges at which the eggs were held. Seventeen eggs which were held at a temperature range of from 28 to 39 degrees F. hatched approximately 100 days after being laid. Three other clutches were held at somewhat higher average temperatures but with minimum readings of 27 and 25 degrees F. These three clutches failed to hatch, thus suggesting a lethal minimum temperature of near 27 degrees F. In this study four clutches of eggs held at average temperatures of from 71 to 73 degrees F. hatched in ten to 15 days with the largest hatch on the 12th day. This is in marked contrast to eggs held at similar average temperatures by Frömming which took 17 to 20 days to hatch. The disparity may be the result of varietal differences in European and American *D. laeve* but also could result from the use of average temperature figures. If one uses only maximum and minimum readings to compute averages, identical temperature means can occur while actually the intermediate temperatures, at which the eggs are held most of the time, can differ greatly from each other.

Milax gagates (Drap.)

Only fragmentary observations were made of the mating of this species. Excitatory organs, similar in appearance to those of *Deroceras*, were used by the slugs in mutually stroking each other. It is likely that a lengthy courtship period occurs. The longest fraction of a courtship period observed by the authors, however, lasted for only 12 minutes and did not culminate in sperm interchange.

Eggs were laid by large specimens of *M. gagates* on 15 occasions. The slugs had been in captivity from ten to 45 days when the eggs were laid. Mating may have occurred in captivity although it was never observed. Two slugs which were born and reared together in the laboratory laid fertile eggs 100 days after birth, thus providing a possible indication of mating. The slugs which laid the eggs measured 19 mm. or less than one-third of their potential full size. Another *M. gagates*, which had been kept in isolation from birth, also laid fertile eggs 71 days later, thus indicating that self-fertilization or parthenogenesis must have occurred. *Milax gagates* laid eggs singly or in clusters of as many as 16 eggs. The eggs were laid below the soil surface in tunnels which were approximately the diameter of the extended slug's body with which they were shaped. The bottom of a tunnel was generally about three cm. below the surface and the maximum length of a tunnel, which descended as a gradual incline, was five cm. After the tunnels were formed, the lowest lying eggs were laid first, others being piled upon the preceding ones as the slug withdrew from the tunnel. Generally, egg-laying ceased before the tunnel was filled but on one occasion eggs were deposited upon the soil surface

around the mouth of an egg-filled tunnel. The tunnel mouth was plugged with a dark-colored, mucoid material on several occasions. When dried, this material camouflaged the entrance to the tunnel and may have been produced for that purpose. Occasionally, eggs were found under a clod of soil rather than in a tunnel.

Generally, freshly laid eggs were moist and adhered to each other, but the eggs were never attached to each other in the manner of those of *D. reticulatum*. The eggs were oval and measured from 1.5 to four mm. in length. Within most clutches, the eggs were of uniform size. Large slugs laid larger eggs than small slugs did. When laid, the eggs were somewhat translucent and of a watery appearance but they were more opaque than most slug eggs. Those eggs which were most watery and translucent showed very few bumps or malleations of any sort on the shell surfaces. In contrast, some quite opaque and whitish eggs were found which most closely resembled frosted window glass. All eggs of a single cluster were similar in appearance.

Forty-four of 119 eggs or 38 percent developed until eclosion. Eggs, held at approximately 72 degrees F., hatched on the 11th to the 24th day after being laid. The longest hatching span for eggs from a single clutch extended from the 11th to the 19th day. In all other instances the eggs of a single clutch hatched within two days of each other.

Limax poirieri Mab.

The authors believe that this name has not been reported previously in the American literature. Consequently, a short explanation of the reasons justifying its use appears to be desirable. Henrik Walden of the Zootomiska Institute in Stockholm has two papers in press in which he discusses differences in the morphology of *L. marginatus* Müller and *L. poirieri* Mab. He considers, as did Van Regteren Altena (1950), that the slugs illustrated by Pilsbry (*l.c.*) under *L. marginatus* are *L. poirieri*. At the same time that Walden was making his anatomical studies, the senior author became convinced on the basis of behavioral characteristics that *L. marginatus* is probably not found in the eastern United States. *Limax marginatus* is considered to be synonymous with *L. arborum* Bouchard-Chantereaux and, as the latter name implies, is reportedly strongly arboreal in its habits in Europe. Karlin (1957) indicated that *L. marginatus* (really *L. poirieri*) from New York State was a slug of the soil surface rarely climbing even low plants in greenhouses. The apparent contradiction in observed behavior is resolved as a result of Walden's studies. Mating of this species was never observed although it may be inferred from the foregoing discussion that it takes place on the ground.

One hundred and eighty-six eggs were obtained in clutches of one to 63 eggs. Of these, seven eggs hatched in 13 to 14 days at an average temperature of 68 degrees F. while the length of the incubation period is unknown for other eggs which hatched over a span of eight days. The eggs were all between two and 2.5 mm. in length and oval in shape. Some were slightly asymmetrical, being more pointed at one end than the other. A distinct sculpturing of wavy wrinkles formed a definite lined pattern which encircled the eggs and was visible under low magnification. The eggs were watery in appearance and translucent.

Limax maximus L.

Gerhardt (1933, 1934) described and pictured the complex aerial mating of European specimens of *L. maximus*. Observations made during this study showed no major behavioral variations in American specimens. Preliminary courtship actions consisted of circular crawling motions, repeated body contacts and the pugnacious or slime-eating actions described for *Deroceras reticulatum*. After a period of excitation, accomplished without sarcobela which are not found in the genus, the slugs intertwined their bodies and crawled over the edge of a ledge. A thick slime thread supported both slugs which were hanging suspended several inches below the point of attachment of the slime thread. The penes were extruded and intertwined. These measured three inches or more in length. Ten to 15 minutes were spent with the slugs thus suspended. Copious slime production accompanied rotating motions of the penes which were then gradually withdrawn as the slugs climbed the mucous thread following copulation. Some authors have also reported the slugs to drop to the ground following copulation.

A survey made by the senior author in more than 100 greenhouses indicated that *L. maximus* is quite solitary in habits, single specimens often being collected. Cannibalism has also been reported for this species. These factors, plus its size and its mating habits, make *L. maximus* difficult to rear in the laboratory where eggs of this species were never obtained. Desiccated eggs of this species were collected, however, after five days of exposure to the sun. They were shrivelled and hard and made an audible noise when dropped. Within three to six hours, after moistening, all had regained their shape and gelatinous consistency. The eggs were held for 43 days after which they collapsed without developing. Each egg was globular, five mm. in diameter and translucent with a watery appearance of the shell and contents.

Limax flavus L.

The complete mating behavior of *L. flavus* has never been observed by the authors. Limited observations indicate that it is terrestrial in nature although it may occur on tilted or vertical surfaces as well as horizontal ones. Excitatory, circular crawling as well as pugnacious movements have been observed. Frömming (*l.c.*) indicated that courtship may endure only seven or eight minutes. Thus, this is the shortest duration of such behavior amongst the species discussed.

Most slugs have been observed to lay eggs at night. This habit apparently is related to environment conditions however, which, in turn, initiate periods of activity and quiescence. Under suitable conditions, slugs will lay eggs at any time of the day and those clutches produced by *Limax flavus* during this study were always deposited, at least in part, during daylight hours.

Over 138 eggs were laid by captive specimens in clutches of 12 to 32 eggs. One *L. flavus* laid 12 eggs during a period of three hours. The eggs became visible at the genital orifice and were deposited in a few seconds but the intervals between the appearance of eggs averaged 15 minutes in length, a much longer time than that noted for *D. reticulatum*.

Egg clutches of *L. flavus* were frequently partially buried. Unlike *M. gagates*, which created distinct tunnels in which to deposit its eggs, *L.*

flavus appeared to utilize fissures and depressions which were already present in the soil. Those eggs which were deposited last were usually laid above the soil surface.

Eggs of *Limax flavus* measured 6.3 by four mm. and varied slightly in shape. In some, the outer gelatinous layer was a little thicker at the apices of the egg, causing it to appear more elongate. The albumen and embryo, however, enclosed by the inner membrane were uniform in size in each egg. The eggs of *L. flavus* were joined together by strands of mucous which were continuations of the outer membranes. During the laying process the strands sometimes were broken, but as many as ten eggs attached together in this manner have been found.

Discussion, Conclusions, and Summary

The mating and egg-laying behavior of six species of limacids has been described. Three of these, in the genera *Deroceras* and *Milax*, possessed evertible excitatory organs or sarcobela which were used in preliminary courtship actions. Members of the genus *Limax* lacked these organs but engaged in similar patterns of early courtship. *Limax maximus* copulated in mid-air while suspended from a slime thread. *Limax poirieri* probably mated on the soil since it is terrestrial in its behavior. This behavioral characteristic, plus anatomical differences, has been used as a basis for the introduction of this name into the American literature to replace *L. marginatus* of Pilsbry (*l.c.*). *Deroceras reticulatum* mated on open soil areas whereas *D. laeve* followed similar behavioral patterns in concealment. Courtships involving three individuals occurred occasionally in *D. reticulatum* but were never noted in the other species.

An instance of parthenogenesis or self-fertilization was noted in *Milax gagates*. Similar records for other species are scattered throughout the literature suggesting that this is a rare but regular occurrence.

All of the slugs showed cryptic propensities in the choice of egg-laying sites. *Milax gagates* completely buried its eggs while *L. flavus* partially did so. All of the other species almost invariably laid their eggs beneath stones, boards, soil clods or decaying vegetation. In greenhouse and laboratory situations, eggs were also laid in contact with the sides of tanks, jars and inverted flower pots.

Slug eggs showed many interspecific differences and even the eggs of a single species varied in size, shape and opacity. Eggs of a single clutch were usually similar, however. When differences did occur they were associated with the outer gelatinous envelope and the albumen and embryos within the inner egg membranes were uniform in appearance. Calcium particles in the egg shells produced varying degrees of opacity and eggs tended to become more translucent as the developing embryos within utilized the calcium granules to form their internal shells.

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HYPERTHERMIA AS A FACTOR IN CYTOLOGICAL FIXATION OF THE OYSTER MANTLE

RALPH M. COMBS
Gulf Coast Research Laboratory, Ocean Springs, Mississippi
and
Northwestern State College of Louisiana, Natchitoches, Louisiana

While conducting studies on the cytology of the storage of glycogen and lipids in the pallium of the oyster, the author (1959) found that the literature pertaining to the effects of elevated temperature on the action of killing fixing agents was very limited. This suggested the use of the oyster mantle, the epithelial cells of which are very responsive to alterations in technique, as a favorable tissue to evaluate hyperthermic fixation.

Some investigations have shown that temperature increases enhance certain forms of cytological fixation. Sakamura (1927) found that boiling water gave good results in removing the chromosomal matrix thus exposing the chromonema. Elftman (1954) described the improvement of lipoid oxidation in tissues by dichromates when carried out with heat at certain concentrations and pH. In the study of the fixation of the ganglia of