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THE SHAPE OF CALCIUM CARBONATE DEPOSITS AS AN EXTERNAL MARKER FOR FEMALE REPRODUCTIVE STATUS IN TERRESTRIAL ISOPODS

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ABSTRACT

The shape of sternal calcium carbonate deposits (CCD) preceding moult was used as a diagnostic character to distinguish between reproductive and non-reproductive females in six species of terrestrial isopods belonging to different families. Samples in the wild at the beginning of the reproductive season revealed females with CCD of incomplete shape, with a diamond-shaped opening at the level of central furrow. All the females showing this pattern made a parturial moult a few days later, while all females showing complete CCD made a normal moult. A laboratory experiment confirmed that these characteristics were diagnostic for females performing a secondary vitellogenesis. These females were receptive to mating at that time, provided the stage of the intermoult cycle was not too early. Screening the CCD shape is therefore a non-destructive method that can be used for routine diagnosis of females' reproductive status in terrestrial isopods.

In most crustaceans, moulting continues during the whole of adult life, a process required for growth, and often for reproduction (Skinner, 1985). In terrestrial isopods, there is a perfect coordination between moulting and reproduction in females (Steel, 1980). Female reproduction is synchronous to a parturial moult (PM), during which there is a differentiation of lamellar structures (oostegites) at the first five pereion segments. These oostegites form a brood pouch (the marsupium) in which eggs are laid and incubated during one month. Before this special moult, ovarian maturation occurs due to the accumulation of vitellogenin (secondary vitellogenesis, oocyte diameter > 250 µm) (Besse, 1976). Females are receptive to mating only during this stage in the majority of species (Mead, 1976; Lefebvre and Caubet, 1999; Moreau and Rigaud, 2000). Males and non-reproductive females undergo normal moult (NM) for growth. In females, during the normal intermoult stages, oocyte maturation is blocked (oocyte diameter < 250 µm) by the Vitellogenesis-Inhibiting Hormone (Gohar *et al.,* 1984).

Until now, the only reliable means to distinguish breeding and non-breeding females before parturial moult was to measure their oocyte diameter. However, a simple and non-destructive tool allowing identification between these female types would be very useful to manage diverse experiments on the reproductive biology in terrestrial isopods. For example, during one of our studies of the mating capacity of males (Moreau and Rigaud, 2000), we lost a considerable amount of time and biological material by sometimes supplying to males some females not receptive to mating.

In terrestrial isopods, the premoult period can be estimated by the appearance of ventral white calcium carbonate deposits (CCD) in the first four sternites of the pereion (Zidar *et al.,* 1998). These deposits are the principal reservoir of calcium salts (Steel and Campbell, 1977), and have a function similar to gastroliths in crayfishes or terrestrial crabs (Stevenson, 1985). They can be used to approximate externally the different stages of the moulting cycle, because there are major changes in their size and shape with the approach of the ecdysis (Steel, 1982; Zidar *et al.,* 1998). Steel (1982), in laboratory rearing of the Oniscidae *Oniscus asellus,* noted a difference in shape of CCD between the female in normal premoult period and the females in parturial premoult period.

The aim of this study was to extend Steel's result to determine a general non-harmful identification between the reproductive and non-reproductive females for species of woodlice representative of various families.

MATERIALS AND METHODS

Species investigated belong to different families of Oniscidea (Isopoda) (Vandel, 1962): *Armadillidium nasatum* (Budde-Lund, 1879) and *Armadillidium vulgare* (Latreille, 1804) (Armadillidae); *Oniscus asellus* (Linné, 1758) and *Philoscia muscorum* (Scopoli, 1763) (Oniscidae); *Cylisticus convexus* (De Geer, 1878) (Cylisticidae); and *Porcellio scaber* (Latreille, 1804) (Porcellionidae).

Isopods were collected in the wild at the beginning of reproductive season (April and May 1999), near Poitiers (46°40′N). On each site, all visible animals under stones or leaf litter were gathered without size discrimination. They were sorted immediately after collection according to sex and stages of moulting cycle. Females with white calcium carbonate deposits (CCD) on sternites were carefully checked, and the shape of their CCD noted and drawn. They were then maintained individually at 20°C ± 1 until ecdysis, that occurred between 1 and 10 d following the collection. The type of moult (normal vs. parturial) was noted.

A second experiment was conducted in the laboratory. Individuals used in this trial were born at room temperature (20°C ± 1) during spring 1999 in the laboratory at Poitiers. No *P. scaber* and *C. convexus* were available for such a study. Four months after their birth, young were sexed, and males and females were reared separately to avoid sibling mating in rearing boxes (26 × 13 × 8 cm). Food (dead leaves and a slice of fresh carrot) was provided *ad libitum*. When animals reached their reproductive size (Moreau and Rigaud, 2000), females were separated in two sets. One set was maintained under natural photoperiod (October decreasing photoperiod) for females continuing growth moults (McQueen and Steel, 1980; Mocquard *et al.,* 1989). The second set of females was reared under the simulating photoperiod of LD 18:6, to promote the onset of reproduction and induce the appearance of parturial moults (McQueen and Steel, 1980; Mocquard *et al.,* 1989). After one month of these treatments, each female differentiating CCD was paired with one male to control her mating receptivity (size homogamy respected). Before this breeding experiment, the shape of CCD of each female was noted. Pairing was done in a cylindrical box (diameter 8 cm), with moistened soil and a piece of dead leaf, at 20°C, at night, during 12 h (time sufficient for mating to occur; see Mead, 1976). Each female was then dissected to check her insemination status. Inseminated females were characterised by the presence of a white ball of sperm in their genital ducts, while oviducts of non-mated females were thin and transparent. The diameters of 10 oocytes were measured under a microscope (obj. × 10), using an eyepiece micrometer to determine the average oocyte diameter.

RESULTS

The examination of wild-collected females revealed two major categories of calcium carbonate deposits (CCD) according to their shape. These two groups could be distinguished, whatever the species, after the stages 4 or PE1 of the moulting cycle—as defined respectively by Steel (1982) and by Zidar *et al.* (1998) (e.g., Fig. 1). The first group showed large CCDs, where the central zone of each segment (where left and right sternites are in contact) was completely white, leading to the visual impression of a bilateral continuity (Fig. 1b, d). This group will be referred as CCD1. The second group was characterised by CCDs incomplete in the central zone of segments 2 to 4, leading to a visual impression of a diamond-shaped opening at the level of central furrow (Fig. 1a, c). The CCDs were also smaller than those of the first group. This group will be referred as CCD2. The CCD shapes were very similar in *Armardillidium nasatum* and *Armardillidium vulgare,* but those of *Porcellio scaber, Cylisticus convexus, Oniscus asellus,* and *Philoscia muscorum* were slightly different (Fig. 1). The dissection of five *O. asellus* and three *A. vulgare* females with CCD2, at the

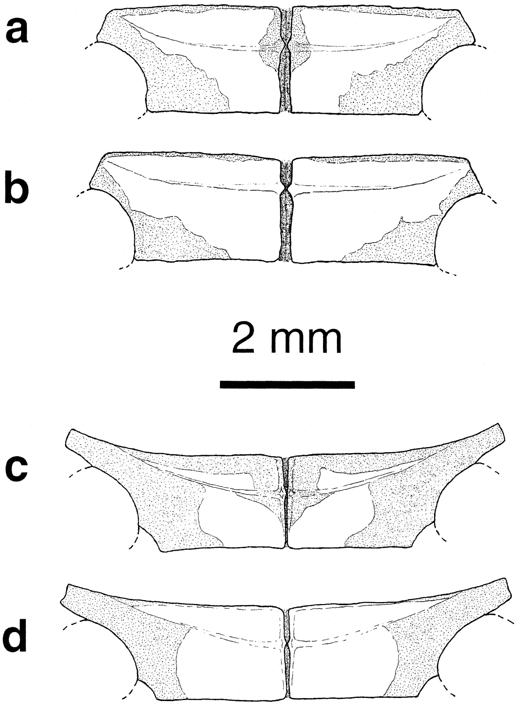

Fig. 1. Schematic drawings of calcium carbonate deposits (CCD) preceding moult in two terrestrial isopod species, at PE3 stage of the premoult cycle (as defined by Zidar *et al.*, 1998). a, b: third pereion segment of *Porcellio scaber;* c, d: second pereion segment of *Armadillidium vulgare*. a, c: incomplete CCD (CCD2 type); b, d: complete CCD (CCD1 type).

Table 1. Correlation between the shape of the calcium carbonate deposits (CCD) and the moult type observed in wild-caught females in six species of Oniscidea. CCD1: females with complete calcium carbonate deposits; CCD2: females with incomplete calcium carbonate deposits; NM: normal moult; PM: parturial moult.

	Number of females			
	CCD1		CCD2	
Species	NM	PM	NM	PM
A. nasatum	41	0	0	30
A. vulgare	38	0	0	23
C. convexus	6	0	0	11
P. scaber	38	0	0	47
O. asellus	64	0	0	40
P. muscorum	67	0	0	35

end of the PE3 stage of the premoult period (Zidar *et al.,* 1998), revealed a very thin and folded epidermis of the new cuticle, at the level of the diamond-shaped opening. After the parturial moult, this zone corresponded to the location of a cotyledon within the marsupium, i.e., the finger-like extension of the intersegmental membrane allowing water and nutrients exchanges between the mother and the embryos (Hoese and Janssen, 1989).

Sorting females using the CCD shape showed that moults following CCD1 were always normal moults whereas those following CCD2 were always parturial moults, whatever the species (Table1). The measure of oocyte diameters in the laboratory confirmed these data. The CCD1 shapes were found in females in primary vitellogenesis (oocyte diameter < 250 µm), whereas CCD2 shapes were found in females undergoing a secondary vitellogenesis (oocyte diameter > 250 µm), even if differences in oocyte diameter between species are significant (Fig. 2) (two-way ANOVA testing the effects of the species and CCD shape on oocyte diameter: Whole model: $F_{7,116} = 48.30$, $P < 0.0001$; Effect of species: $F_{3,116} = 5.97$, $P < 0.0009$; Effect of CCD shape: $F_{1,116} = 305.37$, $P < 0.0001$; Effect of the interaction species*shape: $F_{3,116} = 0.22$, $P > 0.85$). Overall, this pattern was a good indicator of female mating receptivity because most females were mated when they differentiated CCD2, whatever the species (logistic regression testing the effects of the species and CCD shape on the female mating status: Whole model: Likelihood-Ratio $\chi^2_7 = 112.16$, $P < 0.0001$; Effect of the species: L-R $\chi^2_3 = 1 \cdot 10^{-6}$, $P > 0.95$; Effect of the CCD shape: L-R $\chi^2_1 = 108.75$; $P < 0.0001$; Effect of the interaction species*shape: L-R $\chi^2_3 = 1 \cdot 10^{-6}$, $P > 0.95$). However, this relationship was not strict, because between 7.7% and 17.6% of females were not inseminated (3/19 for *A. nasatum,* 3/17 for *A. vulgare,* 2/16 for *O. asellus,* and 2/26 for *P. muscorum*) (Fig. 2). Previous studies have shown that oocyte maturation is a necessary condition for females to be receptive to mating, but also that a minimal oocyte diameter must be reached before female mating. This minimal diameter varied according to the species: 260 µm for *A. vulgare,* 350 µm for *A. nasatum,* 250 µm for *O. asellus,* and 320 µm for *P. muscorum* (Lefebvre and Caubet, 1999; Moreau and Rigaud, 2000). In the present experiment, the minimal diameter was not reached for most of the non-mated females differentiating CCD2. These non-mated females were at the very beginning of the intermoult stage PE2 (Zidar *et al.,* 1998).

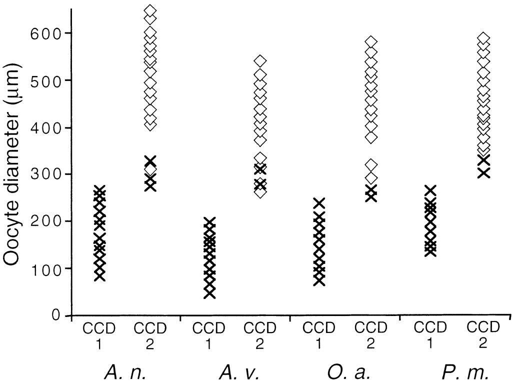

Fig. 2. Relationship between calcium carbonate deposits (CCD) shape, the maturation stage of oocytes and the mating status of females in four species of terrestrial isopods. Crosses denote non-inseminated females; diamonds denote inseminated females. CCD1: complete CCD; CCD2: incomplete CCD. *A.n.*: *Armadillidium nasatum*; *A.v.*: *Armadillidium vulgare*; *O.a.*: *Oniscus asellus*; *P.m.*: *Philoscia muscorum.*

DISCUSSION

This study provided a diagnostic difference in shape of the calcium carbonate deposits (CCD) preceding moult, between breeding and non-breeding females in several species of terrestrial isopods. This difference consisted in less complete CCD in females in preparturial intermoult, showing a diamond-shaped opening at the level of central furrow. This opening was distinguishable at the early stages of the appearance of CCD. These results extend the observation of Steel (1982) to several families of terrestrial isopods, even if differences in general CCD shapes are observed between species. Dissections suggested that the incomplete CCD2 shape is associated with the differentiation of cotyledons. The position of the cotyledons is consistent with this hypothesis: they are always found centrally in segments 2 to 5 within the marsupium, whatever the Oniscidea family (albeit additional cotyledons can be found laterally in some species or families) (Lewis, 1990). They are not present in segment 1 in the species studied here (Lewis, 1990), which match with our observation that CCD on segment 1 were always complete.

This external difference in CCD before the moult will be very useful during studies on the reproduction of terrestrial isopods. The reproductive state of a given female can be routinely determined, before her parturial moult, by a brief inspection (one or two seconds) of the first five sternites of the pereion under a microscope. The difference is even visible by eye on large females. In addition, the CCD shape allows one to rapidly distinguish females receptive to mating, provided the moulting stage is not too early. We used this non-destructive method successfully in a laboratory experiment on sexual selection, where competition between females receptive to mating was required (Moreau *et al.,* 2001). This diagnostic character would also permit one to follow the dynamics of female's onset of reproduction in wild populations, without dissection, and also to calculate the time during which females are receptive to mating in the wild.

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