Fate of glair glands and oocytes in unmated crayfish: a comparison between gonochoristic slough crayfish and parthenogenetic marbled crayfish

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Abstract

In the period before spawning, freshwater crayfish develop glair glands on the underside of the pleon. These glands produce the mucus for a tent-like structure in which the eggs are fertilized and attached to the pleopods. Long-term observation of females of slough crayfish, *Procambarus fallax*, kept in the laboratory without contact to males revealed that glair glands developed in late winter and early autumn of each year. However, in contrast to mated females, unmated females never formed fertilization tents and never spawned. Their glair glands persisted for an unusually long period of time and disappeared only during the next moult. Inhibition of spawning and mucus release from the glair glands suggests that the females had information on sperm availability and saved resources when unmated. Marbled crayfish, *Procambarus virginalis*, a parthenogenetic descendant of slough crayfish, developed glair glands and spawned in spring and autumn as their mother species although they never mated. These findings suggest that on their way from gonochorism to parthenogenesis regulation of spawning and glair gland activity has been decoupled from mating and sperm transfer.

Key words: freshwater crayfish, spawning, glair glands, mating, *Procambarus fallax*, *Procambarus virginalis*.

Introduction

The final phase of ovarian maturation in freshwater crayfish is externally reflected by the development of whitish glair glands on the underside of the pleon (Reynolds 2002). These glands are located in the last thoracic sternal plates, the sterna and pleura of the pleon, the

pleopods and the uropods (Figure 1A) and terminate with many pores on the underside of the thorax and pleon. Glair glands first appear some weeks before spawning as faint creamy-whitish patches and become more and more distinct thereafter. Thus, they are good indicators of forthcoming spawning.

Immediately before egg laying the glair glands release a translucent mucus that forms a tent like structure on the underside of the thorax and pleon. Within this gelatinous mass the sperm is mobilized from the spermatophores that are either externally attached (Figure 1A) or internally stored (Figure 1B), depending on family. The fertilization tent further secures fertilization of the eggs and attachment of the zygotes to the pleopods, where they are brooded until hatching of the first juvenile stage (Reynolds 2002; Vogt and Tolley 2004; Niksirat et al. 2013). Glair glands and fertilization tent are unique features of freshwater crayfish and occur in all three families, the Astacidae, Cambaridae and Parastacidae (Andrews 1906; Mason 1970; Thomas and Crawley 1975; Jussila et al. 1996). Simpler cement glands that facilitate attachment of the eggs to the pleopods only occur in other Macrura and Stomatopoda (Adiyodi and Anilkumar 1988).

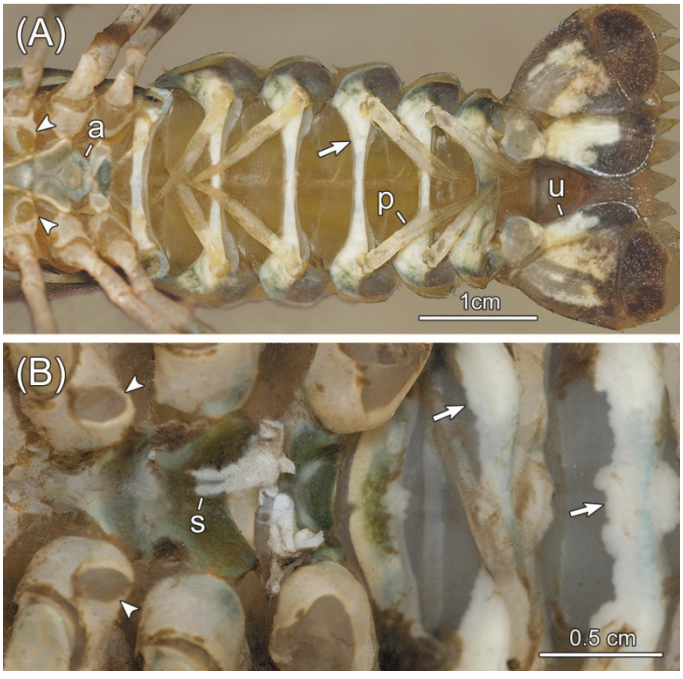

Figure 1. Glair glands and sperm storage in cambarid and astacid crayfish (photos: Lucian Pârvulescu). (A) Cambarid *Orconectes limosus* with mature glair glands in pleonal sternites (arrow), pleopods (p) and uropods (u). Sperm is internally stored in the *annulus ventralis* (a) close to the gonopores (arrowheads). (B) Astacid *Austropotamobius torrentium* showing mature glair glands in pleonal sternites (arrows) and spermatophores (s) externally attached to thoracic sternal plates near the gonopores (arrowheads).

There is only little information on what happens with the glair glands and the mature oocytes if crayfish females remain unmated. One possibility is that unmated females spawn like mated females but the eggs do not develop. Alternatively, the mucus and mature oocytes may be resorbed to avoid wasting of energy and resources. In the present work, these hypotheses were tested in laboratory-reared slough crayfish. Moreover, the fates of glair glands and oocytes in unmated slough crayfish were compared with parthenogenetic marbled crayfish that generally reproduce without males (Vogt et al. 2015).

Materials and methods

Three females of slough crayfish, *Procambarus fallax* (Hagen 1870), were raised in the laboratory from the egg stage for 22 months without contact to males. In the first 7 months they were kept in a female group and thereafter they were kept individually. Another three females from the same batch were paired with males during the reproduction seasons in spring 2015, autumn 2015 and spring 2016. The crayfish were kept in plastic containers of 30 x 25 x 20 cm equipped with gravel and shelter. Tap water was used as the water source and replaced once a week. Water temperature fluctuated from ~15°C in winter to ~25°C in summer and photoperiod was natural. The crayfish were fed with TetraWafer Mix pellets *ad libitum*. The observation period lasted from July 1, 2014, to March 31, 2016.

The data on the fate of the glair glands and oocytes in slough crayfish were compared with my records on these structures in marbled crayfish, *Procambarus virginalis*, a partheno-genetic descendant of slough crayfish (Martin et al. 2010). Marbled crayfish were raised under the same conditions as slough crayfish.

Results

In the three reproduction periods analysed, the unmated females of *Procambarus fallax* never spawned although they regularly developed glair glands (Table 1). In contrast, the mated females generally laid eggs. In both mated and unmated females, the glair glands developed in the three to four weeks before spawning. In mated females, they were emptied immediately before egg-laying to form the fertilization tent but in unmated females they persisted another 3 to 5 weeks until the next moulting. They finally disappeared during ecdysis.

Laboratory reared marbled crayfish usually reproduced twice a year like slough crayfish. They also showed glair glands with increasing intensity 2-4 weeks prior to spawning. Marbled crayfish with prominent glair glands generally produced fertilization tents and spawned,

despite the absence of males. Only in one out of more than one hundred cases a female with glair glands skipped spawning and moulted instead. The glair glands disappeared during ecdysis as in the unmated slough crayfish.

Table 1. Fate of glair glands and oocytes in unmated and mated *Procambarus fallax*.

Females	Spring 2015		Autumn 2015		Spring 2016	
	glair glands	spawning	glair glands	spawning	glair glands	spawning
Unmated (n=3)	7-8 wk	–	6-9 wk	–	7-9 wk	–
Mated (n=3)	2-4 wk	+	3-4 wk	+	3-4 wk	+

Discussion

There is only little information on the fate of the glair glands and oocytes in unmated females of freshwater crayfish. Our findings on the cambarid *Procambarus fallax* revealed that spawning is suppressed in unmated females and that the glair glands are not emptied. The glair glands disappear during the next ecdysis and the oocytes are probably resorbed. Inhibition of spawning was also observed in unmated astacids *Austropotamobius pallipes*, *Astacus leptodactylus* and *Pacifastacus leniusculus* (Holdich et al. 1995). In contrast, Woodlock and Reynolds (1988) observed normal spawning but decay of the attached eggs in females of *Austropotamobius pallipes* held in female pairs. Inhibition of spawning and glair secretion in unmated females is interpreted as an energy saving strategy.

Maturation of the oocytes and glair glands in crayfish is independent from the presence of males as shown by all experiments. It is rather governed by environmental cues, mainly temperature and photoperiod (Reynolds 2002; Vogt 2015). Ovarian maturation is thought to be negatively and positively controlled by two hormones, the vitellogenesis-inhibiting hormone (VIH) from the X-organ-sinus gland system in the eyestalks and the ovary-stimulating hormone (OSH) from the thoracic nerve ganglia (Reynolds 2002; Vogt 2002). Maturation of the glair glands are probably regulated by the same hormones.

Reynolds (2002) assumed that spawning may be triggered by mating. However, there are often many days or even weeks between mating and spawning. Interestingly, in the experiments by Holdich et al. (1995) females paired with males from another species spawned

although the eggs had no chance to develop. This observation suggests that spawning depends either on mating or the availability of sperm. In astacid and parastacid crayfish, the spermatophores are externally attached to thoracic sternal plates, and thus, inseminated females could easily feel the sperm with their pereiopods. In our *Procambarus* species, in which sperm is stored in the *annulus ventralis*, mechano- and chemoreceptors may provide information on the presence of sperm.

It is unknown whether crayfish can voluntarily empty the glair glands and ovaries by deliberate contraction of the respective musculature or if these processes are involuntarily elicited by hormones. The latter possibility is more likely. A candidate for hormonal regulation of spawning and glair secretion is the prostaglandin $F_{2\alpha}$, which increases sharply in the final stage of oogenesis. Spaziani et al. (1995) revealed by *in vitro* tests with *Procambarus paeninsulanus* that this hormone induced contraction of the ovarian tissue in a dose dependent manner (Spaziani et al. 1995)

There are interesting differences between unmated females of the bisexually reproducing slough crayfish and the parthenogenetic marbled crayfish. In the laboratory, marbled crayfish mostly reproduce twice a year like slough crayfish (Vogt 2015) but these events are independent of mating and the availability of sperm, simply because there are no males. In more than 10 years, I have recorded only a single case of spawning inhibition in marbled crayfish with glair glands, suggesting that the information system that allows distinction between mated and unmated conditions has been lost or changed during transition from gonochorism to parthenogenesis. These differences may facilitate the detection of hormones and genes that regulate spawning in freshwater crayfish.

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