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Glair glands and spawning 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 females develop glair glands on the underside of the pleon. These glands produce the mucus for a gelatinous tent-like structure in which the eggs are fertilized and attached to the pleopods. Long-term observation of females of the sexually reproducing slough crayfish, *Procambarus fallax*, kept in captivity revealed that the glair glands developed in late winter and late summer of each year independently of the presence of males. In mated females, they secreted their contents shortly before spawning. In contrast, unmated females of slough crayfish did neither empty their glair glands nor spawn. Their glands persisted for an unusually long period of time and disappeared only during the next moult. Apparently, slough crayfish females use information on sperm availability to either spawn or save the resources. Females of marbled crayfish, *Procambarus virginalis*, a parthenogenetic all-female descendant of slough crayfish, developed glair glands in approximately the same periods of the year but generally spawned despite of the lack of males. These findings suggest that in marbled crayfish glair secretion and spawning is decoupled from mating. Therefore, the species pair *P. fallax* and *P. virginalis* seems to be particularly suitable to investigate the regulation of spawning in freshwater crayfish.
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KEY WORDS: freshwater crayfish, glair gland, spawning, mating, *Procambarus fallax*, *Procambarus virginalis*.

Глиальные железы и вымет яиц раками без спаривания: сравнение раздельнополого болотного и партеногенетического мраморного раков

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РЕЗЮМЕ: Перед выметом яиц у пресноводных раков на нижней стороне плеона развиваются глиальные железы. Эти железы выделяют слизь, формирующую студенистые шатровидные образования, в которых яйца оплодотворяются и прикрепляются к плеоподам. Многолетнее наблюдение за содержащимися в неволе самками болотного рака *Procambarus fallax*, размножающихся половым способом, показало,

что глиальные железы этого рака развиваются в конце зимы и лета независимо от присутствия самцов. У самок после спаривания железы начинали секретировать перед выметом яиц. В отличие от болотного рака, железы партеногенетических самок мраморного рака не секретировали, и вымет яиц не происходил. Их железы сохранялись необычно долго и исчезали только в ходе следующей линьки. Можно предположить, что самки болотного рака используют информацию о наличии половых продуктов самцов для вымета яиц. Самки мраморного рака *Procambarus virginalis*, партеногенетического потомка самок болотного рака, развивают глиальные железы примерно в тоже время года, но, как правило, выметывают яйца при отсутствии самцов. Полученные данные указывают на то, что выделение глиальных желез и вымет яиц мраморного рака не связаны со спариванием. Таким образом, *P. fallax* и *P. virginalis* — пара видов пресноводных раков, удобная для изучения механизмов регуляции вымета яиц.
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КЛЮЧЕВЫЕ СЛОВА: пресноводные раки, яичная железа, нерест, спаривание, *Procambarus fallax, Procambarus virginalis.*

Introduction

In the weeks before spawning, freshwater crayfish develop prominent whitish glair glands on the underside of the pleon (Reynolds, 2002). These glands first appear as faint creamy-whitish patches and become more and more distinct, being good indicators of forthcoming spawning (Jussila et al., 1996). Shortly before egg laying the glair glands secrete translucent mucus that forms a tent like structure on the underside of the thorax and pleon (Andrews, 1904; Vogt, 2016). Within this gelatinous mass the sperm is mobilized from the spermatophores that are either externally attached or internally stored in a sperm receptacle, depending on crayfish family (Reynolds, 2002; Vogt, 2002; Niksirat et al., 2013). The fertilization tent further secures attachment of the eggs to the pleopods, where they are brooded until hatching of the first juvenile stage and beyond (Vogt, Tolley, 2004). 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, Crawley, 1975; Jussila et al., 1996; Vogt, 2016). Simpler cement glands that only facilitate attachment of the eggs to the pleopods also occur in other Decapoda (Adiyodi, Anilkumar, 1988).

During student courses I was often confronted with the question what happens with the glair glands and the oocytes if crayfish females remain unmated. Unfortunately, there is no published data on the fate of the glair glands in unmated females and only little information on the occurrence of spawning in this condition (Woodlock, Reynolds, 1988; Reynolds et al., 1992; Holdich et al., 1995; Tropea et al., 2010; Buřič et al., 2011). One possibility is that unmated females form a fertilization tent and spawn like mated females but the unfertilized eggs do not develop. Alternatively, the mucus precursors in the glair glands and the oocytes in the ovary may be reabsorbed to avoid wasting of resources. In the present communication, I report on the fate of the glair glands and the occurrence of spawning in mated and unmated slough crayfish, *Procambarus fallax* (Hagen, 1870), and unmated females of its close relative marbled crayfish. Marbled crayfish, *Procambarus virginalis* Lyko, 2017, is a parthenogenetic all-female species that originated from slough crayfish by autotriploidization and concomitant transition from gonochorism to parthenogenesis (Scholtz et al., 2003; Martin et al., 2010, 2016; Vogt et al., 2015). It was earlier named *Procambarus fallax* forma *virginalis* Martin et al., 2010 and has recently been raised to species rank due to reproductive isolation from *P. fallax*, significant genetic differences, and superior growth and fecundity (Vogt et al., 2015; Lyko, 2017).

Materials and Methods

Females of slough crayfish *Procambarus fallax* (Hagen, 1870) (Decapoda: Cambaridae) were separated from their male batch-mates in juvenile stage 5, the first life stage that enables external sex determination. In the following five months they were kept communally in a female group and thereafter individually. Three of these females were mated and another three females remained unmated during the reproduction seasons in late winter 2015, late summer 2015, and late winter 2016. Mating was achieved by pairing individual females with glair glands and individual males with hooks on the ischia of the 3rd and 4th pereopods, which indicate sexual active forms. Copulation was considered successful when the male had turned the female on the back and inserted its copulatory organ into the sperm receptacle (*annulus ventralis*) of the female for at least 30 minutes. All mated and unmated females were then kept individually 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 the natural ambient regimen of Heidelberg (Germany). The crayfish were fed daily ad libitum with TetraWafer Mix pellets. The observation period lasted from January 20, 2015, to May 31, 2016.

The time periods in which glair glands were visible were recorded. In Table 1, I give the time in weeks only because at the beginning of glair gland maturation it is difficult to decide whether development has already started or not. All specimens were checked daily for spawning, which was best visible by the appearance of eggs on the pleopods. The data on *P. fallax* were compared with my corresponding records on glair glands and spawning in the parthenogenetic marbled crayfish, *Procambarus virginalis*, which I have collected over the last 13 years. Marbled crayfish were raised under the same conditions as slough crayfish, either individually or communally.

Results

The females of both crayfish species developed glair glands in the reproduction seasons. These glands were located in the last thoracic sterna, the sterna and pleura of the pleon, the uropods, and the pleopods (Fig. 1A, B). The glair glands developed autonomously in the absence of males.

In the three reproduction periods analysed, the mated slough crayfish females generally laid eggs. Their glair glands were visible for about three to five weeks (Table 1) and the contents were secreted shortly before egg-laying to form

Table 1. Presence of glair glands and occurrence of spawning in unmated and mated females of *Procambarus fallax.*

Таблица 1. Наличие глиальные желез и вымет яиц у неспаренных и спаренных самок *Procambarus fallax.*

Females	Winter 2015		Summer 2015		Winter 2016	
	glair glands	spawning	glair glands	spawning	glair glands	spawning
Unmated (n=3)	7–8 wk	no	6–9 wk	no	7–12 wk	no
Mated (n=3)	3–4 wk	yes	3–4 wk	yes	3–5 wk	yes

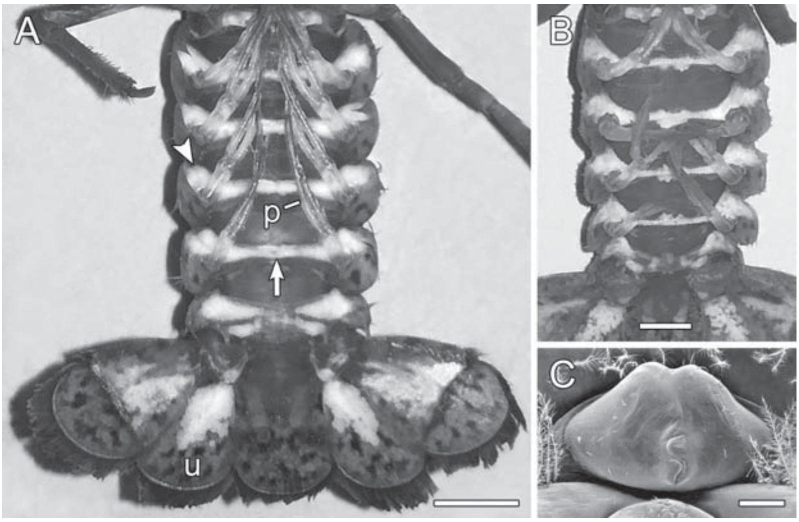

Fig 1. Morphology of glair glands and sperm receptacle. A — mature glair glands on underside of pleon of parthenogenetic *Procambarus virginalis* female. The whitish glands are located in the sterna (arrow), pleura (arrowhead), uropods (u) and pleopods (p). Scale bar 5 mm. B — pleon of unmated *Procambarus fallax* female showing glair glands 12 weeks after their first appearance. Scale bar 3 mm. C — scanning electron micrograph of *annulus ventralis* of young *P. virginalis* female. Scale bar 200 µm.
Рис. 1. Морфология глиальных желез и семеприемника. A — зрелые глиальные железы на нижней стороне плеона партеногенетической самки *Procambarus virginalis*. Беловатые глиальные железы расположены в стерне (стрелка), плевре (короткая стрелка), уроподах (u) и плеоподах (p). Масштаб 5 мм. B — плеон неспаренной самки *Procambarus fallax* с глиальными железами через 12 недель после их появления. Масштаб 3 мм. C — сканирующая электронная микрофотография семеприемника (*annulus ventralis*) молодой самки *P. virginalis*. Масштаб 200 мкм.

the fertilization tent. In contrast, the unmated slough crayfish females did not spawn although they regularly developed glair glands (Table 1). Their glair glands persisted up to 12 weeks and disappeared only during the next ecdysis.

Laboratory reared marbled crayfish mostly reproduced twice a year like slough crayfish (Vogt, 2015) but some individuals reproduced only once or three times per year. They showed glair glands with increasing staining intensity 3–6 weeks prior to spawning. In contrast to unmated slough crayfish females, unmated parthenogenetic marbled crayfish females generally produced fertilization tents and spawned. Only in one out of 103 documented cases a female with glair glands skipped spawning and moulted instead. The glair glands of this specimen disappeared only during moulting as in unmated slough crayfish.

Discussion

Comparison of unmated females of the sexually reproducing slough crayfish and parthenogenetic marbled crayfish revealed interesting differences with respect to spawning and the fate of the glair glands. Mated slough crayfish females and unmated marbled crayfish females usually spawned twice a year (Vogt, 2015). These spawnings were associated with emptying of the glair glands. In contrast, unmated slough crayfish females neither emptied their glair glands nor spawned. Their glair glands persisted for many more weeks, perhaps in expectation of a late mating chance. These differences between *P. fallax* and *P. virginalis* suggest that the information system that allows distinction between mated and unmated conditions has been lost during the evolution of marbled crayfish, i.e. during the transition from gonochorism to parthenogenesis.

Complete inhibition of spawning was also observed in unmated females of the astacids *Astacus leptodactylus* Eschscholtz, 1823, *Austropotamobius pallipes* (Lereboullet, 1858) and *Pacifastacus leniusculus* (Dana, 1852) by Holdich et al. (1995). In contrast, Woodlock and Reynolds (1988) observed normal spawning but decay of the attached eggs in unmated females of *A. pallipes* that were held in female pairs. In unmated females of laboratory-raised *Cherax destructor* Clark, 1936 (Parastacidae) the spawning behaviour was quite diverse: about 20% spawned when raised at 30 °C but none spawned at 27 °C (Tropea et al., 2010). Spawning of unmated females also occurred in facultatively parthenogenetic females of the cambarid *Orconectes limosus* (Rafinesque, 1817) (Buřič et al., 2011, 2013). Out of 30 unmated parthenogenetic females, 28 spawned and only one female did not spawn (the remaining individual died) (Buřič et al., 2011), resembling the situation in marbled crayfish. The reasons for the observed differences in spawning behaviour of unmated females of sexual reproducers are unknown but may rely on species differences, environmental conditions, pre-experimental experience, and the nutritional status of the individuals. Information on the fate of the glair glands is lacking for all of the cited gonochoristic species. The inhibition of glair secretion and spawning in unmated females of freshwater crayfish may be interpreted as an energy saving strategy.

Maturation of the oocytes and glair glands in crayfish does not require the presence of males as shown by this and other studies. Both processes are apparently closely linked and regulated by environmental cues, mainly temperature and photoperiod (Reynolds, 2002; Vogt, 2015). These factors modulate the antagonistic activity of the ovarian maturation inhibiting VIH (vitellogenesis inhibiting hormone) from the X-organ-sinus gland system and the ovarian maturation stimulating OSH (ovary stimulating hormone) from the thoracic nerve ganglia (Reynolds, 2002; Vogt, 2002). It is not yet known how maturation of the glair glands is regulated but perhaps the same environmental cues and hormones are involved.

Mating is regarded as a possible trigger for spawning (Reynolds, 2002). This assumption is supported by experiments by Holdich et al. (1995) who paired females with males from different species. Such females generally spawned but the eggs did not develop due to incompatibility of the gametes. Under natural conditions there are often many days or even weeks between mating and spawning suggesting that spawning could also depend on the remembrance of mating by the female or sensing of the presence of sperm. In astacid and parastacid crayfish, the spermatophores are externally attached to the thoracic sternal plates and the tail fan (Reynolds, 2002; Vogt, 2002; Niksirat et al., 2013), and thus, females could easily sense the presence of sperm with their pereopods. In Cambaridae, sperm is stored internally in the *annulus ventralis* (Fig. 1C) (Reynolds, 2002; Vogt et al., 2004), and in this case, mechanoreceptors might give information on its filling status.

It is unknown whether crayfish can voluntarily empty the glair glands and ovaries by active contraction of the glandular and ovarian musculatures. It is more likely, that both processes are involuntary and regulated by hormones like prostaglandin $F_{2\alpha}$, which was shown to sharply increase shortly before spawning in *Procambarus paeninsulanus* (Faxon, 1914) (Spaziani et al., 1995). In vitro tests revealed that this hormone elicits contraction of the ovarian tissue in a dose dependent manner.

The present study is the first report on the persistence of glair glands in unmated crayfish. The differences in glair gland and spawning behaviours between gonochoristic *P. fallax* and parthenogenetic *P. virginalis* makes this crayfish pair an interesting model system to investigate the regulation of spawning in crayfish. The availability of a complete draft genome of the marbled crayfish and full genomes of *P. fallax* females and males (Gutekunst et al., 2018) should facilitate research in this direction substantially.

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