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Mating rate influences female reproductive investment in a simultaneous hermaphrodite, *Lymnaea stagnalis*

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Multiple mating often imposes direct fitness costs on females but can provide indirect benefits such as enhanced genetic diversity and offspring quality. The costs and benefits of multiple mating have been investigated extensively in separate-sex species but less so in simultaneous hermaphrodites, despite being highly relevant given their flexible resource allocation and ability to compensate for costs experienced in one sexual role by gains in the opposite role. At high mating rates, the promiscuous hermaphroditic pond snail *Lymnaea stagnalis* experiences depressed female fecundity mediated by seminal fluid compounds. By experimentally manipulating mating opportunities, we tested for effects on female reproductive investment over 10 weeks. As expected, continuous access to mating partners resulted in decreased female investment, in terms of both total number of eggs (fecundity) and egg mass dry weight. Total investment in offspring increased over time for all treatments but was significantly less pronounced in treatments with continuous access to partners, and this was irrespective of partner identity. Investment per offspring was positively correlated with higher mating rates across treatments. Thus, multiple mating resulted in higher investment in egg masses at low than at higher mating rates. In contrast, at higher mating rates the investment per egg was higher. We conclude that, in *L. stagnalis*, mating multiply can severely impact female reproductive success and, although we cannot entirely exclude reallocation of resources to the male function from this study, we argue that this is probably the result of sexual conflict caused by previously identified seminal fluid components.

As a consequence of differential investment in gametes, males and females adopt different strategies to maximize their fitness. Females, by definition, produce large, costly gametes and fitness is limited mainly by resources that can be allocated to eggs and maternal care. In contrast, males often produce millions of sperm, which, in theory, could fertilize all the eggs in a population. Therefore, the fitness of males is limited by access to eggs carried by females and selection will favour males that can obtain, besides many copulations, the highest proportion of fertilizations from copulations (Bateman 1948; Anthes et al. 2010). As a result of male strategies aimed at increasing paternity, females often experience costs of mating multiply (Chapman et al. 2003). Such mating costs can include impaired immunity (Rolff & Siva-Jothy 2002), exposure to parasites, increased predation risk and physical injuries (Crudgington & Siva-Jothy 2000). In addition, males often transfer not only sperm during copulation but also seminal fluid compounds that can influence the recipients' behaviour and physiology, for instance by increasing current investment in offspring at the expense of future reproduction (Chapman et al. 1995). Such male seminal fluid compounds can prevent females from reaching their reproductive optima (Arnqvist & Rowe 2005).

Despite these negative effects of mating, females generally seem to copulate more often than appears to be necessary to fertilize all eggs. Why then do females mate multiply? Evidence reveals that not only is multiple mating driven by male promiscuity, but it can also provide benefits to females that can counterbalance costs (Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Simmons 2005). These benefits may be either direct or indirect. Direct benefits to females can be accrued through, for example, guarding behaviour by sires or nutritious nuptial gifts. In the field cricket *Gryllus lineaticeps*, for instance, females that mated multiply and thus received multiple spermatophores lived 32% longer and produced almost twice as many offspring as singly mated individuals (Wagner et al. 2001). A meta-analysis of the effects of polyandry in insects also found evidence for positive effects of mating with multiple males on female fitness, including increased egg production and higher percentages of hatched eggs (Arnqvist & Nilsson 2000). Indirect benefits may result from an improved genetic make-up or genetic diversity of offspring (Møller 1997).

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The bulk of work on benefits and costs of polyandry has focused on separate-sex species, even though hermaphroditism is present in 20 of the 28 more speciose animal phyla (Michiels 1998; Anthes 2010) and is estimated to be present in 5% of animal species (Jarne & Auld 2006) and most plants. Although sexual selection was once thought to be absent from the lower animal classes (Darwin 1871), its presence is now well established in hermaphrodites (Anthes et al. 2010; Pelissié et al. 2012 and references therein) and considered an important evolutionary force (Charnov 1979; Michiels 1998; Arnqvist & Rowe 2005; Koene 2012). Many hermaphroditic species mate promiscuously even when damaging mating tactics are employed, for example stabbing mating partners with so-called 'love-darts' in land snails (Koene & Schulenburg 2005), piercing the body with copulatory setae in earthworms (Koene et al. 2005) and hypodermic insemination in marine flatworms (Michiels & Newman 1998). This is thought to occur because individuals can compensate for fitness losses in one sexual function via their other sexual function and it has therefore been suggested that sexual conflicts between mating partners can become more costly for hermaphrodites than for separate-sex species (Michiels & Koene 2006).

Similar to the situation in separate-sex species, in hermaphrodites the extent to which benefits can counterbalance mating costs depends on many variables, such as food and mate availability and whether or not individuals can phenotypically adapt to current conditions (Schärer 2009). For example, in the hermaphroditic marine slug *Chelidonura sandrana* investment per offspring is increased in polyandrous animals compared to animals that have repeatedly mated with the same individual, and maternal investment peaks at intermediate mating rates (Sprenger et al. 2008a, b). In this study, we focused on the simultaneously hermaphroditic pond snail *Lymnaea stagnalis*. This species mates unilaterally and has the option to reciprocate in the other sexual role (i.e. role alternation: Koene & Ter Maat 2005). It fertilizes internally, prefers outcrossing, and its egg production has been shown to be influenced by mating rate (Koene et al. 2009a, b, 2010; Jarne et al. 2010). Intermediate mating rates (animals grouped once a week) resulted in enhanced female reproductive output compared to snails that mated only once (Koene et al. 2006). However, even higher mating rates (continuous access to one or more partners) led to strongly depressed oviposition rates (Van Duivenboden et al. 1985; De Visser et al. 1994). The negative effect of copulation on the number of eggs laid by the female copulation partner (hereafter recipient) is due to repeated receipt of ejaculates, which contain bioactive male accessory gland products (Koene et al. 2009a, 2010), but also to male (hereafter donor) expenditure on expensive ejaculates (Hoffer et al. 2010). In addition, received sperm (allosperm) can be stored and used for months in *L. stagnalis*; hence female fertility can be maintained at low mating rates (Cain 1956; Y. Nakadera, C. Blom & J.M. Koene, unpublished data).

The above-mentioned results suggest a sexual conflict in *L. stagnalis*, since mating rates exceed those optimal for recipients. However, it remains unclear whether decreased reproductive investment in terms of egg production is ameliorated by possible benefits accrued through multiple mating and polyandry. The main goal of the present study was therefore to integrate previous treatments into one comprehensive study to assess the effect of mating opportunity on fecundity and female reproductive investment. To that end, we subjected pond snails to treatments that provided either no mating (self-fertilization) or various mating opportunities that comprised one-time mating, and repeated and continuous access to either the same or different potential partners. Under these conditions, paired snails copulate in both sexual roles and mating rates are highest when continuously paired (Koene &

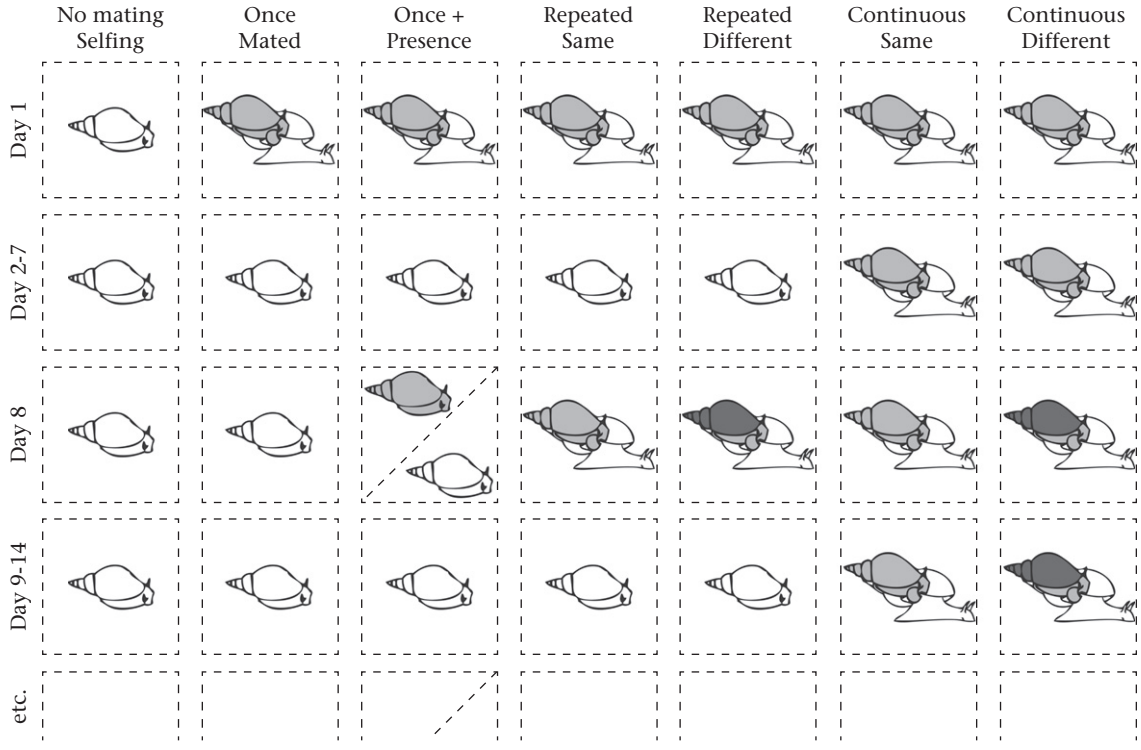

Figure 1. Schematic overview of the experimental set-up and procedures. Only the first 2 weeks of a total of 10 weeks are shown, as later weeks are a repetition of days 8–14. Treatment labels are explained in the main text. Focal animals are shown in white. Grey-coloured snails indicate either the same partner (light grey shell) or a different partner (dark grey shell). Dashed squares indicate experimental jars and dashed diagonal lines indicate a physical separation in the jar. Focal snails are shown as copulating as a female, but in reality individuals mated in both sexual roles. Snail outlines were redrawn after De Boer et al. (1996).