ANIMAL BEHAVIOUR, 2006, 72, 1119–1124
doi:10.1016/j.anbehav.2006.03.009

Pheromonal regulation of reproductive success in female zebrafish: female suppression and male enhancement

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(Received 15 August 2005; initial acceptance 4 January 2006; final acceptance 30 March 2006; MS. number: A10227)

Competition among females represents an important but rather neglected variable in studies of sexual selection. Probably to avoid injury to self and offspring, competition among females is often displayed without much physical interaction and therefore remains harder to observe. Here, we show for the first time in a teleost fish, the zebrafish *Danio rerio*, that females can use waterborne pheromones to suppress reproduction by other females. Female zebrafish that had been exposed to another female's pheromones for 4 days prior to mating spawned significantly fewer viable eggs than females held in isolation or exposed to male pheromones. Male pheromones not only stimulated female reproduction but also increased the quality and viability of eggs. In grouped females, reproductive success correlated with dominance rank. These results indicate that fish pheromones function to control mates and competitors in addition to serving as reproductive timing signals. Differences in female reproductive success observed in many fish species might be explained by this mechanism.

In many species, female members of a group differ in their reproductive output. However, competition among females has received less attention than competition among males because the latter is considered a primary component in sexual selection. Males compete for mating opportunities because female gametes are the limiting resource (Trivers 1972). However, females compete for resources, such as shelter, nest or food, for themselves or their offspring (Creel 2001), and may also compete for sperm (Nakatsuru & Kramer 1982; Olsson et al. 1997).

Physical interaction, visual and species-specific olfactory cues (pheromones) are hypothesized to trigger a cascade of hormonal responses that influence maturity and successful reproduction. These effects have been well studied in insects, such as ants (Heinze & Oberstadt 2003) and honeybees (Wossler 2002), and in mammals (primates: Abbott 1987; van der Walt et al. 2001; meerkats: O'Riain et al. 2000; rodents: Lawton & Whitsett 1979; Drickamer 1984; Bujalska 1985; Abbott 1987; Wolff 1992; Bennet 1994; van der Walt et al. 2001). For instance, urinary cues of dominant female house mice delay sexual maturity in subordinate females, whereas urinary cues of males accelerate maturity and induce ovulation (Whitten 1956; Drickamer 1984).

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In aquatic animals, waterborne pheromones are important in communication, with influences on dominance, individual recognition, courtship and synchronization of mating behaviour (Atema 1988; Stacey 2003). Among teleost fish, the sex pheromones of the goldfish, *Carassius auratus*, are best understood (Defraipont & Sorensen 1993; Sorensen & Goetz 1993; Sorensen et al. 2000; Poling et al. 2001): sex pheromones of goldfish function primarily as timing signals to synchronize male and female spawning (Sorensen et al. 1995b).

Male/female synchrony in mating behaviour appears to be facilitated by at least two chemical cues derived from circulating female hormones: a preovulatory steroidal cue and a postovulatory prostaglandine cue (Poling et al. 2001). Exposure to the female pheromone not only enhances spawning behaviour, but also sperm production and sperm motility of males (Defraipont & Sorensen 1993). In various fish species, ovarian fluid discharged immediately before spawning sharply increases sexual activity of conspecific males (*Bathygobius soporator*: Tavolga 1956; *Poecilia reticulata*: Gandolfi 1969; *Plectoglossus altivelis*: Honda 1979).

Studies on female–female competition in fish are rare; competition has been documented in the angelfish *Centropyge potteri*, where dominant females physically interfere with the mating of subordinate females, who spawn significantly less often than dominant females (Lutnesky & Kosaki 1995). In our study, we addressed the social and

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pheromonal mechanisms that determine reproductive success in female zebrafish.

Frequent spawning and the potential for genetic analysis make the zebrafish *Danio rerio* a convenient study object. Zebrafish are native to ponds, rivers and streams in India and Bangladesh, although nothing is known about their reproductive behaviour in the wild. In the laboratory, both sexes reach sexual maturity at about 3 months. Females show a 2–5-day ovulatory cycle when held in mixed-sex groups (Eaton & Farley 1974; Nagel 1986) and will defend territories with nest sites from other females. Home ranges of males overlap those of several females (Delaney et al. 2002). Synchronization of mating behaviour and receptivity is achieved by exposing mating partners to each other's pheromones for several (up to 24) hours before spawning. Histological studies of zebrafish ovaries indicate that oocytes are not released from the ovarian stroma into the central lumen of oviducts (ovulation) until they are stimulated by the male (Hisoka & Firlit 1962). Male holding water, testis homogenates and testis fractions containing steroid glucuronides induce ovulation in female zebrafish (Chen & Nartinich 1975; van den Hurk et al. 1987); deglucuronidation of these fractions leads to a loss of ovulation-inducing potency. Pheromones are perceived by means of olfaction, as anosmic females do not have an ovulatory response to male holding water (van den Hurk & Lambert 1983; van den Hurk et al. 1987; van den Hurk & Resink 1992). Mating is initiated at the onset of light. The number and viability of eggs spawned is a reliable and quantitative measure of reproductive success.

Here we examined whether female reproductive success in zebrafish is regulated by pheromones and whether female dominance affects female reproductive success.

METHODS

Experimental Animals

Zebrafish originated from a local pet supply and were kept in mixed-sex groups of 10–12 animals in 9-litre aquariums (AHAB Aquatic Habitat recirculation system with carbon filters; Aquatic Habitats, Inc., Apopka, Florida, U.S.A.) under a 14:10 h light:dark cycle with light on at 0900 hours. Animals were fed twice daily with brine shrimp (*Artemia*) and standard fish food. Water temperature in all tanks was kept at 27°C. For each experiment we used only unrelated and unfamiliar fish.

Experimental Design

To study the influence of male and female chemical interaction on female egg production, animals were randomly assigned to one of three experiments conducted in parallel (see Fig. 1). A 9-litre aquarium was divided into two parts by a dark mesh screen (width 0.5 mm) to prevent physical contact. A steady flow of tank water (2 ml/min) led from the first compartment, which contained a female (experiment 1 FF; $N = 25$), no animal (experiment 2 F; $N = 17$) or a male (experiment 3 FM; $N = 31$), to the second compartment, which contained a test female. Animals used in experiments 1–3 were of similar age (6 months $\pm$ 2 weeks) and were size matched (total length $\overline{X} \pm \mathrm{SD} = 35 \pm 2$ mm).

To study whether female reproductive suppressibility varied with social rank we conducted a fourth experiment (3F; $N = 11$) where we kept three size-matched females together in a 9-litre tank for 4 days. These females were 13 months old. We chose three females rather than two so that a true subordinate nonterritorial female would emerge; when using only two females, the subordinate female frequently established a small territory (subdominant female). Behavioural interactions were observed daily for 10 min in the morning (0900–1100 hours) and in the afternoon (1400–1600 hours), during which we recorded all aggressive interactions between females to determine their dominance hierarchy. Each chase that was followed by rapid escape of the other individual was counted as one aggressive interaction. Individual females could be distinguished by natural differences in stripe pattern.

In the afternoon of the fourth day all test females in experiments 1–4 were individually paired with an unfamiliar male in new 9-litre tanks provided with specially designed egg dishes. In experiment 3 FM a new male was used for mating. The next day, 90 min after the onset of

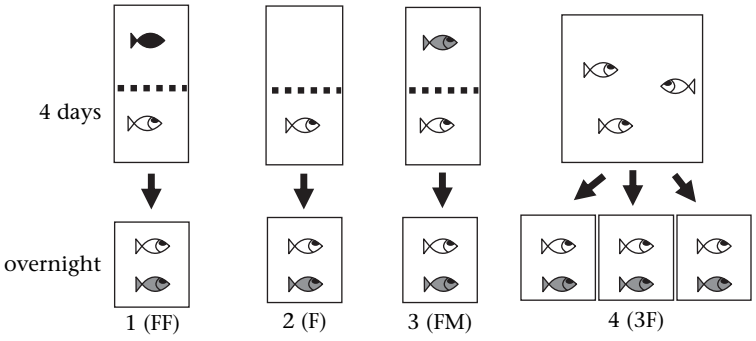

Figure 1. Experimental design. Experiment 1: a female was exposed to another female's pheromones (FF); experiment 2: a female was isolated (F); experiment 3: a female was exposed to a male's pheromones (FM); experiment 4: three females were together in a tank (3F). Each experiment lasted for 4 days. On the fourth night, all females were isolated and an unfamiliar male was introduced with an egg dish. Eggs were counted the next day. Male stimulus fish = grey fish; female stimulus fish = black fish, female test fish = white fish.