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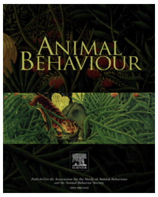

Sperm-limited males save ejaculates for future matings when competing with superior rivals

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Adjusting ejaculates to sperm competition can lead to sperm limitation. Particularly in polygynous species, males may face a trade-off between investing sperm in current or future mating opportunities. The optimal sperm allocation decision should depend on the relative intensity of sperm competition experienced in a mating sequence. Here we asked how males respond to this trade-off in polygynous fish with alternative male mating tactics, intense sperm competition and sperm limitation. Large bourgeois males of the shell-brooding cichlid *Lamprologus callipterus* build nests consisting of empty snail shells, in which females spawn and raise offspring. During spawning, nest males release ejaculates into the shell opening. Genetically distinct, parasitic dwarf males enter shells during spawning to fertilize the eggs from inside the shell. These dwarf males were previously shown to be superior sperm competitors to nest males. Here we showed that when spawning with several females simultaneously, nest males reduced the spawning duration for each clutch and the number of ejaculations per female tended to decrease, reflecting sperm limitation. Experimental exposure of nest males to sperm competition with dwarf males reduced the number and duration of ejaculations by roughly half. Hence, when exposed to competition with a superior rival, nest males did not increase their sperm expenditure as predicted by sperm competition risk models, but in fact saved sperm for future mating opportunities as predicted by sperm competition intensity theory. This seems to be adaptive because of the considerable sperm demands in this species, which is partly due to their high degree of polygyny.

Sperm competition, where sperm of two or more males compete for the fertilization of eggs (Parker, 1970), can cause behavioural and morphological adaptations in males. Adaptive adjustment to sperm competition includes prolonged copulations (Schöfl & Taborsky, 2002), mate guarding and an increase in copulation frequency (Birkhead, 1998), sperm displacement (Parker & Simmons, 1994), the development of copulatory plugs (Dunham & Rudolf, 2009) and breakage of copulatory organs (Snow, Abdel-Mesih, & Andrade, 2006). Males may monopolize either limited resources for breeding or females directly to prevent rival males from gaining access to females (bourgeois tactic; Taborsky, 1997; Taborsky & Brockmann, 2010). As a consequence of this mating monopolization, male reproductive success is typically strongly skewed, and alternative reproductive tactics (ARTs; Oliveira, Taborsky, & Brockmann, 2008) may evolve, where parasitic males invest relatively more in testes than bourgeois males due to their higher risk of sperm competition (Byrne, Roberts, & Simmons, 2002; Stockley, Gage, Parker, & Møller, 1997; Taborsky, 1998).

Adaptations to sperm competition have been modelled extensively, with two types of game theoretical approaches considering how males should respond to either sperm competition risk (SCR; whether it occurs or not, Kelly & Jennions, 2011; Parker, 1998) or sperm competition intensity (SCI; number of ejaculates competing for a set of ova, Parker, Ball, Stockley, & Gage, 1996, 1997). SCR models predict that ejaculate expenditure increases when males are exposed to a single competitor, which has been confirmed by empirical data (for review see Wedell, Gage, & Parker, 2002). SCI models predict that ejaculate expenditure decreases with an increasing number of competitors because males save sperm for better future spawning opportunities, which has received less empirical support (for reviews see Engqvist & Reinhold, 2005; Kelly & Jennions, 2011; Wedell et al., 2002). These models imply that, in general, if males can succeed in sperm competition they should increase ejaculate expenditure, whereas if they have little chance of succeeding against superior competitors, they should reduce ejaculate expenditure to save sperm for future matings. This may be

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particularly important in polygynous species, where both mating frequency and sperm competition can lead to sperm limitation (Wedell et al., 2002). Additional causes of sperm limitation (Shapiro & Giraldeau, 1996) include low fertilization efficiency, large clutch size and high reproductive costs for males (Wedell et al., 2002).

It is generally little understood to what extent variation in sperm and ejaculation characteristics result from either sperm competition or sperm limitation. In bitterling fish, for example, differences in the mating system (Pateman-Jones et al., 2011) or breeding resource distribution (Reichard, Ondrackova, Bryjova, Smith, & Bryja, 2009) significantly affect ejaculate characteristics under high levels of both sperm competition and sperm limitation. An interspecific comparison showed that the species with the shortest spawning season (*Rhodeus amarus*), which corresponds to a high probability of sperm limitation, showed the greatest level of investment in sperm production, the highest ejaculation rate, the smallest clutch size, and spermatozoa apparently adapted to fast swimming (Pateman-Jones et al., 2011). With a clumped rather than an even breeding resource distribution, stronger selection on traits that evolve due to sperm competition was detected (Reichard et al., 2009). In sea urchins, high population densities result in selection on sperm traits associated with sperm competition, whereas low population densities result in selection on sperm traits associated with sperm limitation (Levitan, 2002).

It is possible to disentangle the influence of sperm competition and sperm limitation on sperm and ejaculate characteristics by manipulating the degrees of sperm competition and polygyny independently of each other in species that combine polygyny with the existence of ARTs. To test for an influence of sperm limitation, one can determine how males adjust ejaculation characteristics to the number of females they mate with at a time. To test for an influence of sperm competition, ejaculation characteristics of bourgeois males mating either alone or together with parasitic males can be compared. However, in externally fertilizing species releasing their gametes into the water, it is difficult to determine ejaculation characteristics under different experimental conditions (Shapiro, Marconato, & Yoshikawa, 1994).

In the cichlid fish *Lamprologus callipterus*, large bourgeois males construct and defend nests consisting of empty snail shells, in which the much smaller females breed (henceforth called ‘nest males’; Schütz & Taborsky, 2005). Females lay clutches containing on average 95 eggs, which are deposited one by one inside a shell at intervals of more than 2 min (Schütz, Heg-Bachar, Taborsky, & Heg, 2012). Each egg needs to be fertilized by a separate ejaculate, leading to a total spawning duration of nearly 7 h on average (Schütz et al., 2012) and to severe sperm limitation of nest males (Schütz, Pachler, Ripmeester, Goffinet, & Taborsky, 2010). The latter reduce the number of sperm released per ejaculate drastically after 5 h of continuous spawning, even when spawning with only one female (Schütz et al., 2010) and even though eggs are deposited at a similar rate over the whole duration of a spawning (Schütz et al., 2012).

In *L. callipterus*, two distinct parasitic male types exist: sneaker males opportunistically try to enter territories where nest males are spawning to fertilize eggs by releasing ejaculates into the shell opening when the nest male is inattentive. This tactic is transitional and performed by males of the nest male type (Schütz, Parker, Taborsky, & Sato, 2006). Parasitic dwarf males constitute a genetically distinct male morph (Wirtz Ocana, Meidl, Bonfils, & Taborsky, 2014) remaining small throughout life (Taborsky, 2001). They try to enter shells in which a female is spawning to fertilize the eggs from inside the shell. If they enter successfully, dwarf males stay in the shell during the whole spawning event, and therefore they are in much closer vicinity to the female and eggs during laying than nest males, i.e. they are in a privileged position to fertilize the eggs (Sato, Hirose, Taborsky, & Kimura, 2004). This contrasts with most other species, where bourgeois males are usually much closer to eggs than parasitic males (Taborsky, 2008; Taborsky, Oliveira, & Brockmann, 2008). Thus, spawning of a nest male with a parasitic dwarf male resembles a loaded raffle (Parker, 1990a) where dwarf males have a fertilization advantage, which is revealed also by the much greater fertilization success of dwarf males than nest males in nature (Wirtz Ocana et al., 2014). Theory predicts that the unprivileged male type (here the nest male) should compensate for his disadvantage by investing more in the present ejaculate than the privileged male type (here the dwarf male; Parker, 1990a). Hence, in accordance with sperm competition risk models, nest males should increase their reproductive effort when spawning with a parasitic dwarf male (Parker, 1998). Alternatively, nest males might decrease ejaculate expenditure in such competitive situations to save sperm for future spawning opportunities without participating dwarf males, as predicted by sperm competition intensity models, especially if they involve highly loaded raffles (Parker et al., 1996, 1997).

In species with external fertilization, ejaculate sizes can hardly be determined exactly because sperm diffuse into the water right after release. This is different in *L. callipterus*, since males ejaculate into a snail shell, which allows collection of their sperm and determination of ejaculation characteristics. Additionally, the perceived risk of sperm competition for nest males can be manipulated directly by adding a dwarf male into a shell where the nest male is spawning. Thus, physiological responses of males to the perceived risk of sperm competition before a test spawning are prevented, avoiding this pitfall in testing predictions from sperm competition models (Engqvist & Reinhold, 2005).

Here we aimed to clarify the relative roles of sperm limitation and sperm competition for shaping ejaculation characteristics of *L. callipterus* nest males. Specifically, we asked how much they invest in ejaculates in relation to increasing sperm limitation and sperm competition risks. We compared nest male sperm and ejaculation characteristics when spawning (1) with one or more females simultaneously to test for the influence of sperm limitation, and (2) with or without a parasitic dwarf male present to test for the influence of sperm competition.

We investigated how nest males deal with the apparent trade-off regarding sperm allocation in relation to the current sperm competition risk and future mating opportunities (Wedell et al., 2002). When facing sperm competition with a superior dwarf male, nest males may either increase ejaculate expenditure to compensate for their ‘devalued’ fertilization opportunity (Parker, 1990b, 1998) or decrease it to save sperm for future matings without dwarf males (cf. Parker et al., 1996, 1997; Wedell et al., 2002). In the laboratory, we experimentally added a dwarf male into a shell where a female was spawning with a nest male and analysed nest male behaviour and ejaculate characteristics before and after this manipulation.

To estimate the risk of sperm competition and sperm limitation in the natural situation, we determined the number of females, intruders of the nest male type (mainly territorial neighbours), sneakers and dwarf males entering a nest per day from long-term video recordings obtained in the field. Sperm limitation of nest males should be even higher when nest males spawn with more than one female at a time. Thus, from these long-term video recordings we determined the total spawning duration for each clutch and the number of ejaculations per female for nest males that were spawning simultaneously with different numbers of females.

In addition, we determined the relationship between ejaculation characteristics and sperm numbers in laboratory experiments in which nest males spawned with a female alone, since it is