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Strategic sperm allocation in response to perceived sperm competition risk in a lekking insect

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Sperm competition has been demonstrated to be a strong selective force shaping male mating behaviours and ejaculate traits, particularly in highly polyandrous species. Its effect, however, is expected to be limited in lek mating systems, where female mating decisions appear relatively unconstrained and thus female remating rate is generally low. Hence, testing for male adaptations to sperm competition in lek mating species is of particular importance in order to understand whether and how sperm competition, as a selective force, may also have shaped male phenotype in such systems. Here, we investigated copulation behaviour and strategic sperm allocation in the lekking lesser wax moth, *Achroia grisella*, a well-established model for studying precopulatory sexual selection as males produce elaborate ultrasonic courtship songs targeted by female choice. Recent evidence suggests that females do sometimes remate, thus creating a selective potential for sperm competition. To test the hypothesis that sperm competition also selects for male adaptations in ejaculate traits in lek mating systems, we staged experimental matings after males had experienced either a competitive (one competitor present) or a noncompetitive (no competitor present) social environment during early adulthood. Males increased sperm transfer rate and thereby allocated higher proportions of available sperm reserves during virgin copulations when experiencing the elevated sperm competition risk environment. Our study provides the first evidence of strategic sperm allocation in relation to sperm competition risk in a lekking insect, demonstrating that sperm competition might represent an important selective force in such mating systems.

Sperm competition occurs when spermatozoa from more than one male compete for a defined set of ova (Parker, 1970; Parker & Pizzari, 2010; Simmons, 2001). Under sperm competition, male reproductive success depends not only on the number and quality of females with which a male can mate, but also on his ability to fertilize the eggs of the females with which he successfully copulates. As a male's share of paternity usually increases with relative ejaculate size (Martin, Reimers, Lodge, & Dziuk, 1974; Parker & Pizzari, 2010), the question of strategic investment in sperm production and/or strategic sperm allocation at a given mating has been central to sperm competition models. In particular, models predict that males should respond differently to sperm competition risk (i.e. the probability that at least one rival ejaculate is present) compared to sperm competition intensity (i.e. the number of ejaculates in competition). While male ejaculate expenditure, in terms of both sperm production and sperm allocation, is predicted to increase with higher sperm competition risk, males are expected to reduce the number of sperm allocated when sperm of more than one competitor is involved (Engqvist & Reinhold, 2005; Kelly & Jennions, 2011; Parker, Ball, Stockley, & Gage, 1996, 1997).

In lekking species, males gather in a display arena where they compete for females while females visit the lek in order to choose freely the highest quality male with which to mate (Höglund & Alatalo, 1995; Kirkpatrick & Ryan, 1991). Lek mating systems are well known to exhibit, in exemplary fashion, the distinctive Darwinian sex roles of pronounced female choosiness and strong male–male competition (Darwin, 1871; Höglund & Alatalo, 1995). For decades, it has been assumed that strategic allocation of male reproductive resources was of minor importance in these systems. First, because reproductive investment of lekking males consists solely in courtship display and sperm delivery and does not involve male parental care, males were expected to mate rather indiscriminately with as many females as possible. The benefit of male

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mate choice is therefore unlikely to compensate for the cost of losing mating opportunities (Deutsch & Reynolds, 1995; Johnstone, Reynolds, & Deutsch, 1996; Trivers, 1972). Second, because females do not derive direct fitness benefits from mate choice and can express unconstrained mating preferences, female remating behaviour and thus the risk of sperm competition was thought to be rare or absent (Avery, 1984; Birkhead, Atkin, & Møller, 1987), which in turn would decrease the prospect of male adaptations to sperm competition (Hunter, Harcourt, Wright, & Davis, 2000; Parker, 1990a, 1990b).

However, this classical view of what constitutes the male sex role in a lek has been recently questioned, because sperm production is costly and might considerably limit male reproductive rate through permanent or temporary sperm depletion (Boivin, 2012; Dewsbury, 1982; Galvani & Johnstone, 1998). In addition, courtship might be energetically demanding (Andersson, 1994; Höglund, Kålås, & Fiske, 1992). For both reasons, strategic allocation of male reproductive resources, including strategic sperm allocation, may well be expected; and it has indeed been demonstrated, for example, that males of lekking species adjust sperm transfer to female reproductive quality in birds and mammals (Bro-Jørgensen, 2007; Saether, Fiske, & Kålås, 2001). Moreover, although sperm competition is often predicted to be low or absent, evidence for multiple mating (and multiple paternity) in leks suggests that sperm competition could also play a non-negligible role in these systems (Hess, Dunn, & Whittingham, 2012; Lank et al., 2002; Petrie, Hall, Halliday, Budgey, & Pierpoint, 1992). Yet, despite the tremendous amount of theoretical and empirical work on sperm competition and strategic sperm allocation, and the fact that the potential for postcopulatory processes to affect male fitness has recently been emphasized in lekking species (Leftwich, Edward, Aphey, Gage, & Chapman, 2012; Sardell & DuVal, 2014), no study has yet directly addressed the evolution of strategic sperm allocation in response to sperm competition in a lek mating system.

The lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae), is a well-established model system for studying precopulatory sexual selection due to its remarkable mating system in which males gather in leks and broadcast ultrasonic courtship calls (Greenfield & Coffelt, 1983; Spangler, Greenfield, & Takessian, 1984; see also Methods below). Given that approximately 20% of females remate with another male (Engqvist, Cordes, Schwenniger, Bakhtina, & Schmoll, 2014; Greenfield & Coffelt, 1983), which creates a sperm competition risk of more than 30% (Engqvist et al., 2014), we may expect selection for male adaptations to sperm competition. Females that remate only very rarely do so more than once (one female out of 130; A. Jarrige, personal observation, 2014); thus competition between sperm of more than two males is very rare and both average sperm competition risk and intensity are relatively low, which are prerequisites to fit sperm competition risk models (Engqvist & Reinhold, 2005; Parker, 1990a; Parker et al., 1997 and see above). Indeed, because manipulation of perceived sperm competition risk might also affect male perception of sperm competition intensity, clear predictions for sperm allocation would be difficult in more strongly promiscuous systems as sperm competition risk and sperm competition intensity are predicted to have opposite effects on ejaculate size (Engqvist & Reinhold, 2005). Moreover, adult males emerge with a fixed amount of eupyrene (i.e. fertilizing) sperm (spermatogeny index of 1; Boivin, Jacob, & Damiens, 2005; Friedländer, 1997; Friedländer, Seth, & Reynolds, 2005) aggregated in bundles of 256 spermatozoa (Fernandez-Winckler & da Cruz-Landim, 2008). Thus sperm production (in the larval stage) and sperm allocation (in the adult) are separated in time and can be singled out by suitable experimentation, rendering the lesser wax moth an ideal model system to investigate strategic sperm allocation in response to sperm competition risk.

In the present study, we investigated whether male mating behaviour and sperm allocation in the lesser wax moth is affected by experimentally manipulated sperm competition risk by exposing males to the presence of a competitor during 72 h prior to and during virgin mating. We predicted that males experiencing social cues indicative of sperm competition risk (i.e. with one competitor present) would transfer a greater proportion of their sperm reserves to females during their first copulations than males of the same age experiencing no such cues (no competitor present, control). To test this prediction, we compared: (1) copulation duration; (2) number of eupyrene sperm transferred at virgin copulations; and (3) number of eupyrene sperm retained in the male genital tract between the two groups.

METHODS

Study Species

The lekking lesser wax moth is a cosmopolitan symbiont of the western honeybee, *Apis mellifera*, in whose colonies the moth larvae feed on honeycomb and other organic material (Kunike, 1930). Both male and female adult moths have atrophied mouthparts and neither feed nor drink. Hence, all resources for somatic maintenance and reproduction are acquired entirely at the larval stage (Greenfield & Coffelt, 1983). Adults have a markedly short life span as males and females typically only live for 7 or 10–14 days in the laboratory, respectively. Mating activities of moths usually take place in the vicinity of their natal honeybee colony. For 6–10 h every night males gather in leks and broadcast ultrasonic courtship calls by wing fanning while remaining stationary on the substrate (Greenfield & Coffelt, 1983; Spangler et al., 1984). Receptive females in the vicinity orient and move towards singing males up to 1 m distant, usually by running. Female precopulatory mating preferences based on evaluation of male song traits have been studied in several populations, showing that females prefer songs delivered with greater power (expressed as the product of mean amplitude by calling rate), greater peak amplitude and amplitude fluctuations (Jang & Greenfield, 1996, 1998; Limousin & Greenfield, 2009). Males respond to surrounding males by temporarily accelerating the pulse pair rhythm in their songs (Jia, Greenfield, & Collins, 2001). Males exhibit the dichotomous spermatogenesis typical of Lepidoptera by producing eupyrene (nucleate) and apyrene (anucleate) spermatozoa (Friedländer, 1997; Friedländer et al., 2005). Both types of spermatozoon reach the female spermatheca, but only eupyrene spermatozoa fertilize eggs while the function of apyrene spermatozoa is still to be clarified (Friedländer et al., 2005; Silberglied, Shepherd, & Dickinson, 1984).

Laboratory Stock Population and Experimental Animals

We maintained a stock population of lesser wax moths derived from several hundred individuals collected in Departement of Indre et Loire, France, in October 2007. Moths were reared in an environmental chamber at 24 ± 2 °C, 45% relative humidity and under a 12:12 h light:dark (LD 12:12 h) photoperiod. Larvae were cultured on a standard ad libitum diet containing wheat, corn and rye flour, water, glycerol, nutritional yeast, honey and beeswax (modified from Dutky, Thompson, & Cantwell, 1962; see Table A1). These laboratory conditions closely mimic natural conditions for this species. Under these conditions, generation times as measured from oviposition to oviposition range from 45 to 55 days. In each generation, individuals were bred such that close inbreeding (full-sibling matings) was avoided and a large population size was