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Number of Sperm in Human Ejaculates Varies in Accordance With Sperm Competition Theory

One of the major predictions of sperm competition theory (Parker 1970, 1982) is that there should be a positive association between number of sperm inseminated and risk of the female double-mating (i.e. mating with a second male while containing fertile sperm from a previous male). A number of across-species studies on mammals support this prediction. For example, polyandrous species of primates have larger testes and may inseminate more sperm per ejaculate than monandrous species (Short 1979; Harcourt et al. 1980). However, such studies tell us only that sperm competition may have shaped the general reproductive anatomy and physiology of species during mammalian evolution. They do not show that sperm competition has shaped the variation in copulatory behaviour shown by individual males. Yet the theory would predict that at each copulation the ejaculate should be adjusted according to the risk of the sperm finding themselves in competition with sperm from another male. We have tested this prediction using humans.

Our assumption that modern humans are suitable for testing the predictions of sperm competition theory is based on the following consider-ations. Double-mating occurs in both overtly promiscuous species and apparently monogamous species (McKinney et al. 1984), including humans (Smith 1984). In consequence, sperm competition may have been a selective force in shaping many aspects of human anatomy, physiology and behaviour (Smith 1984). The minimum level of double-mating necessary for sperm competition to generate and maintain selection for competitive ejaculates in humans may be calculated (from formulae in Parker 1982) to be about one copulation in every 7700. In a recent (1987–1989) survey of women in London, Rotherham and Manchester, U.K., more than one in every 1000 copulations was a double-mating (R. R. Baker & M. A. Bellis, unpublished data), at least eight times greater than the threshold level. Demonstrable levels of cuckoldry and frequent cases of legally disputed paternity (Smith 1984) show that double-matings do lead to reproductive reward for the male whose sperm prevail in such competition, despite modern methods of contraception.

The males of many 'monogamous' species spend much of their time between copulations close to their female partner, thus reducing the risk of that female double-mating. We assume that, for such species, the level of risk is inversely proportional to the percentage of time that the pair are together. Sperm competition theory would predict, therefore, that the males of such pairs should adjust the number of sperm ejaculated according to the percentage of time the pair have been together since their last copulation.

Fifteen male–female human pairs with average sexual activity (one–three copulations per week; Smith 1984) were recruited from staff and post-graduates in the School of Biological Sciences at the University of Manchester. Each pair was asked to collect an ejaculate by condom during copulation and to return the sample along with a completed questionnaire.

One potential problem in using ejaculates collected by condom is that the appropriate psychological and other stimuli may not be present. Except through accident, the ejaculated sperm are not destined for competition: a scenario that could militate against a male responding in accordance with sperm competition theory. Of course, the problem is conservative making it less, not more, likely that a negative correlation between sperm number and the percentage of time together will emerge. Nevertheless, we sought some control by asking each pair also to return a masturbatory ejaculate, again collected in a condom but with no genital contact. Such ejaculations should be even further divorced from the stimuli associated with normal copulation and thus even less likely to fit the predictions of the theory.

An additional advantage of having more than one ejaculate from each pair was that it facilitated a double-blind protocol: (1) subjects could be told only that the aim of the experiment was some form of comparison of copulatory and masturbatory samples; and (2) sperm could be counted by an experimenter unaware of the type of sample being counted.

Subjects were provided with a 'kit' containing instructions and all necessary equipment, including lubricated (non-spermicidal) condoms. After ejaculation, subjects suspended the condom from a cardboard stand for 8 h or more while the ejaculate decoagulated. With scissors, subjects then cut the condom so that the end containing the ejaculate fell into a screw-top jar containing 52 ml of fixative (2% glutaraldehyde in a phosphate buffer, pH 7·2). The jar was shaken to disperse the ejaculate and was then labelled with a cryptic code. The same code was written on a questionnaire and only here was the sample identified as masturbatory or copulatory. Among other information requested for each sample was: time since last ejaculation; time since last copulation; and the percentage time together since last copulation. The completed questionnaire was placed in an envelope which was then sealed.

Pairs were asked to return at least two ejaculates (one copulatory; one masturbatory) collected, as nearly as possible, during their normal sexual activity. Samples were taken from April to October 1988. Between pairs, there was no pattern to the sequence of collection and, in most cases, samples were not consecutive and were interspersed with other ejaculations that were part of the pair's normal activity. Five of the 15 pairs failed to return any sample. The remainder returned a total of 34

(18 copulatory; 16 masturbatory). Median time interval between ejaculation of the sample and the male's previous ejaculation was 56 h (IQR:18–71) for copulatory samples and 54 h (22–78) for masturbatory samples. Ejaculates were returned for sperm counts to MAB; questionnaires to RRB. Estimates of the number of sperm per ejaculate were based on procedures in the *Human Semen Manual* (Belsey et al. 1980) except that we used an 'Improved Neubauer Haemocytometer' and obtained a mean ($\pm$ SE) from eight (not two) samples per ejaculate.

To avoid problems of independence, we restricted analysis to the 10 copulatory and 10 masturbatory samples first provided by each pair. As predicted, the copulatory samples show a highly significant ($r_s = -0.948$, $P_{one-tailed} < 0.001$) rank-order correlation between the estimated number of sperm in an ejaculate and the percentage of time a pair have spent together since their last copulation (Fig. 1). The masturbatory samples show no significant rank-order correlation ($r_s = -0.195$, $P_{one-tailed} = 0.295$). The rank-order correlation coefficients for copulatory and masturbatory samples are significantly different ($z = 3.020$, $P_{one-tailed} = 0.0013$; z-transformation test; Chambers 1958).

Regression analysis showed that the percentage of time a pair spent together was a strong (79% of variance explained) and significant ($F_{1,8} = 30.5$, $P_{two-tailed} < 0.001$) predictor of the number of sperm ejaculated during copulation. In contrast, time since last ejaculation was a weak (32%) and non-significant ($F_{1,8} = 3.7$, $P_{two-tailed} > 0.2$) predictor. Moreover, multiple regression analysis and analysis of residuals failed to find even a secondary influence of time since last ejaculation. Thus, we
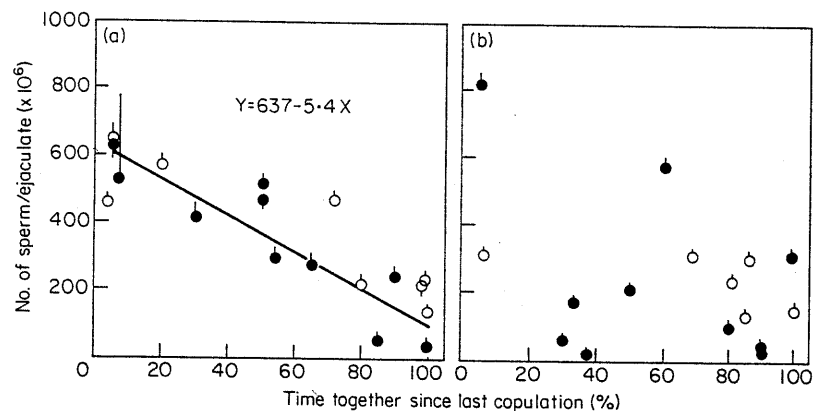

Figure 1. Variation in number of sperm in human ejaculates in relation to the percentage of time the male and female have spent together since their last copulation. (a) Copulation; (b) Masturbation. Data points show the estimated number of sperm in a single ejaculate ($\bar{X} \pm$ SE based on eight separate counts per ejaculate). ●: first sample of each type provided by a pair; ○: subsequent samples.

found no evidence of physiological constraint imposed on number of sperm ejaculated during copulation by rate of sperm production (approximately 300×10^6 per day; Johnson et al. 1980). Of course, this conclusion may not hold at higher levels of sexual activity. Multiple regression analysis of the masturbatory samples showed that the best predictor (70% of variance explained) of the number of sperm ($N \times 10^6$) was time (T h) since last copulation ($N = 3 \cdot 806T + 22 \cdot 30$; $F_{1,8} = 18 \cdot 47$, $P_{\text{two-tailed}} < 0 \cdot 01$).

This study has shown that the number of sperm inseminated into a human female during copulation varies in a way that fits the predictions of sperm competition theory. No part of our data allows us to identify the mechanism of adjustment. Pheromonal effects, short- and/or long-term psychological effects or even variation in the sexual stimuli given by the female are all possible. However, whatever the mechanism, adjustment persists despite, and may even be unaffected by, the wearing of a condom, at least as long as copulation is otherwise normal with full genital penetration. When ejaculation is the result of masturbation, such adjustment is not apparent. Instead, the number of sperm ejaculated relates primarily to the time interval since last copulation.

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Effects of Experience on Use of Orientation Cues in the Giant Tropical Ant

Animal orientation generally involves use of a variety of navigation cues arranged in hierarchical fashion (Able 1980). However, how these cue hierarchies might vary within individuals has received little attention. The relative importance of orientation cues varies with experience in honeybees (von Frisch 1967) and homing pigeons (Keeton 1974). We tested the relative importance of visual and pheromonal cues in orientation to food sources by the giant tropical ant, *Paraponera clavata*. Ant species use a variety of cues during orientation, including landmark memorization, dead-reckoning from celestial cues, and pheromonal trails (Wehner 1981). We report that the cue hierarchy used during orientation changes with experience in *P. clavata*.

Paraponera clavata workers use pheromone trails in recruitment to food and as orientation guides by individual foragers (Breed et al. 1987). This suggests that pheromone trails might be of primary importance for orientation to food sources in this species. *Paraponera clavata* are also highly responsive to visual cues, however, so orientation may involve a combination of visual and pheromonal information.

Our experiments were performed at the La Selva Biological Station, Costa Rica, in January 1988. Ants foraging 1–4 m from nests were baited from trunk trails on tree buttresses to 1-M sucrose feeding stations at the end of one of the branches of a wooden Y-shaped apparatus. The sucrose solutions elicit the same feeding and recruitment response as nectar, a natural food source for *P. clavata* (Breed & Bennett 1985). The stem of the Y measured 0·75 m and each branch measured 1·0 m. The Y was supported a few cm above the ground by