

## LINEAR MODELS FOR ASSESSING MECHANISMS OF SPERM COMPETITION: THE TROUBLE WITH TRANSFORMATIONS

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**Abstract.**—Although sperm competition is a pervasive selective force shaping the reproductive tactics of males, the mechanisms underlying different patterns of sperm precedence remain obscure. Parker et al. (1990) developed a series of linear models designed to identify two of the more basic mechanisms: sperm lotteries and sperm displacement; the models can be tested experimentally by manipulating the relative numbers of sperm transferred by rival males and determining the paternity of offspring. Here we show that tests of the model derived for sperm lotteries can result in misleading inferences about the underlying mechanism of sperm precedence because the required inverse transformations may lead to a violation of fundamental assumptions of linear regression. We show that this problem can be remedied by reformulating the model using the actual numbers of offspring sired by each male, and log-transforming both sides of the resultant equation. Reassessment of data from a previous study (Sakaluk and Eggert 1996) using the corrected version of the model revealed that we should not have excluded a simple sperm lottery as a possible mechanism of sperm competition in decorated crickets, *Gryllodes sigillatus*.

**Key words.**—Crickets, *Gryllodes sigillatus*, ideal lottery, sperm competition, sperm displacement, spermatophore.

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Sperm competition is the competition between the sperm of two or more rival males for the fertilization of a single female's eggs, and leads to the evolution of traits in males designed to incapacitate the sperm of their rivals or to deter recent mates from remating (Parker 1970). Although sperm competition is widely recognized as a pervasive evolutionary force shaping reproductive tactics of males (Smith 1984; Birkhead and Møller 1998), the mechanisms underlying different patterns of sperm precedence remain obscure, with few exceptions (e.g., Waage 1979; von Helversen and von Helversen 1991; Gack and Peschke 1994). Parker et al. (1990) derived a series of linear models designed to identify two of the more basic mechanisms of sperm competition: sperm lotteries (also termed sperm raffles), in which the sperm of different males are recruited for fertilization in direct proportion to their relative abundance in the female's sperm stores; and sperm displacement, the physical displacement of one male's sperm from the female's stores by the sperm of a more recent mating partner. By experimentally manipulating the number of sperm transferred by each of two males mated to the same female, Parker et al. (1990) showed how it is possible to infer which of these two mechanisms may be operative based on the relative numbers of offspring sired by the two males. Such an experimental approach can provide a first approximation of the processes that may be at play, although it should not preclude more intensive investigations of the physiological or morphological basis of sperm competition.

In a previous study (Sakaluk and Eggert 1996), we used

Parker et al.'s (1990) linear models to analyze sperm competition data in an attempt to identify the underlying mechanisms determining paternity in doubly mated females of the decorated cricket, *Gryllodes sigillatus*. Crickets offer an ideal subject on which to test these models because after mating, the ejaculate of a male typically remains attached outside the female's genital opening in the form of an externally attached spermatophore, which enables an experimenter to manipulate the duration of sperm transfer by removing the spermatophore. In *G. sigillatus*, the spermatophore consists of two distinct components, a small sperm-containing ampulla surrounded by a much larger gelatinous portion, the spermatophylax, that is devoid of sperm. After mating, females typically detach the spermatophylax from the ampulla and spend up to 60 minutes or more consuming it, after which time they remove and consume the ampulla. While the ampulla remains attached to the female, sperm are evacuated from the ampulla and enter the female's reproductive tract at a more or less constant rate for the first 50 minutes of ampulla attachment, after which time the ampulla is usually depleted of sperm (Sakaluk 1984).

Our experimental design followed the recommendations of Parker et al. (1990) in allowing the first male to transfer a full ejaculate (i.e., the ampulla remained attached for 50 minutes), whereas the ampulla from the second male to mate with the female was experimentally removed at different times after mating (i.e., 5, 10, 20, 30, 40, or 50 min), thus curtailing sperm transfer to the female. We subsequently tested our data against the linear models derived by Parker et al. (1990). The results of our linear regression analyses were inconsistent with both the lottery model and a model of sperm displacement in which no sperm mixing occurs until displacement is complete (Sakaluk and Eggert 1996). In testing

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TABLE 1. Conformance of the paternity data of Sakaluk and Eggert (1996) to the ideal lottery assessed using the basic form of the linear model (Parker et al. 1990).  $S_1$  and  $S_2$  designate the number of sperm transferred by the first and second males mated to a female *Gryllodes sigillatus*, respectively.  $P_2$  represents the proportion of offspring sired by the second male.

Linear model		$P_2 = S_2/(S_2 + S_1)$
Slope:	Predicted slope	1
	(b)	
	Observed slope	$1.124 \pm 0.336$
	$\pm$ SE	
	Model supported?	yes
Rationale	$b$ different from zero; $b$ not different from one	
	$t$ -test	$t = 3.349, P = 0.0027; t = 0.369, P > 0.5$
Intercept:	Predicted intercept	0
	(a)	
	Observed intercept	$0.041 \pm 0.121$
	$\pm$ SE	
	Model supported?	yes
Rationale	$a$ not different from zero	
	$t$ -test	$t = 0.337, P = 0.7389$

the lottery model, we plotted our data according to the generalized linear lottery equation  $1/P_2 = b(S_1/S_2) + a$ , where  $P_2$  is the proportion of offspring sired by the second male, and  $S_1$  and  $S_2$  are the numbers of sperm transferred by the first and second males, respectively (or an acceptable surrogate, such as the time of sperm transfer if it occurs at a constant rate). The slope of the regression was not significantly different from zero, leading us to reject the lottery model. While this result is technically correct, a subsequent reanalysis of the same data using the equation for an ideal lottery in its initial and most basic derivation,  $P_2 = S_2/(S_1 + S_2)$ , revealed that our data are actually consistent with an ideal lottery (Table 1; Fig. 1a). Our original paper showed that the same was true when we considered all data (including five instances in which sperm transfer after the first mating was incomplete; see Fig. 2 in Sakaluk and Eggert 1996).

Although these conflicting results seem paradoxical at first, their cause becomes evident upon closer inspection. The equations tested were derived from one another by performing identical operations on both sides of the equation, a standard technique in algebra that results in equivalent equations. One of these operations involved inverting fractions on both sides of the equation. This created two problems. First, the transformation changed the distribution of independent values significantly. Our experimental protocol allowed the first male to transfer a full ejaculate, whereas the second male's sperm transfer was interrupted after varying amounts of time. We attempted to apportion the values for  $S_2$  (sperm transferred by the second male) as evenly as possible between zero and a full ejaculate, creating a relatively even distribution of values of  $S_2$ , or  $S_2/S_1$ , or  $S_2/(S_2 + S_1)$  between zero and 50, zero and one, and zero and 0.5, respectively (Fig. 1a; see also Figs. 3 and 4 in Eggert and Sakaluk 1996). When the equation was assessed using the inverted  $S_1/S_2$  as the independent variable, however, the majority of observations became clumped between one and three (Fig. 1b), thus vi-

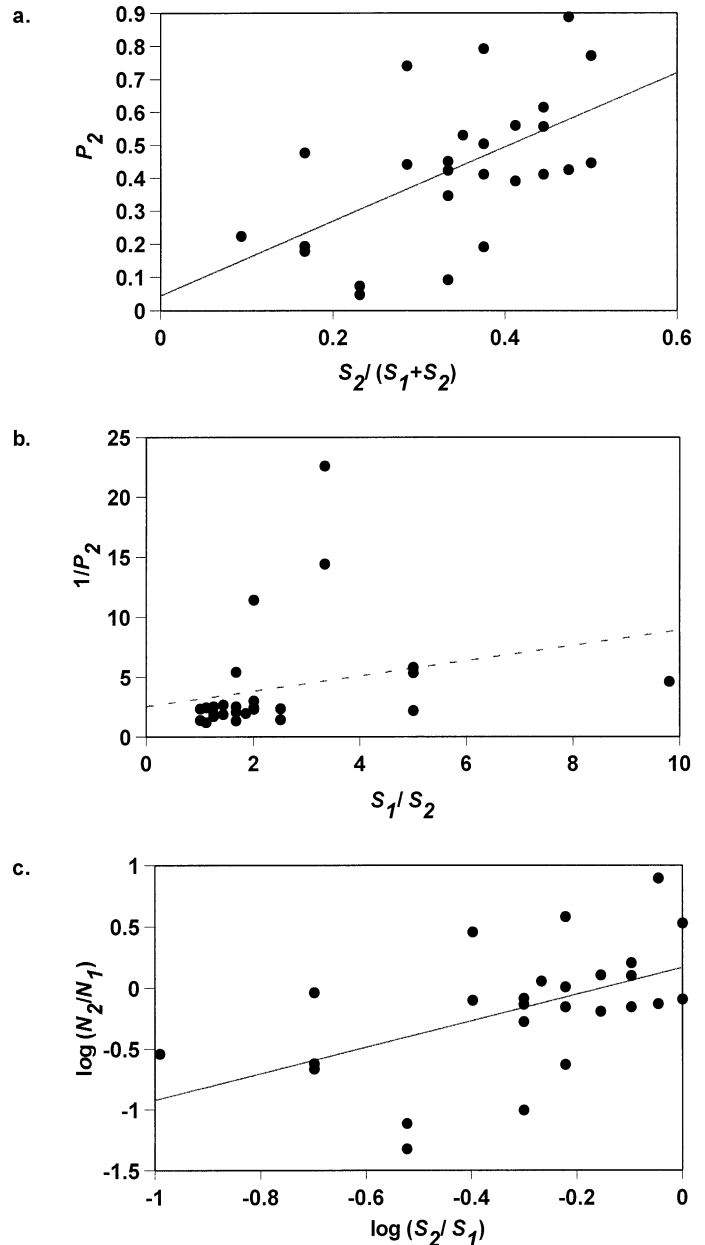


FIG. 1. Paternity data of Sakaluk and Eggert (1996) plotted according to various linear models of a sperm lottery. (a) Basic equation for testing the ideal lottery (Parker et al. 1990):  $P_2 = S_2/(S_2 + S_1)$ ; (b) transformed equation for ideal/loading lottery (Parker et al. 1990):  $1/P_2 = b(S_1/S_2) + a$ ; (c) recommended equation for ideal/loading lottery (this paper):  $\log(N_2/N_1) = \log(S_2/S_1) + \log(P_2/P_1)$ .

olating the assumption of normality that is required of linear regression.

The other problem arising with the transformation of our data stems simply from the fact that they are "real" biological data. As such, they have a certain amount of variation associated with them, whether this is due to measurement error or actual biological variation. Our original data were  $P_2$ ,  $S_1$ , and  $S_2$  values. Some  $P_2$  values were fairly close to zero, but variation around the mean was normal. Inverting the equation resulted in variances that were severely skewed

in many instances. This distortion of the variance distribution was caused by the inversion of small  $P_2$  values, whose inverse values,  $1/P_2$ , became quite large, resulting in the skew. These large values depart sufficiently from the regression line to render it nonsignificant. This non-normality of variances represents a violation of another one of the fundamental assumptions underlying linear regression (Fig. 1b).

Transforming equations by inversion thus may create problems by altering the overall distribution and skewing the original variances to a degree that the data are no longer suitable for a regression analysis. Interestingly, however, this problem arises only with the equations for lottery models (ideal and loaded), but not with the displacement models in Parker et al. (1990). The two equations for displacement models use  $S_2/S_1$  as the independent variable, which has an even distribution across its range of values. As the dependent variable they use  $P_2$ , which is unproblematic, and  $-\ln(P_1)$ , which can become fairly large when  $P_2$  is extremely close to one, but this did not happen in our experiment.

We suggest that the problem with the lottery models can be remedied by reformulating the problematic equation using the actual numbers of offspring sired by each male ( $N_1$  and  $N_2$ ), instead of  $P_2$  values. As in Parker et al.'s (1990) paper, we use  $S_1$  and  $S_2$  to describe the number of sperm transferred by each male,  $s_1$  and  $s_2$  to describe the number remaining in the female sperm stores, and  $p_1$  and  $p_2$  for the probability that a male's sperm remain in the female's storage organs. Hence,

$$P_2 = \frac{s_2}{s_1 + s_2}. \quad (1)$$

The basic equation for a loaded raffle then becomes,

$$\frac{N_2}{N_1 + N_2} = \frac{p_2 S_2}{p_1 S_1 + p_2 S_2}, \quad (2a)$$

$$\frac{N_1}{N_2} + 1 = \frac{p_1 S_1}{p_2 S_2} + 1, \quad (2b)$$

$$\frac{N_1 + N_2}{N_2} = \frac{p_1 S_1 + p_2 S_2}{p_2 S_2}, \quad \text{or} \quad (2c)$$

$$\frac{N_2}{N_1} = \frac{p_2}{p_1} \frac{S_2}{S_1}. \quad (2d)$$

The last equation shows that the ratio of the number of offspring sired by the second male to the number of offspring sired by the first male should be equal to the relative number of sperm transferred by the two males, multiplied by the degree of "loading" as expressed by  $p_2/p_1$ . However, the problem with this equation is that it also uses ratios, which in statistics is known to have rather serious drawbacks (e.g., a non-normal distribution; Sokal and Rohlf 1981). For example, if the true value of  $N_1/N_2$  is one, but there is variation around this value such that there are cases in which  $N_2$  is twice as large as  $N_1$ , and other cases in which  $N_1$  is twice as large as  $N_2$ , then the ratio in these instances will be 2 and 1/2, respectively, and a calculation of the mean from these values yields 1.25 instead of 1.0. This problem can be overcome, however, by using the logarithms of the ratios, which

should follow an approximately normal distribution. Equation (5) derived above, can be transformed to

$$\log \frac{N_2}{N_1} = \log \frac{p_2}{p_1} + \log \frac{S_2}{S_1}. \quad (3)$$

With this equation, we can graph  $\log(N_2/N_1)$  against  $\log(S_2/S_1)$  for a test of the fair or loaded lottery. If the outcome of sperm competition is consistent with a raffle, the regression should yield a line with a slope of one. The y-axis intercept indicates the loading: when the raffle is fair,  $p_2 = p_1$  and thus  $\log(p_2/p_1) = 0$ . When applied to our original data, this version of a linear model is also consistent with a fair lottery (Fig. 1c, slope =  $1.088 \pm 0.358$ ; slope is different from zero:  $t = 3.037$ ,  $P = 0.0057$ , but not different from one:  $t = 0.245$ ,  $P > 0.5$ ; intercept =  $0.159 \pm 0.141$  is not different from zero:  $t = 1.129$ ,  $P = 0.2699$ ).

These results show that we should not have excluded a simple fair raffle as a prospective mechanism of sperm competition in our previous study (Sakaluk and Eggert 1996). The mechanism we favored in our original paper was one in which only a portion of the sperm in the store is displaced, and sperm of the second male mixes instantaneously with that of the first throughout the displacement process. To decide which of these two mechanisms are most consistent with our paternity data, we require accurate information about the total number of sperm remaining in the sperm stores after two successive matings. At the very least, we require more information about properties of the female sperm storage organ. The fair raffle requires that the spermatheca must either be perfectly expandable, or large enough to accommodate two males' ejaculates; displacement with instantaneous mixing suggests that the capacity of the spermatheca limits the total number of sperm remaining in storage inside the female.

To approach a more complete understanding of the processes and mechanisms involved in sperm competition in *G. sigillatus*, it would be necessary to have information about the actual number of sperm in the spermatheca after one and two matings. Theoretically, it would be necessary to have information about both the number of sperm from each of the competing males that enter the female sperm storage organs, and the number that remain in the sperm stores. However, in practice, this is rarely going to be feasible, and an assessment of relative sperm numbers in the sperm stores will certainly be more helpful than no information at all. If the fair raffle is indeed the mechanism underlying the pattern of sperm competition in *G. sigillatus*, we would expect the number of sperm in the spermatheca to approximately double after a second mating. On the other hand, if previously transferred sperm are displaced during a mating, then the number of sperm in the spermatheca after two matings should be significantly less than twice the number found after a single mating.

Because such data were not previously available, we carried out an experiment in which we determined the number of sperm transferred by the same male in single and double matings with virgin females. Each of 25 males was allowed a single mating with one virgin, and two matings on consecutive days with another virgin female. Thirteen males were assigned to the single-mating treatment first, whereas the remaining 12 males were assigned to the double-mating treat-

ment first to control for any order effect. Sperm numbers were determined using methods described in Schaus and Sakaluk (2001). The spermathecae of doubly mated females contained more sperm than those of singly mated ones (mean  $\pm$  SE of sperm counts:  $66.99 \pm 8.54$  for singly mated females,  $111.8 \pm 15.97$  for doubly mated females;  $N = 25$ , paired  $t$ -test,  $t = 3.355$ ,  $P = 0.0026$ ). We then determined the ratio of sperm numbers for each pair of females mated to the same male (one doubly, one singly). The logarithm of each ratio was used to calculate a mean logarithm, which was then back-transformed. Logarithms were used because of the problems arising with the statistical treatment of ratios as discussed above. The mean ratio of sperm counts thus obtained was 1.604; the limits of the 95% confidence interval were 1.114 and 2.312, respectively (for estimation of the confidence interval, see Zar 1984).

The experimental design of this study assumes that the number of sperm transferred by males in matings on consecutive days remains the same. There is some evidence that sperm numbers are indeed constant, or at least do not vary systematically, in such repeat matings of male *G. sigillatus* on consecutive days (Gage and Barnard 1996). If this is true, then our results can be taken at face value. However, a recent study in which males from our own laboratory population were mated to three different females on consecutive days (Schaus and Sakaluk 2001) suggests that sperm numbers decline over successive matings. A repeated-measures ANOVA revealed significant differences between males in sperm number ( $df = 23$ ,  $F = 3.27$ ,  $P = 0.0003$ ), but also between first, second, and third matings ( $df = 2$ ,  $F = 6.11$ ,  $P = 0.004$ ), with mean sperm counts (per 10- $\mu$ l sample) of 185 for first, 155 for second, and 138 for third matings. If these values are representative of the actual relative numbers of sperm transferred in successive matings, then the expected ratio of sperm numbers in doubly and singly mated females depends on the order in which males mated with females. For males that mated once with the first female, and twice with second female, the ratio should not be 2, but 1.58 [(155 + 138)/185] if no displacement occurs. If displacement does occur, the value should be even lower; if two new sperm displace one that is already in the female's spermatheca, the ratio should not be 1.5, but 1.21 [(155 + 0.5  $\times$  138)/185]. When males mate with females in the reverse order (twice with the first female, once with the second), and no displacement occurs, the expected ratio is 2.46 [(185 + 155)/138]. With partial displacement (0.5 old sperm displaced per new one), the expected value is 1.90 [(185 + 0.5  $\times$  155)/138]. Partitioning our data into the two order groups, we find that we are again unable to differentiate between the two possible mechanisms: for males mating once with the first female ( $N = 13$ ), the back-transformed mean ratio was 1.393 (95% CL: 0.820–2.367); for males mating twice with the first female ( $N = 12$ ), the mean ratio was 1.869 (95% CL: 1.063–3.287). In each case, both the expected value for partial displacement and that for no displacement are well within the 95% confidence limits of the means.

Consequently, we must concede that neither the determination of the  $P_2$  values in our original paper (Sakaluk and Eggert 1996) nor the sperm counts presented here enable us to make a definitive choice between partial displacement and

a fair raffle as the mechanism of sperm competition in *G. sigillatus*. Our estimate of relative numbers of sperm after two matings (1.604) agrees surprisingly well with our estimate of the relative volume of the spermatheca after two matings (1.49), which was obtained independently based on linear measurements of spermathecae (Sakaluk 1986; Sakaluk and Eggert 1996). This agreement suggests that in *G. sigillatus* there may indeed be a linear relationship between spermathecal volume and number of sperm stored (cf. Simmons and Siva-Jothy 1998). If such a linear relationship exists, spermathecal measurements of singly and multiply mated females could contribute significantly to an improved understanding of the mechanism of sperm competition in this species.

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