

Pitfalls in experiments testing predictions from sperm competition theory

L. ENGQVIST & K. REINHOLD

Institut für Evolutionsbiologie und Ökologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

Keywords:

ejaculate size;
sexual selection;
sperm allocation;
sperm competition intensity;
sperm competition risk.

Abstract

As females of many species mate with more than one male, ejaculates often face competition from the sperm of other males. In recent years, numerous papers have been published on theoretical predictions of evolutionary, behavioural and physiological responses to variation in the strength of sperm competition (SC). These theoretical predictions have also been extensively tested. However, although predictions from SC theory are relatively straightforward, extra caution has to be paid in the design of experiments testing them. One difficulty is for example to disentangle immediate and mean SC risk and intensity. Without carefully designed experiments, it is also very easy to simultaneously increase SC risk and the probability of intense SC – a situation for which we currently have no clear predictions, as the theoretical models to date only assume variation in either SC risk or intensity. In this paper, we discuss these and some other pitfalls related to manipulations of SC risk and intensity and suggest how to avoid them.

Introduction

In the more than three decades that have passed since Parker's (1970) influential work on sperm competition (SC) was published, the interest in this topic has continuously increased (for reviews see Smith, 1984; Birkhead & Møller, 1998; Simmons, 2001). In particular, optimal sperm allocation strategies have been studied intensively, through theoretical analyses as well as empirical tests of the theoretical predictions. We here discuss problems that can arise in experiments testing the predictions from the theoretical models (Parker, 1990, 1993; Parker *et al.*, 1996, 1997) on how males should respond to variation in sperm competition risk (SCR) and sperm competition intensity (SCI). SCR is defined as the probability that the male's sperm will compete against the sperm from other males for a given set of ova (Parker, 1998). Assuming that several ejaculates typically compete for each set of ova, SCI is defined as the number of competing ejaculates. The logic behind these models is easy to understand. If females mate multiply and sperm

compete numerically, males can increase sperm competitiveness, and hence the probability of siring many offspring by transferring more sperm during copulation. However, an increased sperm production is likely to have some costs. Accordingly, the models assume that males that produce more sperm must decrease their remaining reproductive effort, and are thus assumed to have lower mating success or provide less paternal care. Among other things, these models (Parker *et al.*, 1996, 1997) make two sets of specific predictions.

The first set predicts how much of its energy budget a given male should allocate to sperm production. If, on average, males have an increased probability of mating with females that will mate more than once, males should invest a larger proportion of their reproductive effort in sperm production (Fig. 1a). The same is predicted if the average number of competing ejaculates increases (Fig. 1b). Thus a male's investment in spermatogenesis is expected to increase with both the *mean* risk and *mean* intensity of SC that males are subjected to.

The second set of predictions concerns the question how much of its current sperm reserves a male should allocate to a specific copulation. This will be affected by the *immediate* (equivalent to local) risk and intensity of SC perceived by the male in this particular mating (Fig. 2). If males are able to distinguish between matings

Correspondence: Leif Engqvist, Institut für Evolutionsbiologie und Ökologie, Rheinische Friedrich-Wilhelms-Universität Bonn, An der Immenburg 1, D-53121 Bonn, Germany.
Tel.: +49 228 735754; fax: +49 228 735129;
e-mail: lengqvist@evolution.uni-bonn.de

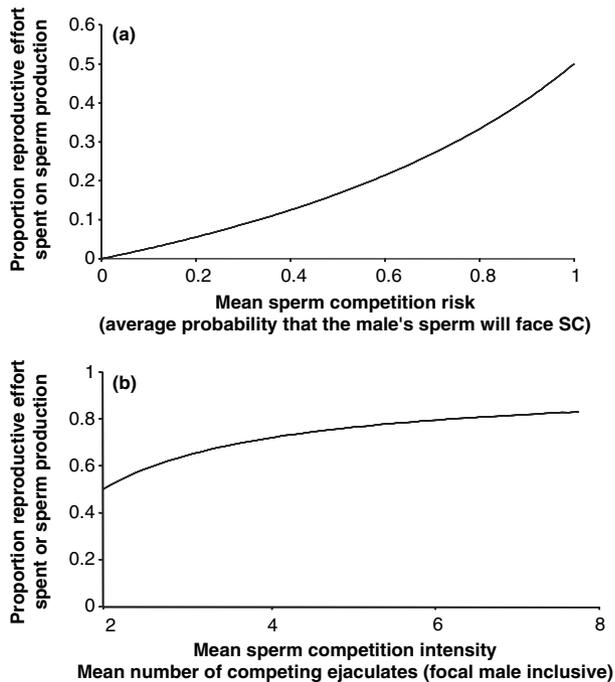


Fig. 1 Theoretical predictions on how much of its total reproductive effort a male should allocate to sperm production in relation to (a) the average risk of sperm competition and (b) the average intensity of sperm competition. (a) is modified from Parker *et al.* (1997), and (b) is from Parker *et al.* (1996).

with high and low SCR, they should always allocate a larger amount of their present sperm reserves to the matings with high SCR (Fig. 2a). Males' expected response to variation in SCI is more complicated: males are expected to allocate least sperm in matings with no competitors and most sperm in matings with exactly one competitor. With SCI above one competing ejaculate, however, males are expected to allocate a decreasing amount of sperm with increasing SCI (Fig. 2b). The cause of this counterintuitive result is that with an increasing number of competitive sperm, the marginal fitness increase (fertilization probability per sperm) of any additional sperm investment constantly declines.

As in the original work of Parker *et al.* (1996), the terms 'SCI between species' and 'SCI within species' have often been used in the sense of mean and immediate SCI, respectively. These terms are unfortunately somewhat misleading, as what is described as 'SC between species' (i.e. mean SC risk and intensity) can vary between species (Harcourt *et al.*, 1981; Svård & Wiklund, 1989; Kappeler, 1997; Stockley *et al.*, 1997; Hosken, 1998; Byrne *et al.*, 2002), but also between populations (Evans & Magurran, 1999a), environments (Gage, 1995; Stockley & Seal, 2001; Brown & Brown, 2003) and even between individuals. An illustrative example of the latter kind of variation is the difference in sperm production

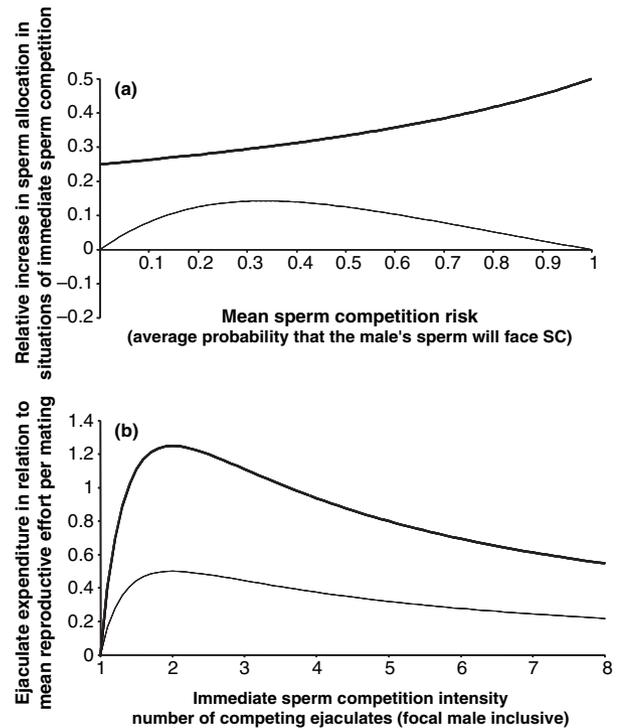


Fig. 2 Theoretical predictions on relative male ejaculate size in a mating in relation to (a) increased perceived immediate sperm competition risk and (b) increased immediate sperm competition intensity. The upper bold line in (a) gives the difference between a male certain to face sperm competition and a male certain not to face sperm competition, and the lower thin line the difference between a male certain to face sperm competition and a male with no information of sperm competition other than the average risk of sperm competition. Note that when males are certain of sperm competition (high immediate sperm competition risk), they should *always* increase ejaculate size. The upper bold line in (b) gives the ejaculate expenditure in relation to immediate sperm competition risk for males with a mean sperm competition intensity of five and the lower thin line for males with a mean sperm competition intensity of two. Figures are modified based on published models (a: Parker, 1990; Parker *et al.*, 1997; b: Parker *et al.*, 1996).

between sneakers and dominant males in several species (e.g. Gage *et al.*, 1995; Taborsky, 1998; Simmons *et al.*, 1999). Sneakers, which are certain to face SC in all matings (high mean SCR), usually develop relatively larger testes than dominants. Immediate SC ('within species'), however, varies between male matings. For example, males copulating with females in the presence of another male (high immediate SCR) usually transfer more sperm than when copulating in the absence of rival males (e.g. Gage, 1991; Nicholls *et al.*, 2001; Olsson, 2001).

What is important about this otherwise purely semantic point is that experimental treatment may also change 'SC between species' – mean SC of individuals. This effect may be intended or unintended. Therefore, there is a risk

that it may be experimentally confounded with immediate SC. These problems will be discussed in the next section.

Pitfall 1: testing mean and immediate responses

There can be major problems with testing the predictions concerning immediate SC risk and intensity. Namely, the experimental manipulation of male perception of immediate SCR and SCI might also affect male perception of mean SCR and SCI. Imagine the following situation: prior to the experiment, focal males are either kept alone, together with one other male, or together with many males for a certain period of time. This is carried out to manipulate male perception of the immediate SCI in the subsequent test copulation. Such an experimental design is encountered frequently (Gage & Barnard, 1996; Oppliger *et al.*, 1998; Evans & Magurran, 1999b; Schaus & Sakaluk, 2001; Candolin & Reynolds, 2002; Evans *et al.*, 2003; Pizzari *et al.*, 2003). However, manipulating male perception of immediate SC in this way possibly also affects male perception of mean SC. Specifically, if the focal male judges future copulations to involve a high risk and intensity of SC (many other males around), he should, if physiologically possible, invest a larger amount of energy reserves into sperm production.

According to this argument, one can therefore expect males to ejaculate more sperm in situations of high risk and intensity of SC but this need not be because of strategic allocation of the male's current sperm reserves, but could result from an increased investment in sperm production. Several empirical studies (e.g. Gage, 1995; Stockley & Seal, 2001) have for instance shown that males reared at high densities develop larger testes than males reared at low densities. This problem may not be great in studies of SCR: predicted sperm allocation goes in the same direction whether mean or immediate risk increases. Nevertheless, it is not possible to conclude that the change in ejaculate size was due to a change in sperm production or due to strategic sperm allocation of male sperm reserves. In contrast, the expected results of an SCI experiment will be completely different if mean and not immediate SCI is being changed. In fact, using the above described experimental design, it is quite possible that *both* properties are affected, a situation for which we have no clear predictions. For instance, do we expect the ejaculates of males perceiving a mean *and* immediate SCI of four ejaculates to be larger or smaller than males' perceiving a mean and immediate SCI of two? For future research we therefore urge *not* to use the experimental design described above. Ideally, perceived immediate SC risk and intensity must not be manipulated until the test copulation (e.g. Fuller, 1998; Pilastro *et al.*, 2002), preventing males from responding physiologically to this new situation. Alternatively or additionally, one can test and eventually control for differences in sperm reserves

(e.g. testis weight) between treatments (e.g. Evans *et al.*, 2003).

Pitfall 2: manipulating immediate SCI

The intensity model of SC (Parker *et al.*, 1996) predicts how males should respond to situations in which the male's sperm compete against the sperm from one to several males. The model was designed to fit the mating situation for group spawners with external fertilization, but in principal the same predictions apply to mating systems with internal fertilization when females mate multiply and the sperm from many males is stored before oviposition, given that there is a fair raffle between sperm from different males. In one specific model, the assumption is made that all males participating in the raffle have perfect information on the number of competing ejaculates in the current competitive situation (perfect information on immediate SCI). In this situation the model predicts that males should invest most sperm when competing with one other male and with increasing SCI above this level, males should invest a decreasing sperm amount.

Several empirical studies have tried to address this question (e.g. Gage & Barnard, 1996; Fuller, 1998; Schaus & Sakaluk, 2001; Candolin & Reynolds, 2002; Pilastro *et al.*, 2002; Pizzari *et al.*, 2003). However, in these studies the *presence* of competing males has been experimentally manipulated in order to simulate different levels of SCI. Thus, males have information on the number of males nearby but here we argue that this can, but must not be identical to having information on the exact number of competing ejaculates (see also Dosen & Montgomerie, 2004).

Imagine that the focal male in the experimental situation estimates the probability that SC will actually occur with sperm from the nearby competitor(s) to be less than one, say 20% (note that the male is likely to estimate this probability more accurately than the experimenter). What consequences would this have for the expected results? In a treatment with no other male, the focal male will of course perceive no immediate SCR and an SCI of zero. In a treatment with one potential competitor, the focal male will estimate the immediate risk of SC to be 20% and a probability of 20% that his ejaculate will compete with another one. In a treatment with two other males however, the male will estimate SCR to be 36%, the probability of competing with one other male 32%, but the probability of competition with two other ejaculates only 4%, assuming no influence of male interactions on the actual probability of competition. The expectations concerning differences in strategic sperm allocation in these situations become less obvious and are probably different from the model situation.

Under the described scenario, we see two main problems. First, the actually expected SCI in the test situation will be less than the intended. Secondly, the

situation in the experimental design described above introduces a new condition which is not part of the original model. From the assumptions of the theoretical model it follows that males assessing SCI to be above one other competing ejaculate will also face *certain* SCR. This is not the case in this experimental design in which increasing the *probability* of competing ejaculates will affect *both* immediate SCR and SCI. Thus, introducing more males will always increase immediate SCR, which has a positive effect on ejaculate size. Simultaneously, the probability of high immediate SCI increases which is predicted to have an opposite effect on ejaculate size, but the magnitude of this effect is difficult to estimate unless one has extensive knowledge about male perception of SC.

Nonetheless, it seems reasonable to assume that the key prediction of the theoretical model is still applicable to this new condition. Namely, in response to an increasing number of males present at spawning, there should be a more or less rapid increase in sperm allocation to a peak followed by a monotonic decrease. However, the point at which male expenditure should be highest may well shift from the situation when one other male is present to a situation when more males are present (Box 1). The magnitude of this shift will depend on the probability that males nearby will participate in the raffle. If this probability is close to 100%, we have the situation that is assumed in the theoretical model and we will expect male expenditure to be highest when one other male is present. On the other extreme, if this probability is very low, increasing male number will initially only affect SCR and we will expect maximum expenditure when many males are present. We strongly recommend that this potential discrepancy between theoretical model and experimental setup should be taken into account when interpreting results and planning experiments.

An obvious way to avoid this pitfall is to ensure that the sperm from each experimental male really encounters the intensity of SC predetermined at the treatment assignment. To be exact, females used in the experiment should actually mate with the given number of males specified by the corresponding treatment. However, in this experimental design extra caution has to be paid to avoid purely correlational results. For instance, more vigorous males may be better in avoiding high SCI (e.g. Elgar *et al.*, 2003; Pizzarri *et al.*, 2003), due to, for instance, a superior mate guarding ability. Therefore it will be more difficult to obtain estimates of ejaculate size from vigorous males assigned to treatments with high SCI, and one may consequently run the risk of obtaining a biased, nonrandom data set. If vigorous males also have larger (or smaller!) sperm reserves, this would for example also confound the results. The possible pitfalls with correlational results in behavioural studies have already been elucidated elsewhere (Milinski, 1997). Again, testing and eventually controlling for differences

in, e.g. testis weight or using a repeated measurements design might prove helpful.

A frequently used method is to use phenotypic differences between females as indicators of immediate SC risk and intensity (Simmons *et al.*, 1993; Simmons & Kvarnemo, 1997; Martin & Hosken, 2002). For instance, larger or older females may be expected to mate or to have mated more often. This is an excellent and nonproblematic method when testing differences in SCR. For tests of the predictions on male response to perceived immediate SCI it may also prove fruitful, but only if the frequency distributions of female number of matings for the different female groups are well known. Alternatively, SCR should be very close to 100%. Otherwise we run the risk of making the same mistake as in the previous example, i.e. that experimental groups differ more in the probability that females will or have mated twice than they do in the probability that they will or have mated more than twice. Furthermore, extra caution has to be paid to differences in fecundity between female groups, as fecundity is also predicted to affect male sperm allocation (Galvani & Johnstone, 1998; Reinhold *et al.*, 2002). This has been empirically demonstrated in for instance dung flies (Parker *et al.*, 1999).

Pitfall 3: ignoring future and past risk in immediate SC risk and intensity studies

Whereas the previous problem predominantly concerned external fertilizers, the next problem is only related to species with internal fertilization. The difference between external and internal fertilization is that males in species with internal fertilization have to take the females' past mating history and future mating probability into account when estimating immediate SCR and SCI. This is not the case in species with external fertilization. Therefore, these factors have to be considered when designing and interpreting studies of immediate SCR and SCI in species with internal fertilization.

In studies of immediate SC effects on sperm allocation it is often desirable to assign male individuals to a 'no competitor', or 'low risk' treatment. An experimental design often seen in experiments of immediate SCR is as follows: a focal male either mates with a female in close vicinity to a rival male (high SCR) or alone (low SCR) (e.g. Gage, 1991; Cook & Gage, 1995; Nicholls *et al.*, 2001). Alternatively, focal males were allowed to observe a female's first copulation with a rival male (high SCR) or not (low SCR) (Olsson, 2001). In the first example, males' perceived future SCR will be elevated; in the second example, males' perceived past SCR will be elevated. However, imagine this experiment performed in a species in which males generally estimate immediate SCR to be high (species with high mean SCR). This 'background SCR' may well add to the experimental SCR, generating unintended effects. For example males in the 'low risk' treatment may generally expect high

SCR and will possibly perceive the risk of SC to be near 100%, with the consequence that males should invest maximally! Any increase in the putative SC risk will not significantly influence the actual risk of SC, but will only increase the perceived intensity of SC above the level of one competing ejaculate, which will decrease male optimal sperm allocation.

Our advice here is to only use this method in SCR studies when the average risk of SC is relatively low. Otherwise the effect of the enhancement of the perceived immediate SCR through the addition of competitors may not be as large as the effect of the enhancement of the perceived immediate SCI. Regarding studies of immediate SCI in species with internal fertilization, the same general problem applies. The SCI in the 'no competitor' treatment may also be affected by the general 'background' SCI. Our pessimistic advice here is to restrict studies on SCI in internal fertilizers to levels of SC above one other competitor, and expect decreasing sperm allocation with increasing SCI (e.g. Simmons & Kvarnemo, 1997); this is unproblematic (Parker *et al.*, 1996).

The last two pitfalls are actually variations of the same problem. Risk models predict sperm allocation in species or situations with relatively low SCI (Parker, 1990; Parker *et al.*, 1997). In fact, in these models females are not assumed to mate more than twice, or rather, sperm from maximally two males compete for the fertilization of the ova (which includes polyandric species with sperm displacement). Hence, immediate SCI is unimportant. In contrast, intensity models predict sperm allocation in situations where SC usually involves two or more ejaculates. In these situations immediate SCR is *always* high and its effect negligible. Ideally, experiments testing male response to immediate SCR should thus be performed with species in which competition between sperm from more than two males rarely occurs, and experiments testing male response to immediate SCI should be performed with species in which female monandry is infrequent. Between these conditions there is a grey area, which is not covered by the present sperm competition theory (Box 1). This is not a criticism of the present models, but a word of advice to researchers planning future research.

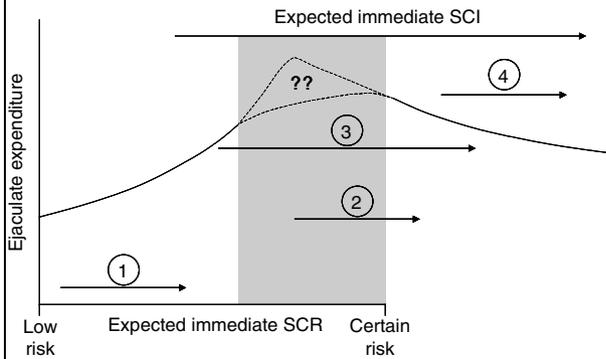
Pitfall 4: the possible consequences of sex ratio manipulation

In many studies, sex ratio manipulation has been used to change male perception of SC risk and intensity (e.g. Oppliger *et al.*, 1998; Evans & Magurran, 1999b; Evans *et al.*, 2003; Reinhardt & Arlt, 2003). With sex ratio manipulation we do not mean the manipulation of immediate SCI by, e.g. number of males immediately present at copulation, but the relatively long-term manipulation of sex ratio affecting the perception of mean SC risk and intensity. The logic behind this design

is that when there are many males per female, SC risk and intensity will be higher than when there are few males per female or even fewer males than females. Thus, male investment in sperm production (mean effects) should increase with increasing sex ratio (proportion of males). If local sex ratio is changed immediately before a test copulation, there are no objections. However, the predictions on energy allocation into sperm production are only valid when the risk and/or intensity of SC changes and *everything else remains equal*. Sex ratio variation, however, may possibly have other additional effects on predicted male sperm investment. If the ratio of males per female increases, the competition between males for female mating partners will also be intensified. Therefore with an increasing sex ratio, male investment in traits favoured by precopulatory sexual selection is likely to increase, too. Not much is known about trade-offs between male investment in traits favoured by precopulatory sexual selection and traits favoured by post-copulatory sexual selection (e.g. sperm number or size), but it is conceivable that such trade-offs do exist (e.g. Warner *et al.*, 1995; Danielsson, 2001; Elgar *et al.*, 2003), and they are actually one of the key assumptions of most theoretical models in sperm competition theory (e.g. Parker, 1998). Therefore, as an effect of the increased strength of precopulatory sexual selection, male investment in sperm production might even *decrease* with increasing sex ratio.

Of course, it may be possible for males to invest more both in sperm production and precopulatory traits, such as weapons used in male–male combat, at the cost of other investments. Nevertheless, the expected average effect of responses to variation of mean SC will be lower in studies using a sex ratio manipulation than in studies manipulating SC alone. Therefore these studies have the potential to be conservative. Thus, when positive results are observed, one can say that these were achieved despite the possibly negative effect from the sex ratio manipulation. On the other hand, negative results from experiments using such a sex ratio manipulation design should be considered with caution.

As mentioned before, this concern does not in general apply to SC studies testing responses to immediate SCR and SCI. There is no reason to suspect that males spending more energy in a fight or to court a female will have less sperm reserves available to expend in the subsequent mating. However, a relatively long-term manipulation of sex-ratio in order to study immediate SC is inappropriate. The reasons are similar to our arguments stated in the section Pitfall 1. This design may affect sperm production both positively, due to an elevated risk and intensity of mean SC, and negatively, due to an increased allocation to precopulatory sexual traits. The outcome of a test experiment measuring ejaculate size in response to the immediate presence of competitors will be difficult to interpret, due to these possibly complex interactions.

Box 1

Sperm competition theory predicts that male ejaculate expenditure should increase with increasing immediate SCR (solid line in left part of the figure). Yet, if males face *certain* sperm competition their ejaculate expenditure should decrease with increasing SCI, i.e. number of competing ejaculates (solid line in right part of the figure). In between, where the SCR and SCI models meet, there is a grey area with unclear predictions. Nevertheless, one prediction is quite clear: we expect a rather rapid increase in ejaculate expenditure to a peak followed by a monotonic decrease. The uncertainty concerns the exact position of the peak. This is illustrated by the two alternative curves (dashed lines).

The figure further illustrates four examples of the hypothetical effects of an increase in the level of sperm competition on male optimal ejaculate expenditure. The arrows depict the enhancement of sperm competition from the level faced on average by males (control), to that experienced by males in experimental treatments with an increased level of immediate sperm competition. These four examples show that at intermediate levels of sperm competition, where the assumptions for both risk and intensity models are to some extent violated, outcomes of an experimentally increased sperm competition are unpredictable and may render unexpected results: an increase or decrease in ejaculate expenditure are both possible.

1. The average SCR of the focal experimental group (species, population, male phenotype, etc.) is relatively low. More importantly, male sperm rarely face the competition of sperm from more than one other male. The theoretical predictions are precise: males should increase their ejaculate size with increasing immediate SCR.

2. An SCR experiment with a high average risk of sperm competition. An increase in the level of sperm competition will potentially also affect the perceived SCI. Predictions are therefore not unambiguous, as intensity and risk of sperm competition have opposite effects on optimal ejaculate size. Hence, an increase in immediate SCR in species (or populations, etc.) with high average SCR must not result in an increase in ejaculate size.

3. An SCI experiment in which males perceive the presence of competitors only and hence males cannot be certain of sperm competition. As the potential immediate SCI increases (number of males nearby), so does immediate SCR. These have contrasting effects on expected ejaculate size and therefore there are no clear predictions for this kind of experiment.

4. Males are certain that their sperm will face sperm competition. An increase in the level of perceived sperm competition will only increase SCI. The theoretical predictions are unambiguous: males should decrease their ejaculate size with increasing immediate SCI.

These examples emphasize how important it is to clearly distinguish between circumstances that can be predicted from SCR models, and those from SCI models. SCR models make predictions for situations where sperm competition is rare and sperm competitions intensity models make predications for situations where the mean number of competing ejaculates is usually two or more (Parker *et al.*, 1996).

In these cases, predictions on male optimal ejaculate expenditure are clear and unambiguous. This is not the case in the grey area in which both properties, risk and intensity, changes simultaneously.

Concluding remarks

Although the predictions from the game theory models on male response to SCR and SCI are quite straightforward, we have described some serious problems in the design of the experiments that may arise when addressing these questions. Most importantly, the accurate test for the set of predictions concerning mean SC risk and intensity is to test for differences in sperm production and for the second set of predictions concerning immediate SC risk and intensity to test for differences in allocation of the males' present sperm reserves. Failure to differentiate

between these two processes (e.g. manipulating mean SCR and measuring ejaculate size or simultaneous manipulation of mean and immediate SC) may make it difficult to interpret the results.

Experiments using a long-term manipulation of sex ratio in order to change mean SC must be interpreted with caution, as traits associated with other aspect of sexual selection will potentially also be affected. Without knowledge of the trade-off between these traits and sperm production, exact predictions are difficult to formulate.

Furthermore, extreme caution should be exercised with regard to the fact that males may estimate SCI

differently than the experimenter. Overall, the problems will be most acute in the design of experiments aiming at testing predictions on variation in immediate SCI. This is the hardest nut to crack. Of the four predictions we have discussed, this is the one with the fewest published experimental tests receiving the highest proportion of contradicting results (e.g. Gage & Barnard, 1996; Fuller, 1998; Schaus & Sakaluk, 2001; Pilastro *et al.*, 2002; Pizzari *et al.*, 2003).

Acknowledgments

We thank Jutta Schneider, David Hosken, Geoff Parker and an anonymous reviewer for valuable comments on a previous version of this manuscript. LE is supported by the Deutsche Forschungsgemeinschaft (EN 469-1/1).

References

- Birkhead, T.R. & Møller, A.P. (eds). 1998. *Sperm Competition and Sexual Selection*. Academic Press, San Diego, CA.
- Brown, C.R. & Brown, M.B. 2003. Testis size increases with colony size in cliff swallows. *Behav. Ecol.* **14**: 569–575.
- Byrne, P.G., Roberts, J.D. & Simmons, L.W. 2002. Sperm competition selects for increased testes mass in Australian frogs. *J. Evol. Biol.* **15**: 347–355.
- Candolin, U. & Reynolds, J.D. 2002. Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (*Rhodeus sericeus*). *Proc. R. Soc. Lond. B* **269**: 1549–1553.
- Cook, P.A. & Gage, M.J.G. 1995. Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). *Behav. Ecol. Sociobiol.* **36**: 261–268.
- Danielsson, I. 2001. Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). *Proc. R. Soc. Lond. B* **268**: 77–81.
- Dosen, L.D. & Montgomerie, R. 2004. Mate preferences by male guppies (*Poecilia reticulata*) in relation to the risk of sperm competition. *Behav. Ecol. Sociobiol.* **55**: 266–271.
- Elgar, M.A., De Crespigny, F.E.C. & Ramamurthy, S. 2003. Male copulation behaviour and the risk of sperm competition. *Anim. Behav.* **66**: 211–216.
- Evans, J.P. & Magurran, A.E. 1999a. Geographic variation in sperm production by Trinidadian guppies. *Proc. R. Soc. Lond. B* **266**: 2083–2087.
- Evans, J.P. & Magurran, A.E. 1999b. Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. *Anim. Behav.* **58**: 1001–1006.
- Evans, J.P., Pierotti, M. & Pilastro, A. 2003. Male mating behavior and ejaculate expenditure under sperm competition risk in the eastern mosquitofish. *Behav. Ecol.* **14**: 268–273.
- Fuller, R.C. 1998. Sperm competition affects male behaviour and sperm output in the rainbow darter. *Proc. R. Soc. Lond. B* **265**: 2365–2371.
- Gage, A.R. & Barnard, C.J. 1996. Male crickets increase sperm number in relation to competition and female size. *Behav. Ecol. Sociobiol.* **38**: 349–353.
- Gage, M.J.G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Anim. Behav.* **42**: 1036–1037.
- Gage, M.J.G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond. B* **261**: 25–30.
- Gage, M.J.G., Stockley, P. & Parker, G.A. 1995. Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **350**: 391–399.
- Galvani, A. & Johnstone, R. 1998. Sperm allocation in an uncertain world. *Behav. Ecol. Sociobiol.* **44**: 161–168.
- Harcourt, A.H., Harvey, P.H., Larson, S.G. & Short, R.V. 1981. Testis weight, body weight and breeding system in primates. *Nature* **293**: 55–57.
- Hosken, D.J. 1998. Testes mass in megachiropteran bats varies in accordance with sperm competition theory. *Behav. Ecol. Sociobiol.* **44**: 169–177.
- Kappeler, P.M. 1997. Intrasexual selection and testis size in strepsirrhine primates. *Behav. Ecol.* **8**: 10–19.
- Martin, O.Y. & Hosken, D.J. 2002. Strategic ejaculation in the common dung fly *Sepsis cynipsea*. *Anim. Behav.* **63**: 541–546.
- Milinski, M. 1997. How to avoid seven deadly sins in the study of behavior. *Adv. Study Behav.* **26**: 159–180.
- Nicholls, E.H., Burke, T. & Birkhead, T.R. 2001. Ejaculate allocation by male sand martins, *Riparia riparia*. *Proc. R. Soc. Lond. B* **268**: 1265–1270.
- Olsson, M. 2001. 'Voyeurism' prolongs copulation in the dragon lizard *Ctenophorus fordi*. *Behav. Ecol. Sociobiol.* **50**: 378–381.
- Oppliger, A., Hosken, D.J. & Ribi, G. 1998. Snail sperm production characteristics vary with sperm competition risk. *Proc. R. Soc. Lond. B* **265**: 1527–1534.
- Parker, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.
- Parker, G.A. 1990. Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. Lond. B* **242**: 127–133.
- Parker, G.A. 1993. Sperm competition games: sperm size and sperm number under adult control. *Proc. R. Soc. Lond. B* **253**: 245–254.
- Parker, G.A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm Competition and Sexual Selection* (T. R. Birkhead & A. P. Møller, eds), pp. 3–54. Academic Press, San Diego, CA.
- Parker, G.A., Ball, M.A., Stockley, P. & Gage, M.J.G. 1996. Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proc. R. Soc. Lond. B* **263**: 1291–1297.
- Parker, G.A., Ball, M.A., Stockley, P. & Gage, M.J.G. 1997. Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond. B* **264**: 1793–1802.
- Parker, G.A., Simmons, L.W., Stockley, P., McChristie, D.M. & Charnov, E.L. 1999. Optimal copula duration in yellow dung flies: effects of female size and egg content. *Anim. Behav.* **57**: 795–805.
- Pilastro, A., Scaggiante, M. & Rasotto, M.B. 2002. Individual adjustment of sperm expenditure accords with sperm competition theory. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 9913–9915.
- Pizzari, T.K.C.C., Løvlie, H., Jakobsson, S. & Birkhead, T.B. 2003. Sophisticated sperm allocation in male fowl. *Nature* **426**: 70–74.
- Reinhardt, K. & Arlt, D. 2003. Ejaculate size variation in the migratory locust, *Locusta migratoria*. *Behaviour* **140**: 319–332.
- Reinhold, K., Kurtz, J. & Engqvist, L. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. *J. Evol. Biol.* **15**: 201–209.

- Schaus, J.M. & Sakaluk, S.K. 2001. Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: not all species play games. *Behav. Ecol.* **12**: 740–745.
- Simmons, L.W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton University Press, Princeton, NJ.
- Simmons, L.W. & Kvarnemo, C. 1997. Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. *Proc. R. Soc. Lond. B* **264**: 1203–1208.
- Simmons, L.W., Craig, M., Llorens, T., Schinzig, M. & Hosken, D.J. 1993. Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. *Proc. R. Soc. Lond. B* **251**: 183–186.
- Simmons, L.W., Tomkins, J.L. & Hunt, J. 1999. Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. Lond. B* **266**: 145–150.
- Smith, R.L. (ed.) 1984. *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, New York.
- Stockley, P. & Seal, N.J. 2001. Plasticity in reproductive effort of male dung flies (*Scatophaga stercoraria*) as a response to larval density. *Funct. Ecol.* **15**: 96–102.
- Stockley, P., Gage, M.J.G., Parker, G.A. & Moller, A.P. 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *Am. Nat.* **149**: 933–954.
- Svärd, L. & Wiklund, C. 1989. Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behav. Ecol. Sociobiol.* **24**: 395–402.
- Taborsky, M. 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. *Trends Ecol. Evol.* **13**: 222–227.
- Warner, R.R., Shapiro, D.Y., Marcanato, A. & Petersen, C.W. 1995. Sexual conflict – males with highest mating success convey the lowest fertilization benefits to females. *Proc. R. Soc. Lond. B* **262**: 135–139.

Received 4 May 2004; revised 4 June 2004; accepted 7 June 2004