

Tactic-specific success in sperm competition

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Sperm competition is a major force in sexual selection, but its implications for mating-system and life-history evolution are only beginning to be understood. The well-known sneak-guard model predicts that sneaks will win in sperm competition. We now provide empirical confirmation of this prediction. Bluegill sunfish (*Lepomis macrochirus*) have both sneak (cuckolder) and guard (parental) males. Guards make nests, court females and provide solitary parental care for the embryos. Sneaks include small cuckolders, which are termed 'sneakers', that dart in and out of nests in order to ejaculate between the spawning pair and larger cuckolders, which are termed 'satellites', that mimic females in order to ejaculate between the spawning pair. Using field behavioural data, genetic data and new mathematical models for paternity analyses, we show, for the first time to the authors' knowledge, that sneaks fertilize more eggs than guards during sperm competition. In addition, we show that sneakers are superior to satellites in sperm competition and, thus, that even among sneaks there are tactic-specific differences in competitive success.

Keywords: sperm competition; alternative mating tactics; sneak-guard model; paternity; bluegill

1. INTRODUCTION

Sperm competition is widespread in nature (Birkhead & Møller 1998). Parker (1998) defined sperm competition as the 'competition between the sperm from two or more males for the fertilization of a given set of ova' (p. 4). The ramifications of sperm competition in the evolution of mating systems and life histories are only beginning to be understood. Parker (1990, 1998) developed the sneak-guard model for understanding sperm strategies in breeding systems where guards (typically larger and older males) attempt to monopolize females against sneaks (typically smaller and younger males), which attempt to steal fertilizations. Sneak-guard breeding systems are widespread among animal species and have often evolved into alternative male mating tactics and strategies (Gross 1996; Taborsky 1998). The model makes two major predictions: (i) the sneak male should have a greater ejaculate expenditure than the guard male, and (ii) the sneak male should have higher paternity per ejaculate than the guard male. Several studies have confirmed the first prediction by showing that sneak males make larger investments than guard males in testes mass and other correlates of expenses, such as ejaculate volume, sperm activity and sperm longevity (e.g. Gage *et al.* 1995; Peterson & Warner 1998; Taborsky 1998; Simmons *et al.* 1999). In contrast, the second prediction has proved more challenging to test, as it requires an assessment of who wins on a per ejaculate or per mating basis. This requires both mating observations and specific analyses of paternity. The only publication that has attempted to test the sperm competition success of sneaks and guards, which was performed in the laboratory using irradiated beetles, did not report any difference (Tomkins & Simmons 2000). We now provide, to the authors' knowledge, the first field test of the sneak-guard model using behavioural observations of natural breeding in bluegill sunfish, genetic markers and

quantitative models of paternity. Our study confirmed the superiority of sneaks over guards in sperm competition.

(a) *The sneak-guard model*

Sperm competition is an evolutionary game between rival males for which the solution will be an evolutionarily stable strategy (ESS) since the probability of winning depends on the strategies played by other males in the population (reviewed in Parker 1998). Parker (1990, 1998) recognized that many mating systems will have asymmetries in information or sperm competition risk and developed the sneak-guard model (and the extra-pair copulation (EPC) model) in order to provide conceptual and predictive underpinnings. In the sneak-guard model, males are either sneaks or guards but not both. It is assumed that either a small proportion of males sneak or that a small proportion of matings involve sneaks. It is also assumed that there is a fair raffle without any predetermined advantage for either male's ejaculate (each sperm counts equally), but that one male has more information about the probability of sperm competition than the other. This asymmetry in information results from guards only knowing the mean probability of sperm competition (p) but not exactly when it will occur, while sneaks always know when they will face competition since they initiate it. There is also asymmetry in risk, as guards will only face competition in a proportion of their matings ($p < 1.0$) while sneaks will always face competition ($p = 1.0$). Thus, the strategy of the guard is shaped by the mean risk while the strategy of the sneak is shaped by the guaranteed risk. The ESS outcome (Parker 1998) is that (i) the sneak will invest more in sperm competition than the guard, and (ii) the sneak will obtain higher paternity in sperm competition.

(b) *Mating system and sperm competition in bluegill sunfish*

Alternative male reproductive tactics and sperm competition are very common in fish and many match

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the behavioural assumptions of the sneak-guard model (Gross 1984; Taborsky 1998). A case in point is the mating system of bluegill sunfish (Gross 1982, 1991). 'Parental' (guard) males breed between seven and ten years of age and compete for nesting sites in densely packed colonies. They attract and spawn sequentially with multiple females and provide sole parental care to the developing embryos in their nest. Females release batches of eggs in distinctive actions called 'dips' and they dip hundreds of times in a nest. Precociously maturing 'cuckolder' (sneak) males employ two alternative mating tactics termed 'sneaker' and 'satellite' tactics. Sneakers first mature at the age of two years and steal fertilizations from parentals by hiding near the edges of nests and darting in and out during female dips in order to release sperm beneath the spawning pair. Once sneakers reach the age of four years, they switch to the satellite tactic and mimic females in order to hold a temporary position in the nest directly between the parental and female during multiple female dips. Thus, while sneakers have a somewhat disadvantaged position during sperm competition relative to both parentals and satellites, satellites have an advantaged position. Cuckolders die before the age of mature parentals and never become parentals themselves. Parental males actively chase sneakers and satellites when these are detected. The alternative life histories of cuckolders and parentals may be part of a single conditional strategy with both genetic and environmental influences on the decision process determining which life-history trajectory is followed (Gross 1996; Gross & Repka 1998).

Sperm competition only occurs when a cuckolder (sneaker or satellite) successfully intrudes on a dip. Intrusion rates vary with the ecology of the colony (Gross 1991), but cuckolders usually accompany less than one-quarter of the dips. Therefore, matings by parentals are typically without sperm competition ($p < 0.25$), while cuckolders are almost always in sperm competition since parentals are rarely absent from a dip ($p \approx 1$) (Gross 1982, 1991).

In this study, we tested the sneak-guard model by determining the paternity of sneakers, satellites and parentals under sperm competition. The first prediction of the model, that cuckolders will invest more in sperm production, is already known through their relatively larger testes and greater ejaculate sperm density (Gross 1982). The second prediction, that cuckolders will have higher success in sperm competition, has not been previously tested because such tests are extremely difficult to conduct methodologically. A single bluegill nest has many thousands of embryos resulting from the spawning of multiple females and males, including the parental and several cuckolders. Although our laboratory has previously determined using molecular markers that the overall paternity of parentals to the embryos in their nests ranges from 40 to 100% (Phillip & Gross 1994; Neff 2001), determining the success of an individual cuckolder per dip was an outstanding empirical and theoretical challenge.

2. CALCULATING WHO WINS IN SPERM COMPETITION

Many challenges exist in evaluating the fertilization success of males in a mating system such as that of the

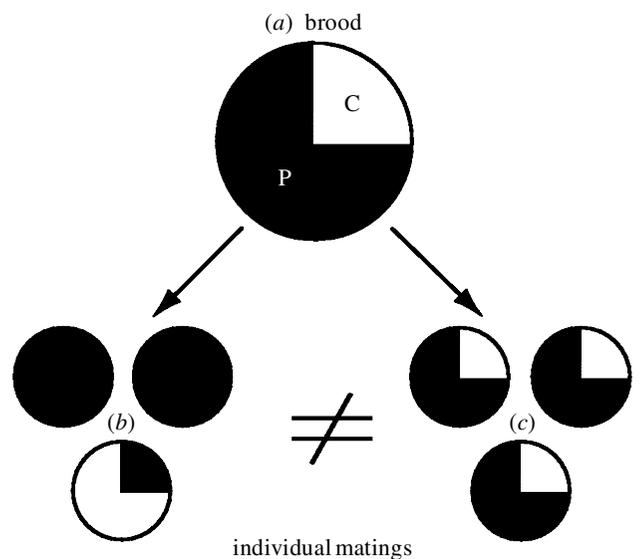


Figure 1. In order to determine the 'winner' of sperm competition in complex mating systems, behavioural data that differentiate matings with and without sperm competition and genetic data that differentiate the relative genetic contributions of sperm competitors in a brood are both needed. This is a figure of a hypothetical brood resembling a nest of the bluegill sunfish in which females release eggs in multiple dips (P, parental and C, cuckolder). (a) Genetic analysis revealed that the parental fertilized 75% of all the eggs in the brood while the cuckolder fertilized the remaining 25%. The paternities could result from either scenario (b) or scenario (c). In (b), 33% of the dips involved sperm competition and the cuckolder fertilized 75% of the eggs per dip in which he was present. In (c), 100% of the dips involved sperm competition and the cuckolder fertilized only 25% of the eggs in those dips. Although (b) and (c) are clearly not equivalent scenarios, either could result in the same overall paternities of the parental and cuckolder in the brood. Therefore, behavioural data on the frequency of sperm competition (i.e. the intrusion rate) must be known in order to calculate who is the superior sperm competitor and test the sneak-guard model.

bluegill. First, the success of cuckolders under sperm competition cannot be readily evaluated from the overall paternity in a parental's brood since they only participate in a fraction of the matings. Second, offspring cannot be assigned to specific dips since thousands occur and eggs cannot be collected from dips without disturbing spawning. Third, only the genotype of a single male, the parental, is available for parentage analyses as cuckolders and females disperse after spawning. Fourth, the large number of cuckolders and females make it difficult to use conventional methods of parentage analysis such as exclusion (e.g. Chakraborty *et al.* 1988). Fifth, the large number of putative parents erodes the resolving power of genetic markers since a parental male is likely to share some alleles with females as well as cuckolders (Chakraborty *et al.* 1988; Neff *et al.* 2000a,b).

We now recognize that calculation of who wins in sperm competition will generally require both behavioural and genetic data (figure 1). The behavioural data provide the frequency at which sperm competition occurs and the genetic data provide the overall paternity of males in mixed parentage broods, while a statistical

Table 1. Summary of the variables and parameters used in the model solving for the average competitive success of different categories of males under sperm competition (see Appendix A)

(Parameters are known quantities that are input into the model, variables are quantities used by the simulation and outputs are the results.)

name	definition
parameters	
D_n	number of dips observed for nest n
I_n^{sn}	number of intrusions by sneakers observed in nest n (expressed as a proportion of D_n)
I_n^{sa}	number of intrusions by satellites observed in nest n (expressed as a proportion of D_n)
F_n	effective number of females genetically contributing to offspring in nest n
M_n	effective number of males genetically contributing to offspring in nest n
C_n	number of offspring sampled from nest n
k_n	number of offspring genetically compatible with putative father in nest n
E	relative number of eggs released in the presence of a cuckold
variables	
Pat_{sn}	proportion of eggs in a single dip fertilized by a sneaker during sperm competition
Pat_{sa}	proportion of eggs in a single dip fertilized by a satellite during sperm competition
Pat_{pa}	proportion of eggs in a single dip fertilized by a parental during sperm competition
Pr_{pat}	probability that the parental male fertilizes the egg during offspring assignment in the simulation (see figure A1b)
outputs	
$\text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}})$	probability distribution relating the probability of observing the input data given the competitive success of sneakers (Pat_{sn}) and satellites (Pat_{sa})
$\overline{\text{Pat}}_{\text{sn}}$	expected paternity of sneakers under sperm competition with parentals (as calculated from equation (1))
$\overline{\text{Pat}}_{\text{sa}}$	expected paternity of satellites under sperm competition with parentals (as calculated from equation (2))

model can link the behavioural data to the overall paternity in order to arrive at the competitive success per mating. A useful statistical procedure is a Monte Carlo simulation (Manly 1997), which can be used for determining the solution that provides the best fit to both the behavioural and genetic data. The solution is that the proportion of eggs fertilized by cuckolders during a single intrusion, when multiplied by the observed intrusion rates, best explains the overall paternity of cuckolders within each nest. The challenge is to develop a rigorous mathematical framework that incorporates the incomplete sampling of candidate parents and uncertainties in genetic offspring assignments. We developed a model based on the two-sex paternity confidence model in Neff *et al.* (2000b) and Neff (2001). Our model provides a framework for calculating the competitive success of all three tactics (sneakers, satellites and parentals) in bluegill sunfish and is applicable to other mating systems as well.

The model (see Appendix A) (variables in table 1) generates a distribution for the probability of the specific paternities of sneakers and satellites when under competition with parentals. Specifically, the probability of observing the proportion of offspring that were genetically compatible with each parental male (i.e. shared at least one allele at each locus) given the observed cuckold intrusion frequency is calculated for each combination of possible sneaker and satellite paternities (0–100%). The resultant distribution is then normalized such that the area under the distribution is unity. From the normalized distribution, the expected paternity of sneakers and satellites under competition with parentals is calculated as a weighted average based on the following equations:

$$\overline{\text{Pat}}_{\text{sn}} = \int_0^1 \int_0^1 \text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}}) \times \text{Pat}_{\text{sn}} \, d\text{Pat}_{\text{sa}} \, d\text{Pat}_{\text{sn}}, \quad (1)$$

and

$$\overline{\text{Pat}}_{\text{sa}} = \int_0^1 \int_0^1 \text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}}) \times \text{Pat}_{\text{sa}} \, d\text{Pat}_{\text{sn}} \, d\text{Pat}_{\text{sa}}. \quad (2)$$

The expected values (which are denoted by the bar) provide unbiased estimates independent of the potential skew or asymmetry in the probability distribution $\text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}})$ and are therefore superior to simply solving for the single most likely paternities (Neff *et al.* 2001a). Our model also enables calculation of the confidence intervals for the sneaker and satellite paternity estimates. The 95% confidence intervals are calculated as the paternity values that divide the lower and upper 2.5% of the area under the normalized probability distribution ($\text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}})$).

3. METHODS

(a) Behavioural data

Behavioural data were collected by divers at natural bluegill colonies in Lake Opinicon and by observers at a nearshore experimental pool facility near Chaffey's Lock, Ontario, Canada, during the breeding season of 1999 (late May to mid-July). The pools were stocked at the beginning of the breeding season with mature cuckolders, parentals and females from Lake Opinicon at densities similar to those in the lake (Gross 1982, 1991).

Three to four divers hovered over each nest and collected data on female dips and the types of males that were spawning during the dip. Satellites are distinguishable from females since satellites are smaller in size and do not fully emulate the dipping motion associated with female egg releases (Gross 1982). Nests with females were randomly chosen for observation and behavioural data were collected continuously until spawning ceased. Due to the constraint on the ability of the divers to observe nests continuously, not every female dip within a nest was recorded. However, as many behavioural observations as possible were made at each nest and a large random sample of data was obtained from which the relative frequencies of sneakers, satellites and parentals at a dip could be determined. Just prior to fry dispersal, some seven days later, the parental male and the fry in his nest were collected by scuba divers. The fry and adult tissue samples were preserved in 95% ethanol for genetic analyses. The same procedure was followed in the pool facility,

except that all mating behaviours were recorded by either direct observation or by video cameras on elevated platforms above the water. All remaining fry not used in the genetic analyses were dried and weighed to provide estimates of clutch size.

(b) Genetic data

Genetic analyses were carried out on 28 nests, including five from the pools. These nests were selected from a total of 44 using two criteria: (i) complete behavioural records from a minimum of 50 dips, and (ii) the offspring were raised through to the fry stage when they could be collected. Each parental male was genotyped at 11 microsatellite loci using the methods of Neff *et al.* (2000c). Following Neff *et al.* (2000a), the parental's allele frequencies within the breeding population were used for determining the optimal number of loci to genotype. In total, we genotyped 46 fry randomly chosen from each nest, thereby generating 5200 genotypes for 1316 individuals. Our paternity calculations assumed similar survivorship from egg to fry for cuckoldler and parental offspring.

Each parental's paternity was calculated using the two-sex paternity model, which allows for the contribution of multiple fathers and mothers in a single brood (Neff *et al.* 2000a). Statistical confidence (95% confidence interval) in each paternity estimate was calculated with the Monte Carlo simulation presented in Neff (2001), which approximates the two-sex paternity confidence model (Neff *et al.* 2000b). The Monte Carlo simulation was used for expediting the calculations of the confidence model, which would have been too computationally intense to solve using today's computers given the large number of loci used. We calculated the confidence in each parental male's paternity in order to show that our estimates had high precision.

(c) Simulation analysis

A key feature of our analysis was the use of linear regression for calculating the relative number of eggs (E) released during competitive and non-competitive dips. The number of eggs released per dip (i.e. $clutch_n/D_n$) was regressed onto the total cuckoldler intrusion frequency (i.e. I_c). We assumed that the number of dips observed (D_n) was a direct measure of the actual number of dips that occurred within a nest. Given the thoroughness of our behavioural survey and the fact that clutch size was highly correlated with the observed number of dips ($r = 0.88$, $n = 28$ and $p < 0.001$), this assumption seemed reasonable.

If females release the same number of eggs during competitive and non-competitive dips, there will be no relationship between the eggs released per dip and cuckoldler paternity. Alternatively, if the slope from the regression is greater than zero, then females release more eggs during competitive intrusions. From the regression equation ($F(I_c)$), the relative number of eggs released could be calculated as $E = F(1)/F(0)$. $F(I_c)$ relates the intrusion frequency of cuckoldlers (I_c) to the number of eggs released per dip. The quotient E therefore represents the number of eggs released when a cuckoldler is present ($F(I_c = 1)$) relative to the number released when only the parental is present ($F(I_c = 0)$).

The egg difference E was input into the model along with the parameters based on the behavioural and genetic data (see table 1 and Appendix A). Since the model requires the number of females and cuckoldlers that may have spawned in each nest, we considered two estimates (see also Neff *et al.* 2000b): (i) the population breeding frequencies of four females and six cuckoldlers, and (ii) a minimum estimate of one female and one

cuckoldler. The expected paternities of sneakers and satellites under competition with parentals were calculated from the model. The 95% confidence intervals for each estimate were also calculated. The statistical difference between the success of sneakers versus satellites was assessed by calculating the exact probability that the two values were equivalent using the following equation:

$$p = \int_{Pat=0}^1 \left(\int_{Pat_{sn}=0}^1 \Pr(Pat, Pat_{sa}) dPat_{sa} \int_{Pat_{sn}=0}^1 \Pr(Pat_{sn}, Pat) dPat_{sn} \right) dPat. \quad (3)$$

4. RESULTS

(a) Behavioural data

A total of 8625 female dips were observed in 20 h and 18 min of spawning observations at 44 nests from seven colonies in both the lake and pools. In total, 10.3% of female dips included sperm competition where both cuckoldlers and parentals spawned during egg release. Sneakers participated in 8.4% and satellites in 1.9% of dips. The lake and pool environments did not differ significantly in intrusion frequencies (lake, average of 9.5% with 6.3% sneakers and 3.2% satellites, and pool, average of 6.2% with 6.0% sneakers and 0.2% satellites) (Kruskal–Wallis test, sneaker $\chi^2 = 1.28$, d.f. = 1 and $p = 0.26$, and satellite $\chi^2 = 2.08$, d.f. = 1 and $p = 0.15$).

(b) Genetic data

The mean (\pm s.d.) paternities of parentals and cuckoldlers per brood were 0.81 ± 0.15 and 0.19 ± 0.15 , respectively. Each paternity estimate had high precision as a result of a narrow confidence interval (see table 2). The paternities were not significantly different in the lake and pool (lake, parental 0.83 ± 0.16 and cuckoldler 0.17 ± 0.16 , and pools, parental 0.73 ± 0.10 and cuckoldler 0.27 ± 0.10) (Kruskal–Wallis test, $\chi^2 = 1.989$, d.f. = 1 and $p = 0.15$). The genetic data, including compatible offspring, parental allele frequencies and paternities and confidence values, are presented in table 2.

(c) Simulation analysis

There was a positive relationship between the number of eggs released per dip and the total paternity of cuckoldlers ($r^2 = 0.15$, $n = 28$ and $p = 0.048$). The resultant regression equation was $F(I_c) = 0.58 \times I_c + 0.25$. The constant was also highly significant ($p = 0.001$). The relative number of eggs released during competitive versus non-competitive dips (E) was therefore 3.3 ($0.83/0.25$) ($F(I_c = 1) = 0.83$ when cuckoldlers were present and $F(I_c = 0) = 0.25$ when cuckoldlers were absent), suggesting there are approximately three times as many eggs when a cuckoldler is present as when the parental male is alone. This number may differ for satellites versus sneakers. However, with the current data we were unable to distinguish this difference and it is not needed for testing the general prediction of the sneak–guard model.

We calculated that, on average, cuckoldlers fertilize 78% of the eggs in a dip during sperm competition and parentals fertilize the remaining 22%. More specifically, sneakers fertilize 89% of the eggs in a dip (95% CI 0.76–0.99)

Table 2. Summary of the behavioural and genetic data used in the model (Appendix A) for analysing the success of sneakers and satellites under sperm competition with parentals

(Each nest-tending parental male's paternity and 95% confidence interval is shown. The behavioural data are the total number of dips observed in a parental nest and the frequency of sneaker and satellite cuckold intrusions expressed as a proportion of the observed dips. The genetic data are comprised of the number of offspring out of the 46 sampled that were genetically compatible (shared at least one allele at each locus) with the nest-tending parental (k_n) and the parental's combined allele frequencies at each microsatellite locus used. Each nest was analysed with two to nine loci. The combined allele frequencies were calculated according to Neff *et al.* (2000a). The paternity and confidence analysis was conducted following Neff *et al.* (2000a,b) and Neff (2001).)

nest	behavioural data			k_n	genetic data	parental male	
	total dips	sneaker intrusion	satellite intrusion		combined allele frequency at each locus	paternity	95% CI
1	119	0.04	0.00	38	0.23, 0.41, 0.56 and 0.40	0.79	0.63–0.89
2	292	0.13	0.01	44	0.39, 0.58 and 0.23	0.94	0.80–0.97
3	188	0.04	0.01	45	0.60, 0.65, 0.59 and 0.42	0.96	0.78–0.99
4	122	0.13	0.02	29	0.41, 0.14 and 0.33	0.60	0.41–0.73
5	79	0.07	0.00	38	0.15, 0.61, 0.39 and 0.45	0.81	0.65–0.89
6	177	0.01	0.00	45	0.48, 0.12 and 0.43	0.98	0.87–0.99
7	443	0.01	0.16	38	0.17, 0.39 and 0.44	0.80	0.63–0.89
8	364	0.01	0.01	42	0.24, 0.39, 0.41 and 0.43	0.90	0.76–0.95
9	114	0.04	0.05	41	0.44, 0.24, 0.06 and 0.41	0.89	0.76–0.94
10	83	0.05	0.00	42	0.39, 0.17 and 0.41	0.90	0.76–0.95
11	140	0.06	0.00	46	0.06, 0.54 and 0.55	1.00	0.92–1.00
12	305	0.01	0.00	46	0.70, 0.65, 0.21 and 0.36	1.00	0.91–1.00
13	376	0.03	0.00	45	0.22, 0.55, 0.52, 0.30 and 0.37	0.98	0.88–0.99
14	111	0.04	0.00	42	0.09, 0.21 and 0.10	0.91	0.79–0.96
15	172	0.09	0.15	26	0.23 and 0.30	0.45	0.17–0.63
16	521	0.09	0.04	38	0.23, 0.33 and 0.22	0.81	0.66–0.89
17	623	0.00	0.11	36	0.24, 0.06 and 0.25	0.77	0.63–0.87
18	245	0.02	0.02	46	0.60, 0.14 and 0.31	1.00	0.92–1.00
19	1244	0.14	0.00	27	0.52, 0.55, 0.47, 0.33 and 0.25	0.54	0.35–0.68
20	188	0.01	0.01	43	0.39, 0.14 and 0.33	0.93	0.80–0.97
21	87	0.10	0.00	31	0.15, 0.21 and 0.33	0.65	0.49–0.77
22	228	0.10	0.06	33	0.69, 0.66, 0.44, 0.77, 0.49, 0.21 and 0.55	0.68	0.50–0.80
23	185	0.18	0.01	38	0.47, 0.23 and 0.30	0.80	0.62–0.89
24	80	0.06	0.00	37	0.45, 0.56, 0.52 and 0.55	0.70	0.41–0.84
25	327	0.08	0.00	34	0.41, 0.44, 0.45, 0.62, 0.76 and 0.77	0.66	0.43–0.79
26	65	0.01	0.00	41	0.46, 0.58, 0.62, 0.66 and 0.66	0.83	0.57–0.92
27	145	0.10	0.00	31	0.16, 0.47, 0.51 and 0.55	0.63	0.43–0.76
28	617	0.03	0.00	41	0.52, 0.55, 0.56, 0.58, 0.65, 0.66, 0.66, 0.66 and 0.76	0.86	0.69–0.93

(figure 2a), which is significantly more than the 67% (95% CI 0.46–0.88) (figure 2b) obtained by satellites (exact test $p < 0.01$). Assuming that only one female and one cuckold spawned in each nest had little effect on the results. Under this scenario, sneakers were estimated to fertilize 92% of the eggs in a dip and satellites the same 67%. Thus, the model is relatively robust to this parameter.

Therefore, it is evident that cuckolders win over parentals in sperm competition.

5. DISCUSSION

We have now confirmed the second prediction of Parker's (1990, 1998) sneak-guard model. Cuckolders, both sneakers and satellites, are superior to parentals in sperm competition. A companion paper (Neff *et al.* 2002) provides detailed biological information on the sperm and ejaculate investment strategies of males and shows that cuckolders invest more than parentals in total spermatogenesis and ejaculate sperm density (sperm have

similar morphology), which supports the first prediction of the sneak-guard model (see also Gross 1982). Thus, both predictions of Parker's (1990, 1998) theoretical model are empirically supported.

The only other study that has tested the outcome of sneak-guard sperm competition is the recent investigation by Tomkins & Simmons (2000) in an internally fertilizing beetle (*Onthophagus binodis*) with dimorphic males (guards are horned major males and sneaks are hornless minor males). They confirmed that sneaks invest more in sperm competition than guards, but found no difference in fertilization success. They acknowledged the artificial nature of the experiment (only two matings per female, irradiation for measuring paternity and a laboratory setting) and encouraged further studies using molecular markers and natural conditions. We have performed such a study here.

In addition to finding that cuckolders are more successful sperm competitors than parentals, as predicted by the sneak-guard model, we found that sneakers are

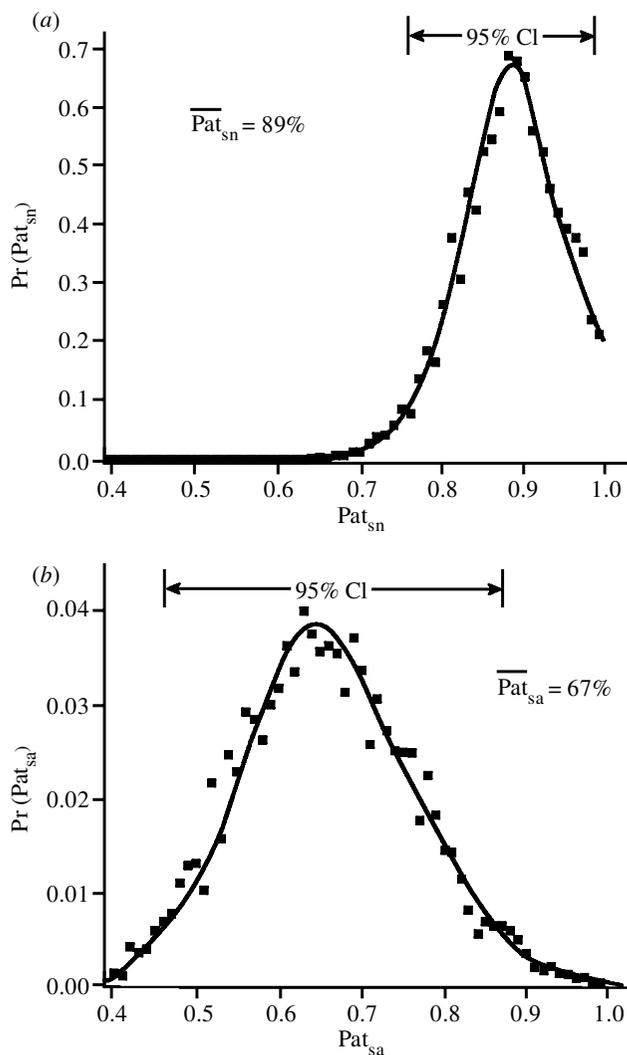


Figure 2. The normalized probability distributions associated with the possible paternity values for (a) sneakers and (b) satellites under sperm competition with parentals as generated by the model. The distributions shown represent slices taken for the original distributions at $\text{Pr}(\text{Pat}_{\text{sn}}, \text{Pat}_{\text{sa}} = 0.67)$ for sneakers and $\text{Pr}(\text{Pat}_{\text{sn}} = 0.89, \text{Pat}_{\text{sa}})$ for satellites. The lines represent higher-order polynomials fit to the data. The expected value and 95% confidence intervals are indicated. Only paternity values from 0.4 to 1.0 are displayed. All other values were approximately zero.

more successful than satellites. We already know that, when milt is given equal proximity to eggs in the laboratory, the fertilization success of bluegill cuckold and parental males is directly proportional to the relative number of sperm in ejaculates of equal volume (Neff *et al.* 2001). Therefore, the differences in success between the sneaker, satellite and parental tactics in the field must be due to differences in (i) position, (ii) the number of sperm released, or (iii) both. In the field, satellites have a favoured position since they ejaculate between the female and parental and the female dips towards the urogenital pore of the satellite. In contrast, sneakers have a disfavoured position since they ejaculate below the parental and female and the female dips towards the urogenital pore of the parental. Since satellites have the best position but are inferior sperm competitors relative to sneakers, the difference in competitive success is probably due to

sneakers releasing more sperm per ejaculate. Another aspect that should influence sperm release is the total number of matings available to each tactic. Parentals have access to more matings and fertilize many more eggs than both sneakers and satellites (Gross & Charnov 1980; Phillip & Gross 1994; Neff 2001; this study). Among sneakers and satellites, satellites take part in a larger number of matings and have higher overall reproductive success than sneakers (Gross 1982). Thus, the number of matings probably also influences the number of sperm released per mating. The larger number of matings satellites can obtain may result in their budgeting a smaller amount of sperm per mating, thereby contributing to their inferior sperm competition success relative to sneakers (see also Alonzo & Warner 2000). Thus, mating dynamics, including position, mate number and the information and risk incorporated into the sneak-guard model, are probably all responsible for the evolution of tactic-specific successes in sperm competition.

Our finding of more eggs in dips with cuckold intrusions could either indicate female preference or cuckold selection for more fecund dips. We do not yet know which process is occurring or whether both are occurring. We also do not yet know whether sneakers and satellites both experience exactly the same number of eggs in each dip. However, this does provide the start of a new avenue of research in this mating system.

Finally, our model for determining success under sperm competition should have broad application to the many mating systems where females release batches of eggs over which males compete (e.g. insects, amphibians and fish).

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APPENDIX A

The input and output parameters of our model are defined in table 1. The simulation involves three randomization routines that are based on a defined parameter set and a variable set. The parameter set includes the behavioural data and the genetic data and the variable set includes quantities that the model calculates (e.g. the relative competitive success of each male tactic). The simulation is repeated 99 999 times for each nest and for each possible paternity from which the expected paternity (see equations (1) and (2)) of each male tactic is calculated (figure A1).

The first routine in the simulation (step 3) randomizes the intrusion frequencies based on the observed data (D_n , I_n^{sn} and I_n^{sa}). This routine accounts for the variance between nests in the number of matings observed and, hence, the precision of the intrusion frequency estimates. The second routine (step 4) randomizes the genotypes of the cuckolders and females that have contributed genetically to the offspring within the nest (Neff *et al.* 2000*a,b*). This routine accounts for the incomplete sampling of the putative parents in the genetic analysis. The third routine (step 5) is the most complex and is described in detail in figure A1*b*. Briefly, it randomizes the genotypes of the offspring that are genetically

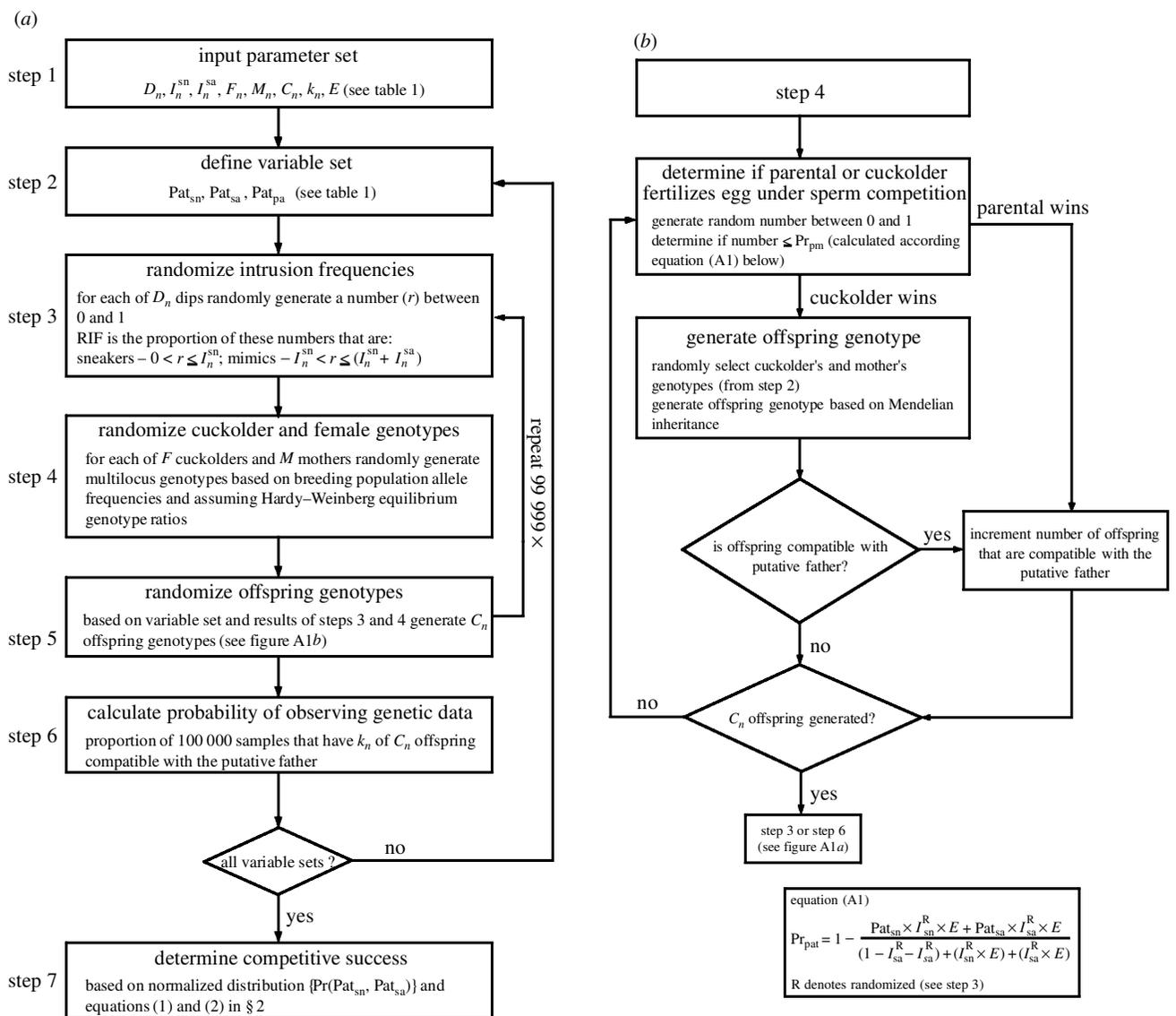


Figure A1. Schematics of the model used to solve the average competitive success of different male tactics under sperm competition. (a) The simulation involves seven steps. In step 1, the behavioural and genetic data are input. Step 2 indexes the paternities of the parental, satellite and sneaker males. Steps 3–5 involve three randomization routines and are repeated 99 999 times for each of the N nests and each possible parameter set. Step 6 calculates the probability of observing the input data given the parameter set and stores the values in the probability distribution ($Pr(Pat_{sn}, Pat_{sa})$). The expected values for the competitive success of sneakers and satellites are calculated using equations (1) and (2) presented in § 2. (b) Detailed schematic of step 5. Equation (A1) is used to determine whether the parental or cuckold fertilizes the egg for each of the C_n offspring sampled from the nest. Equation (A1) incorporates the relative competitiveness of sneakers and satellites (as assigned in the variable set by Pat_{sn} and Pat_{sa}), the intrusion frequencies of sneakers and satellites (I_{sn} and I_{sa}) and the relative number of eggs released by the female (E). If the cuckold is successful at fertilizing the egg then a genotype is generated for the offspring based on those generated in step 4. The offspring genotype is compared to the parental male and, if genetically compatible (offspring shares at least one allele with the parental male at each locus), then it is assigned to the parental male. This mitigates the error in offspring assignments as a result of the possible similar genetic profiles of putative fathers. All offspring that the parental fertilizes are genetically compatible. The routine is repeated for all C_n offspring and the proportion of simulated offspring that are compatible with the parental male is calculated. This proportion is compared to the observed proportion (k_n/C_n) in step 6, based on the genetic data.

sampled from the nest based on the results of the previous two routines (steps 3 and 4) and the variable set (step 2) and determines the proportion of these offspring that are genetically compatible with the parental male. This proportion is then compared to the observed proportion obtained from the microsatellite DNA fingerprinting. The probability of the variable set is determined as the number of matches out of the

100 000 simulated sets. For example, if very few of the 100 000 offspring samples at any of the nests match the observed proportion of compatible offspring, then the given variable set is unlikely. Conversely, if most of the 100 000 samples at each of the nests match the observed proportions, then the given variable set is likely. These values are stored in the probability distribution $Pr(Pat_{sn}, Pat_{sa})$, which is later normalized.

The expected paternities can be calculated from the normalized distribution using equations (1) and (2).

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