

Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.)

CARLO R. LARGIADÈR*†, VALÉRIE FRIES†‡ & THEO C. M. BAKKER†§

†Division of Population Genetics, Institute of Zoology, University of Berne, Baltzerstrasse 6, CH-3012 Berne, Switzerland, ‡Abteilung Verhaltensökologie, Zoologisches Institut, University of Berne, Wohlensstrasse 50a, CH-3032 Hinterkappelen, Switzerland and §Institut für Evolutionsbiologie und Ökologie, University of Bonn, An der Immenburg 1, D-53121 Bonn, Germany

Among the fish species that show exclusive male parental care, the three-spined stickleback represents one of the most intensively studied species with regard to reproductive behaviour. In this species, the most common ‘parasitic’ male tactics in relation to male reproductive behaviour are sneaking and egg thievery, which are often collectively referred to as nest-raiding. However, little is known about the genetic consequences of sneaking and egg thievery in natural populations. Here we assessed the frequency of sneaking and egg-stealing in a natural population, male traits that are associated with the victims of sneaking, and the impact of sneaking and egg-stealing on the reproductive success of nesting males as deduced from the number of offspring in their nests. Fourteen nest-guarding males and a random sample of about 100 eggs/fry of each nest from a natural freshwater population of three-spined sticklebacks were analysed at three microsatellite loci. The analysis revealed a high frequency of genetically successful nest raiding (sneaking or egg thievery), i.e. more than half (57%) of the 14 nests contained offspring (1–94%) which were unrelated to the guardian male. Three of the 14 nests (21%) contained progeny of sneaking males and four of the nests (28%) contained offspring which were unrelated to the guardian male and which probably originated from egg-stealing events. Victims of sneaking were significantly smaller than other guardian males. Moreover, reproductive success correlated positively with male body size.

Keywords: body size, egg thievery, *Gasterosteus aculeatus*, microsatellites, sneaking, three-spined stickleback.

Introduction

Breeding systems are of central interest in evolutionary biology. They influence the strength and consequences of sexual selection (Davies, 1991; Andersson, 1994; Reynolds, 1996), as well as the effective population size which affects the amount of genetic variability that is transmitted to the next generation (Chesser, 1991a,b; Sugg & Chesser, 1994). An impressive range of reproductive tactics is shown by fish, and the usually external fertilization of fish eggs may explain at least partly why in particular sperm competition among different types of males showing alternative mating

tactics is extremely widespread in this group (Taborsky, 1994).

Among the fish species that show exclusive male parental care, the three-spined stickleback represents one of the most intensively studied species with regard to reproductive behaviour (e.g. van den Assem, 1967; Wootton, 1976; Bell & Foster, 1994). The males build a nest, attract females to it, and after a courtship ritual, the female may choose to lay eggs inside the nest which are fertilized by the male that passes through quickly after the female. Several ‘parasitic’ male tactics in relation to male reproductive behaviour have been observed both in the laboratory (e.g. Li & Owings, 1978a,b) and in the field (e.g. Mori, 1995). The most common tactics are sneaking and egg thievery, which are often collectively referred to as nest-raiding.

*Correspondence. E-mail: largiader@zoo.unibe.ch

Sneaking males approach the rival's nest during courtship of the nesting male and after the female has spawned dart through the nest and release sperm. This may occur before or after the nest owner has passed through the nest to fertilize the eggs (e.g. van den Assem, 1967). Attempts to steal a fertilization are often followed by attempts to steal eggs (e.g. van den Assem, 1967). Several studies indicated that by stealing eggs and bringing them into their own nest, males with empty nests increase the attractiveness of their nests to ripe females (Ridley & Rechten, 1981; Belles-Isles *et al.*, 1990; Goldschmidt & Bakker, 1990; Goldschmidt *et al.*, 1993; but see Jamieson & Colgan, 1989). A female preference to spawn in nests that already contain eggs has also been observed in several other fish species, e.g. in the European bullhead *Cottus gobio* (Marconato & Bisazza, 1986) or in the sphynx blenny *Aidablennius sphynx* (Kraak & Groothuis, 1994).

Little is known, however, about the frequency and success of alternative mating tactics in nature and which males are more liable to sneaking and egg thievery. This information is essential for assessing the evolutionary consequences of female mate choice. For example, if sneaking is frequent and successful, the females may lose some of the benefits of choice. Furthermore, the reproductive success of males is usually deduced from the number of eggs in their nests (e.g. Bakker & Mundwiler, 1994; Kraak *et al.*, 1999a). If sneaking is frequent then this measure of reproductive success may be seriously biased.

For three-spined sticklebacks, the only study which applied genetic markers (multilocus DNA fingerprinting) for paternity analysis of offspring, demonstrated the occurrence of unrelated offspring in a male's nest (Rico *et al.*, 1992). However, only 10 fry had been analysed per nest, which, given the large number of eggs that are usually observed (up to several thousands; see Kraak *et al.*, 1999a), did not allow quantitative estimates of the number of unrelated offspring in these nests.

The recent development of highly polymorphic codominant markers such as microsatellites has opened new perspectives for the study of breeding systems. This is illustrated by several studies in various species (Primmer *et al.*, 1995; Clapham & Palsboll, 1997) including fish (Kellogg *et al.*, 1995; Parker & Kornfield, 1996). In a recent study, quantitative information of the genetic consequences of sneaking and egg thievery were obtained for a natural population of another stickleback species, the 15-spined stickleback *Spinachia spinachia*, using microsatellite markers (Jones *et al.*, 1998).

In this study, we aimed at (1) assessing the frequency of sneaking and egg-stealing in a natural population of

the three-spined stickleback using microsatellite makers; (2) identifying male traits that are associated with the victims of sneaking; and (3) estimating the impact of sneaking and egg-stealing on the reproductive success of nesting males as deduced from the number of offspring in their nests.

Materials and methods

Biological material

Fourteen nest-guarding males from a permanent freshwater population of three-spined sticklebacks were caught during May and the beginning of June 1997 at their nests with a dip net. The sampling site (Roche near Montreux, Switzerland, 46°26' N, 6°55' E) is part of the drainage system of the Rhône near Lake Geneva and has been described in detail by Kraak *et al.* (1999a).

The eggs/fry of each nest were counted and a random sample of about 100 eggs/fry of each nest was taken for genetic analyses. This high number provides a reasonable probability (>96%) that the sample includes at least one egg/fry fathered by a sneaking male, which successfully fertilized 5% of the eggs in the nest. Prior to DNA extraction, the developmental stages according to Swarup (1958) of these and the other eggs/fry in the nest were determined.

Immediately after a guardian male had been captured, his anterior half of the ventral side and a randomly chosen lateral side were photographed in a standardized way as described in Bakker & Mundwiler (1994). Based on the resulting slides, the breeding colours of each fish were quantified as a red index for the throat and blue and green indices for the eyes using the methods described in Kraak *et al.* (1999b; for further details see Frischknecht, 1993; Bakker & Mundwiler, 1994; Kraak *et al.*, 1999a). The standard length and the body weight were measured and the condition factor was calculated as $100 \times \text{body weight (g)} \times \text{body length}^{-3}$ (in cm) (Bolger & Connolly, 1989). Finally, a muscle tissue sample of each fish was taken and preserved in absolute ethanol for genetic analysis.

DNA extraction and microsatellite analysis

DNA extractions from eggs and from the muscle tissue of each guarding male were performed as described in Estoup *et al.* (1996). Three microsatellite loci (*Gac1116PBBE*, *Gac7033PBBE*, and *Gac7188PBBE*) were analysed on an automated sequencer (Li-Cor, model 4200). The primer sequences used for amplification of the three microsatellite loci, the PCR conditions and microsatellite fragment detection procedures are given in Largiadèr *et al.* (1999).

Genetic data analysis

In order to obtain a general description for the exclusion power of the microsatellite loci for detecting unrelated embryos in the nest of a guardian male, we calculated several descriptive measures based on allele frequencies of a reference sample ($N=82$) from the Roche population. It is the same sample as in Largiadèr *et al.* (1999), supplemented with some individuals that were previously not analysed. GENEPOP 3.1d (Raymond & Rousset, 1995) was used to test for deviation from Hardy–Weinberg equilibrium and from genotypic linkage equilibrium (Fisher's exact tests) in the reference sample. Mean observed and unbiased expected heterozygosity (Nei, 1978) were calculated with BIOSYS 1.7 (Swofford & Selander, 1989). Average probability of exclusion was calculated for each locus (E_i) according to Chakravarti & Li (1983) and global probability across all loci (E_g) according to Chakraborty *et al.* (1974). The latter gives the average fraction of males that can be excluded from paternity with L loci for a given mother/offspring pair if they are not the fathers.

A three-locus exclusion probability (E_{Ind}) was calculated for each guardian male as follows:

$$E_{\text{Ind}} = 1 - \prod_{i=1}^L (p_1 + p_2) \cdot [2 - (p_1 + p_2)] \quad (\text{Jones } et al., 1998),$$

where p_1 and p_2 are the frequencies in the embryo population of the male's two alleles and L the number of loci, respectively. This measure considers that neither parent is known with certainty and assumes Hardy–Weinberg equilibrium. It can be interpreted as the probability by which a randomly sampled unrelated embryo can be excluded as possible offspring of the guarding male based on the three microsatellite loci.

Eggs not fertilized by the guardian male were diagnosed by simple exclusion procedure. A nest owner was excluded from being the potential father of an embryo collected in his nest if he did not have any allele in common with the embryo at one of the three loci. We used the term *rival fertilization rate* ($= R_{\text{FR}}$) for the

proportion of excluded embryos of a particular nest throughout this paper.

Statistical analysis of male characteristics

The intensity of the breeding colours and the condition of guardian males are expected to decrease with increasing duration of the nesting cycle. Because guarding males had been caught at different stages of their nesting cycle, these variables were corrected for this confounding effect by using the residuals of the linear regressions against the oldest developmental stage of the embryos/fry for further analysis. Standard body length and R_{FR} were, respectively, $\log(x)$ and $\log(x + 1)$ transformed in order to meet the normality assumption of parametric statistical tests. The reported P -values are two-tailed.

Results

Microsatellite amplification success and variation in the Roche population

Amplification success was high; 97.3% of all 1393 analysed eggs/fry yielded an identifiable genotype at all three loci. The average amplification success per nest was 98.9% and the overall amplification success was also 98.9%.

In the reference sample of the Roche population, no significant deviation from Hardy–Weinberg equilibrium and no significant genotypic linkage disequilibrium was found for all loci and pairs of loci, respectively. Observed heterozygosities ranged from 0.5 to 0.8, the number of observed alleles per locus from 4 to 14, the E_i -estimates from 0.33 to 0.65, and the combined exclusion probability (E_g) for all three loci was 0.85 (Table 1).

Sneaking and egg thievery

Eight out of the 14 analysed nests contained eggs/fry that had not been fertilized by the nest owner and corresponding R_{FR} -values ranged from 0.01 to 0.94 (Table 2). Several factors may potentially bias these two estimates. They can be classified into two error sources

Table 1 Genetic variability at three microsatellite loci in a reference sample of three-spined sticklebacks from the Roche population ($N=82$): number of observed alleles, observed and unbiased expected heterozygosities (Nei, 1978), and average exclusion probability (E_i) (Chakravarti & Li, 1983)

Locus	No. of alleles	Observed heterozygosity	Expected heterozygosity	E_i
<i>Gac1116PBBE</i>	14	0.82	0.73	0.65
<i>Gac7033PBBE</i>	4	0.62	0.63	0.35
<i>Gac7188PBBE</i>	9	0.52	0.43	0.33

Table 2 Genotypes for guarding males from the Roche population of three-spined sticklebacks for the loci *Gac1116*, *Gac7033* and *Gac7188*, number of eggs/fry observed in the nests, the number of embryos analysed at these loci, the rival fertilization rate ($= R_{FR}$), the status of eggs (sneaked/stolen) which were not fertilized by the guardian male, the individual exclusion probabilities (E_{Ind}), and the probability of identity based on the three loci

Male	Genotype for locus			No. of eggs/fry	No. embryos assayed	R_{FR}	Sneaked or stolen eggs	E_{Ind}	Prob. of identity
	<i>Gac1116</i>	<i>Gac7033</i>	<i>Gac7188</i>						
1	154/174	215/215	199/199	1063	101	0	No	0.40	6.2×10^{-3}
2	154/174	209/223	199/199	2541	98	0	No	0.46	1.1×10^{-3}
3	124/166	209/209	199/199	1157	90	0	No	0.74	1.3×10^{-3}
4	166/176	215/217	199/199	165	100	0	No	0.77	9.7×10^{-4}
5	154/176	209/215	185/199	2072	105	0	No	0.49	2.4×10^{-3}
6	126/126	209/215	173/199	142	100	0	No	0.78	5.6×10^{-5}
7	158/174	215/217	185/199	1387	100	0.03	Stolen	0.62	6.6×10^{-5}
8	130/174	215/217	199/199	1944	100	0.01	Stolen	0.62	1.3×10^{-3}
9	154/174	209/217	143/185	2833	100	0.05	Stolen	0.83	3.6×10^{-5}
10	166/166	215/217	199/199	1284	100	0.02	Stolen	0.77	4.6×10^{-4}
11	154/166	215/215	199/199	115	100	0.04	Stolen	0.59	1.9×10^{-3}
12	124/154	209/209	155/173	1023	99	0.58	Sneaked	0.88	4.1×10^{-5}
13	130/174	209/217	185/203	849	100	0.94	Sneaked	0.87	6.8×10^{-6}
14	154/174	209/215	143/159	1148	100	0.23	Sneaked	0.91	2.9×10^{-5}

that lead to an underestimation (A) or to an overestimation (B) of the true values.

(A) The first error source depends primarily on the exclusion power of the applied genetic markers and the population frequencies of the nesting males' genotypes (A1) but also on the sampling strategy (A2).

(A1) the higher the population frequency of a nesting male's genotype, the higher becomes the probability that unrelated offspring from a mating with a parent of the same genotype as the nest owner can occur and remain undetected. Such cases may occur if (i) a successful sneaker has an identical genotype, or (ii) if eggs deposited by a female with an identical genotype had been fertilized by a sneaker, or if (iii) the nesting male had stolen eggs from another nest which originated from a mating with a parent of an identical genotype. The probabilities of identity (Table 2), which are simply the expected genotype frequencies in the population under Hardy–Weinberg assumptions, were all below 1%, suggesting that such cases should be rare.

The individual exclusion probabilities (E_{Ind} ; Table 2) for the guarding males ranged from 0.40 to 0.91 with an average value of 0.70. The average value represents the average expected proportion (70%) of randomly sampled embryos/fry that will be excluded as being offspring of an unrelated guardian male. It is also noteworthy, that the lowest values for E_{Ind} (0.40–0.49) belonged to males (1, 2 and 5) with zero estimates of R_{FR} . Thus, we expect that the R_{FR} -values and the number of nests containing eggs fertilized by rivals were probably underestimated.

(A2) In the Roche population, most nesting males had an egg-collection phase of two or three days, and in rare cases, this phase extended up to 10 days (Kraak *et al.*, 1999b). If the frequency distribution of the developmental stages in the sampled nests (Fig. 1) are compared with the duration of the developmental stages under field conditions (S.B.M. Kraak & B. Mundwiler unpublished data) and if an egg-collection phase of three days is assumed, it can be concluded that several males were still collecting eggs (males 2, 3, 6, and 9), and in the case of other males, probably part of the offspring may already have left the nest (nests 4 and 11). Thus, cases of successful sneaking that would have occurred at the end of the egg-collection phase or cases of egg thievery, which had occurred at the beginning of the egg-collection phase, could not be detected in these nests. However, since eggs fertilized by rivals had been detected in two of these cases and since with the exception of nest 6 all males were probably very close to the completion of their egg-collection phase, we conclude that this error source was probably insignificant.

(B) Non-amplifying alleles (Pemberton *et al.*, 1995) and *de novo* mutations may lead to false exclusion. In the presence of non-amplifying alleles, nest-guarding males, which appeared to be homozygous for one allele might potentially have been heterozygous for a non-amplifying allele. An offspring that received the non-amplifying allele from its father and a different amplifying allele from the amplifying paternal allele from its mother, would appear homozygous for the maternal allele, and thus be excluded as offspring of the

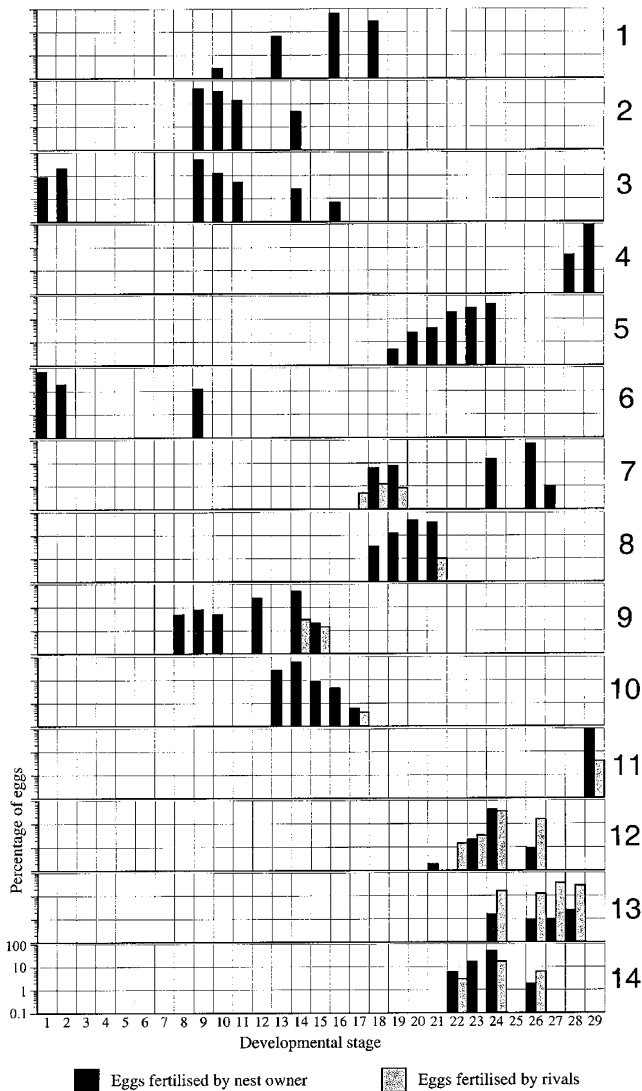


Fig. 1 Frequency distribution of developmental stages (after Swarup, 1958: eggs are fertilized at stage 1 and embryos hatch at stage 25) of embryos and fry in 14 nests of the three-spined stickleback (Roche population). The relative frequencies of offspring fathered by the guarding male and by rival males are shown separately (black and grey columns, respectively). They represent the extrapolated proportions of all embryos and fry counted in a particular nest. Note that the Y-axis has a logarithmic scale to improve the graphic presentation of small values.

true father. *De novo* mutations of the paternal alleles would lead to a similar pattern of incompatibility between the genotypes of offspring and father. Errors due to non-amplifying alleles and *de novo* mutations can be largely excluded if (1) either the father or the offspring have an incompatible heterozygous genotype and if (2) the exclusion could be confirmed at a second locus. These conditions were not met for part of the excluded

offspring. However, cases for which condition (1) was met, were found in all nests but nest 11. Furthermore, in all nests except 7 and 8 exclusions were based on more than one locus. Taking also into account that *de novo* mutations are expected to occur at very low frequencies and that we did not find any indication for the presence of non-amplifying alleles in the reference sample of the Roche population, we conclude that false exclusions had not significantly affected our R_{FR} -estimates.

Based on the occurrence and age distribution of eggs fertilized by males other than the nest owner (Fig. 1), nests could be divided into three categories.

(i) The first category contains nests in which no eggs fertilized by males other than the nest owner had been detected (nests 1–6; Fig. 1, Table 2). Nest 7 may also be classified into this category, in spite the fact that eggs, which had not been fertilized by the guardian male, have been observed. In this case the frequency distribution of the developmental stages of the embryos in the nest strongly suggests that the guarding male had started a second nesting cycle before the first was finished, a phenomenon that, to our knowledge, has not been described in this species so far. For the first completed nesting cycle, no embryo/fry were excluded from being offspring of the guarding male. All eggs that were diagnosed as being fertilized by rivals were found among embryos of stages 18 and 19, whereas the majority of the embryos ($\approx 86\%$) were close to hatching (stage 23) or had already hatched (stages 26 and 27) (Fig. 1). The time gap between stage 18 and 26 is at least 11 days (S.B.M. Kraak & B. Mundwiler unpublished data), which is more than the maximum duration of the egg-collection phase in this population (Kraak *et al.*, 1999b). Furthermore, males generally do not resume collecting additional eggs after an interruption of one or more days in this population (Kraak *et al.*, 1999b). Consequently, for further analysis, we excluded all eggs that belonged to the assumed second egg-collection phase, i.e. all eggs of stages 18 and 19. It is, however, noteworthy that this exclusion did not affect any result of the subsequent analyses.

(ii) The second category consists of four nests (nests 8–11) with low R_{FR} -values ($= 0.05$) (Fig. 1, Table 2). All eggs fertilized by rivals in this category were among the oldest developmental stages found in each nest with the exception of nest 11, which contained only fry of one stage. Such a pattern is expected when the guarding male had stolen eggs in the beginning of his egg-collection phase (e.g. Mori, 1995). Because of its low R_{FR} -value, nest 11 could also represent a case of egg theft.

(iii) Three nests (nests 12–14) that showed R_{FR} -values above 20% represent the third category (Fig. 1, Table 2). Eggs that were not fertilized by the nest-owner were evenly distributed among all developmental stages

of embryos/fry in the nest, which is a second feature that characterizes this category. Probably, these guardian males had repeatedly been victims of sneaking during their entire egg-collection phase.

Guardian male traits related to R_{FR} -values

None of the measured male traits was significantly correlated with the R_{FR} -values (all $r < 0.58$, $P > 0.031$) after applying a sequential Bonferroni correction (Rice, 1989) for multiple tests. In a multiple regression analysis of R_{FR} -estimates on the various male traits, two traits were retained in the model after a backward elimination procedure (model: $N = 14$, $r^2 = 0.49$, $F = 5.31$, $P < 0.03$) and showed significant standardized regression coefficients: -0.67 for body size ($t = 2.95$, $P = 0.013$) and 0.52 for condition ($t = 2.29$, $P = 0.043$). Thus the results of the multivariate analysis suggest that the proportion of eggs fertilized by rivals in a nest increase with decreasing body size and increasing condition of the guardian male. In accordance to this result, category 3 males (that is, males with high R_{FR} -values; see above) were significantly smaller than guarding males with zero or low R_{FR} -values (categories 1 and 2; see above) (t -test, $t = 2.19$, d.f. = 12, $P < 0.05$; Fig. 2).

Reproductive success related to male traits and sneaking/egg thievery

As was pointed out earlier, the nests had been collected at different stages of the nesting cycle of the guardian

male. In order to be able to compare the reproductive success among males with variable stages of their nests, a standardized reproductive success was estimated by partialling out the effect of the stage of the nest on the number of eggs present in the nest (Kraak *et al.*, 1999a). Standardization was done by taking the residuals of egg number to its fitted quadratic function of developmental stage of the oldest egg present in the nest (Fig. 3). The fitted function reflects the initial increase in the number of eggs in the nest with time as the nesting male is collecting eggs during 2–3 days after his first clutch, followed by a decrease through subsequent embryo mortality. A value of total eggs/fry in a nest below the fitted value represents a reproductive success which is lower than the average success of nesting males, and a value above the curve a reproductive success which is higher than the average. It is noteworthy that the Y-intercept of the fitted function, which theoretically predicted equals zero, was not significantly different from zero ($t_{df2} = -1.89$, $P > 0.085$). Thus, we concluded that standardization was not biased by the rather isolated leftmost data point in Fig. 3 representing the nest containing the youngest egg stages.

None of the male traits that we measured were related to reproductive success (all $r < 0.57$, $P > 0.037$) after applying a sequential Bonferroni correction (Rice, 1989) for multiple tests. However, when the standardization of reproductive success was based on only those eggs/fry that had not been excluded from being offspring of the corresponding guardian-male, a positive relationship

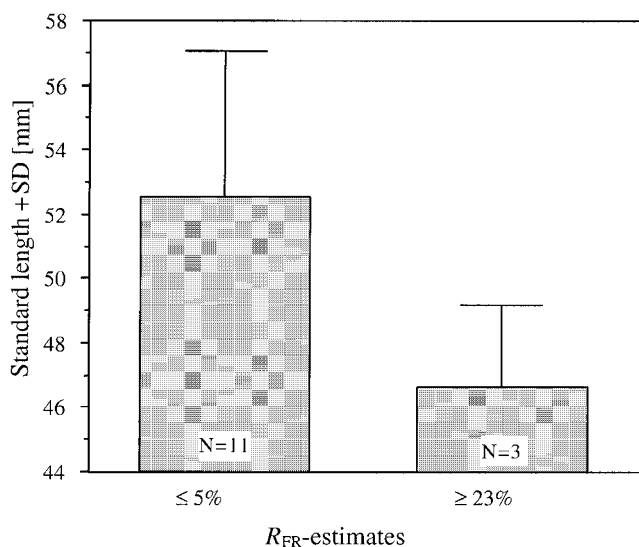


Fig. 2 Body size (mean standard body length in mm + SD) of males of the three-spined stickleback with high ($N = 3$) and low ($N = 11$) R_{FR} -values. R_{FR} stands for rival fertilization rate. See text for further explanation.

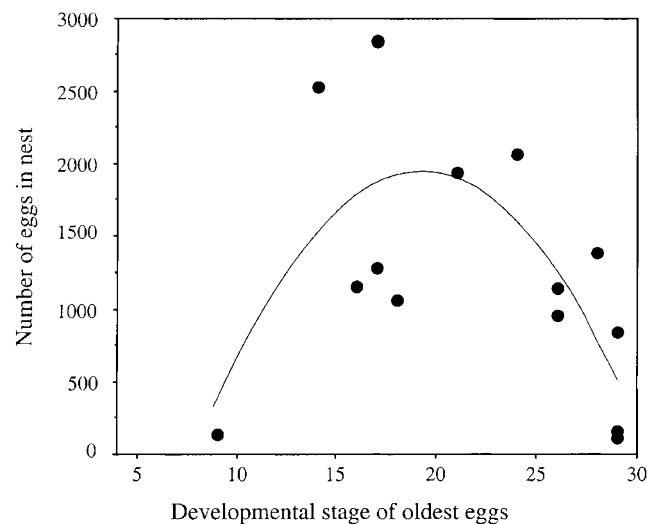


Fig. 3 The relationship between the number of eggs/fry and the developmental stage of the oldest eggs/fry in the nests of 14 males of the Roche population of the three-spined stickleback. The curve is the fitted quadratic function ($y = -75.17x^2 + 584.30x - 3678.2$, $r^2 = 0.51$, $F = 5.80$, $P < 0.02$).

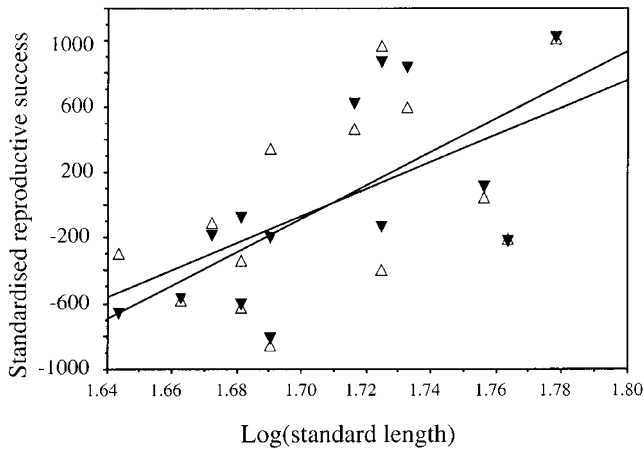


Fig. 4 Relationship between standardized reproductive success and body size (log of standard body length in mm) in 14 males of the Roche population of the three-spined stickleback when all eggs/fry in the nest were taken into account (open triangles; $y = 8264.2x - 14117.5$) or when only eggs/fry fertilized by the guardian male were considered (closed triangles; $y = 10144.1x - 17328.8$). Reproductive success was standardized for the stage of the nest by taking the residuals of quadratic regression (see Fig. 3).

was found between the standardized reproductive success and body size ($r = 0.67$, $N = 14$, $F = 9.80$, $P < 0.01$; Bonferroni corrected P -value = 0.01; Fig. 4; for all other traits all $r < 0.58$, $P > 0.033$). It is noteworthy, that this relationship was, as expected, less pronounced when the analysis was based on all eggs/fry found in the nests ($r = 0.56$, $N = 14$, $F = 5.52$, $P < 0.04$; Fig. 4), but not significantly so (test for homogeneity of slopes, $F_{df1} = 0.155$, $P > 0.697$, ANCOVA, $F_{df1} = 0.002$, $P > 0.963$).

Discussion

Microsatellite analyses revealed a high frequency of genetically successful nest raidings in a natural population of three-spined sticklebacks: 21% of the nests contained progeny of sneaking males and 28% of the nests contained offspring which were unrelated to the guardian male and which probably originated from egg-stealing events. Taking into account a further case of eggs fertilized by rival males (nest 7) that could not unambiguously be attributed to either category, in more than half (57%) of the 14 nests analysed we found evidence for sneaking or egg thievery. This estimate is about twice as high as was found by Rico *et al.* (1992) for 17 nests of a Canadian three-spined stickleback population using multilocus DNA fingerprinting. They analysed 10 fry per nest and found three nests (23%) that contained offspring that were unrelated to the

guardian male. Two of the nests were interpreted as being sneaked and a third as having received both sneaked and stolen eggs. Based on microsatellite markers, lower rates of egg thievery (17%) and sneaking (18%) were also reported for 24 and 28 nests, respectively, of the 15-spined stickleback (Jones *et al.*, 1998).

However, one must be cautious when comparing the sneaking and egg thievery rates among these studies, because there are substantial differences in the sampling strategies and in the *a priori* criteria for distinguishing between sneaking and egg-stealing events among studies. It should be emphasized that without direct information on where the females have laid questionable eggs, these two cases cannot be distinguished unambiguously by means of genetic markers. In the study of Rico *et al.* (1992), a case of egg thievery was assumed, if the genotype of the questionable fry was neither compatible with guardian male nor with the inferred maternal genotypes of compatible fry. Considering that they only analysed 10 fry per nest and that stickleback nests usually contain eggs from several females (e.g. Kraak *et al.*, 1999a), the assumed stolen eggs may also be eggs fertilized by a sneaking male. Jones *et al.* (1998) reported for the 15-spined stickleback that they were able to distinguish distinct egg clusters in the nests, and that egg thieves steal such egg clusters and place them in their own nest. Egg thievery was assumed if all or nearly all embryos of a cluster were excluded from being offspring of the guardian male. Observations indicate that in the three-spined stickleback, immediately after sneaking, the sneaker returns to the raided nest and steals eggs (e.g. van den Assem, 1967). Thus, it seems likely that also eggs that were actually stolen from another nest and that had been fertilized by the guardian male can occur in his nest. If this also is the case in the 15-spined stickleback, the assumed cases of sneaking of both aforementioned studies may also partly represent cases of egg thievery.

In our study, we based our *a priori* criteria for distinguishing between sneaking and egg-stealing events on information about the age of the eggs, i.e. developmental stages of the eggs and the frequency distribution of these stages in the nests, and on field and laboratory observations in the three-spined stickleback. Firstly, almost all egg-stealing is performed by males that have nests but no eggs (van den Assem, 1967; Wootton, 1971; Mori, 1995). Secondly, females show a preference for spawning in nests that already contain eggs (Ridley & Rechten, 1981; Goldschmidt & Bakker, 1990). Thus, if we assume that males steal eggs in order to make their nest more attractive for spawning females we would expect that a male steals eggs at the beginning of his egg-collection phase. We would further predict that a guardian male aims at minimizing the proportion of

unrelated offspring in his nest, and that thus the proportion of unrelated offspring of a successful egg thief, which has successfully prevented other males from sneaking in his own nest, should be low. Victims of sneaking, on the other hand, could receive their unrelated offspring in their nest at any stage of their egg-collection phase. However, it is noteworthy that even if our *a priori* assumptions hold, it is not possible to distinguish between guardian males that were victims of sneaking with relatively small genetic consequences in an early stage of their egg-collection phase and egg thieves. Also cases of egg thievery remain undetected if the egg thief had been a victim of sneaking at a later stage of his egg-collection phase. Nevertheless, because all males attributed to the victims of sneaking category had considerably higher R_{FR} -values (23–94%) than males of the egg thief category (1–5%), these categories still reflect guardian male classes that showed a pronounced difference in their ability to prevent other males from sneaking. The strong difference between these two categories suggests that three well separated nesting male categories exist in the Roche population. In other words, the capability of egg stealers for successful nest raiding is apparently strongly correlated with the capability for preventing other males from raiding their own nests.

In the 15-spined stickleback, R_{FR} -estimates for the victims of sneaking ranged from 13 to 63% (deduced from Table 4 in Jones *et al.*, 1998), also indicating, as is reported here for the three-spined stickleback (23–94%), a large variation in the genetic consequences for the nests of victims of sneaking. However, the R_{FR} -estimates for the nests of suspected egg thieves differ considerably between the two studies. It seems surprising that egg thieves in the 15-spined stickleback (cf. Table 4 in Jones *et al.*, 1998) have a higher proportion of unrelated offspring in their nests than the assumed victims of sneaking. If so, the hypothesis assumed in our study to explain egg thievery cannot hold in the case of the 15-spined stickleback. This would suggest that there are considerable differences in the biological meaning of the same behaviour in two closely related species.

The significantly smaller body size of the inferred victims of sneaking compared to other nesting males suggests that there is a greater chance for small males to become victims of sneaking. Thus body size may be a potential male trait for female mate choice in order to minimize her risk of losing some of the benefits of choice. Interestingly, a similar body size difference between victims of sneaking and other nesting males (excluding cases of assumed egg-stealing) can be extracted from the data in Jones *et al.* (1998; Table 3) for 15-spined sticklebacks (*t*-test, $P_{\text{directed}} = 0.05$; Mann–

Whitney *U*-test, $P_{\text{directed}} = 0.01$; $\gamma/\alpha = 0.8$ for both tests, cf. Rice & Gaines, 1994). If the males that were classified by Jones *et al.* (1998) as egg thieves are included into the 'not sneaked' category as was done in our study, for both tests we obtained even smaller *P*-values.

The observation that small nesting males, in spite of their greater chances of becoming victims of sneaking, still received eggs from females, could be explained by mutual mate choice. Kraak & Bakker (1998) found that in the Roche population attractive males chose larger females in order to obtain more and larger eggs. Thus it can be speculated that less attractive females (i.e. smaller females) were forced to mate with less attractive, smaller males. The frequency of such events would depend on the ratio of ripe females/nesting males in the population. Furthermore, if it is assumed that embryo survival decreases with increasing egg number in a nest (cf. Perrin, 1995), female choice for large attractive males could be modified in favour of smaller males during the reproductive season when these males already have collected large numbers of eggs (cf. Perrin, 1995).

We speculate that the increase of the condition factor of nest owners with increasing R_{FR} -values reflect an increasing intensity of egg cannibalism. In species with paternal care and several brood cycles within a season, such as sticklebacks, the males may dramatically improve their chances of re-nesting by eating some of their own offspring (Rohwer, 1978). Furthermore, when given a choice, an individual should always prefer heterocannibalism to filial cannibalism. So far, laboratory studies indicated that male three-spined sticklebacks do not recognize their own eggs (van Iersel, 1953; van den Assem, 1967; Fitzgerald & van Havre, 1987). Even though this may also be true under natural conditions, our data suggest that victims of sneaking and egg thieves may use the experience of being raided as an indication of the presence of unrelated embryos in their nest. It may also be possible that they memorize the position of eggs in the nest that could have potentially been fertilized by rivals. Thus, we hypothesize that the more nest raidings a guardian male has experienced the more he will eat of the embryos in his nest, because of an increased number of unrelated embryos in his nest.

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