

Alternative reproductive tactics in male rose bitterlings: Effect of density on interaction between frequency -and status- dependent selection

Yoshihiko KANOHI

ABSTRACT

The reproductive success of alternative reproductive tactics in male rose bitterlings, *Rhodeus ocellatus*, was estimated by field observations and experiments under artificial conditions. The average number of males per territory was 3.0 within a study area in a pond. Three tactics are used; territoriality, sneaking, and grouping. The mating tactics of the males changed depending on their size relative to their opponents and on local male density. Larger males usually employed the territorial tactic, and medium-sized males sometimes employed a temporary territorial but also used sneaking and grouping tactics, while small males used only sneaking and grouping tactics. The average fertilization success in the territorial and sneaking tactics was equal when the male density was approximately 3.0 in the tanks. Considering an interaction between the size- and the density-dependence, the average success rate per spawning for each tactic was: territorial, sneaking and grouping tactic are 0.61, 0.31 and 0.11, respectively. The average success rates of the tactics were not equal, but the success rates of the alternative tactics at an ESS switchpoint of 36 mm standard body length were equal. There was significant difference in mating success of marked males among the three size groups. Accordingly, although the alternative reproductive tactics of male rose bitterlings would be a conditional strategy, average success of alternative male tactics might be adjusted to be equal above a male density threshold which was approximately 3.0.

Key words: isozyme analysis, status-dependent selection, switch-point, group spawning.

INTRODUCTION

Not all members of a sex behave in the same way. It is well known that alternative reproductive tactics are employed by individuals within

a sex and even by an individual (Field, 1992; Taborsky, 1994, 2008; Mank and Avise 2006). Social interaction generate the negative frequency-dependent and/or status-dependent selection that are the driving forces in the evolution of alternative reproductive strategies and tactics. Alternative mating behaviors may evolve through several distinct mechanisms (Gross, 1996). In genetically polymorphic species, alternative reproductive strategies coexist in an evolutionarily stable state at the population level (ESS: e.g. Shuster and Wade, 1991; Ryan et al., 1992; Lank et al., 1995). In genetically monomorphic species, alternative male mating tactics may evolve into a mixed evolutionarily stable strategy (mESS) or as conditionally determined tactics in a conditional strategy. Alternative tactics in the conditional strategy may depend on such factors as the relative body size of competing males and the number of males competing. The average fitnesses of the alternative tactics are not equal, but the fitnesses of the tactics at a switchpoint are equal. It would be useful to identify the existence of the switchpoint, because although equal fitnesses are hard to demonstrate, one would ideally test the equality of fitnesses between tactics at the switchpoint.

The literature contains hundreds of examples of alternative reproductive phenotypes that are most readily interpreted as alternative tactics within a conditional strategy (Gross, 1984; Cooke, 1990; Danforth, 1991; Reynolds et al., 1993; Emlen, 1994; Blanchfield *et al*, 2003). A common conditional strategy is the use of fighting or sneaking as alternative mating tactics depending on body size. For example, in scarab dung beetles, *Onthophagus binodis*, male alternative mating tactics depend on body size and horn size (Cooke, 1990); in the ground-nesting bee *perdita portalis*, a large fighter phenotype mates within the nest, while small males mate outside the nest (Danforth, 1991). For both the beetle and bee it is thought that this small-male tactic results

in less average fitness than the large-male tactic.

Alternative male reproductive tactics are found in many species of fish where male compete for access to female mates (Taborsky, 1994, 2008). In most studies, on an individual basis, success rates of small parasitic males are much fewer than those of large territorial males (Warner et al., 1975; Warner and Lejeune, 1985; Hutchings and Myers, 1988; Chan and Ribbink, 1990; Rico et al., 1992; Philipp and Gross, 1994; Taborsky, 1994). However, these have not yet been well quantified any fitness of the alternative tactics at the switchpoint.

Males of the rose bitterling, *Rhodeus ocellatus*, which spawn into the gill cavity of a living freshwater mussel, form a territory around the mussel. Not all males can occupy the mussel as a spawning bed, because the number of matured males is larger than that of the mussels (the proportion of males to the mussels was approximately 2 : 1). Thus competitively subordinate small males participated in mating by sneaking tactics. Generally, in most externally fertilizing fishes, males using alternative reproductive tactics spawn simultaneously with the dominant or territorial males. In the rose bitterling, males spawn into freshwater mussels, and can release sperm over the inhalant siphon of the mussel both before and after egg-laying. I term ejaculating behavior by males before egg-laying 'pre-oviposition ejaculation' (Kano, 1996, 2000). Potential of sperm to fertilize eggs is lost almost completely at 4 min after release of the sperm. Small sneaker males intrude frequently into the territory of dominant males and perform pre-oviposition ejaculation (Kano, 1996).

Females deposit their eggs into the gill cavity from the exhalant siphon of the mussel through their ovipositor. This spawning by the females is repeated at intervals of 6-9 days (Shirai, 1962). The female deposits 10-20 eggs during one spawning cycle, with 1-3 eggs per egg-laying (Nakamura,

territoriality, sneaking, and grouping tactics (KANOH)

1969; Kanoh, 1996). Each mussel is used by plural females; chasing movements are observed between the females. Although the mussels sometimes contain more than 200 eggs of the rose bitterling in their gills, they are not killed by the eggs (Nagata, 1985; Kanoh, 1996).

Field observations and experiments were conducted from 27 April to 22 July, 1989. I have previously scored through behavioral data the success rate per spawning in each tactic, on an individual basis. The average reproductive success per spawning for each tactic was: territoriality (0.61), sneaking (0.31) and grouping (0.11), and thus the success rates of the tactics were not equal. Accordingly, it was suggested that the reproductive strategy of the rose bitterling was presupposed a conditional strategy (Kanoh, 1997, 2000).

However, there existed a negative size-dependent advantage in the fertilization success of the sneaker males. It was shown that sneakers gain almost equivalent success to territorial males on an individual basis in pair spawnings with sneakers, and that smaller sneakers have higher fertilization rates than larger sneaker males in tanks (Kanoh, 1996).

In the present study, I investigate the reproductive behavior of male rose bitterlings under natural conditions, and analyze the fertilization success of sneaking and territorial tactics using isozymes as genetic markers under artificial conditions. From this I am able to; 1) estimate fertilization success for each mating tactic depending on male density; 2) compare success measured at both individual and tactic levels; and 3) discuss the evolutionary mechanism favoring this reproductive strategy.

METHODS

Field site

The field site was a small pond (about 700 m²) in Yao city, Osaka, Japan

with a natural population of rose bitterlings (see also Kanoh, 1996). The main study section was a 2×5 m stretch in water ranging in depth from 40–60 cm. The bottom of the pond consisted of a mud surface over clay. To maintain observations, I arranged 13 mussels, as they might be found on other natural spawning sites, at intervals of 15–120 cm along the shore line. I secured each mussel to a plastic vessel buried in the bottom of the pond. Cylindrical nets (60 cm in diameter and 30 cm in height) under each mussel were used to capture mating fish near the mussel. The water temperature ranged from 16.5 °C to 25.0 °C over the breeding period.

Data collection

Field observations and experiments were conducted from 27 April to 22 July, 1989. I have previously demonstrated the following field observation data (Kanoh, 1997). The frequency of the three spawning patterns—pair, pair with sneakers, and group—were observed at a small pond in Yao city, Osaka. Of 229 spawning acts examined over one breeding period, 82 (35.8%) involved a single spawning pair (territorial male plus female); 106 (46.3%) had a spawning pair accompanied by one or more sneakers; and 41 (17.9%) were group spawnings. The most common pattern had pair spawnings with sneakers, next pair spawning, and finally group spawning (X^2 -test, $X^2 = 28.32$, $df = 1$, $p < 0.001$). In the peak breeding season, when the most males participated in mating, the group spawnings were observed at only local spawning site. These spawning patterns did not change significantly through the breeding period. The local average number of operational males for each spawning pattern was: pair spawning (1.0), pair spawnings with sneakers (3.2), and group spawning (9.0). The operational sex ratio also differed significantly among the three spawning patterns: pair spawning (1.0; male/female), pair spawnings with sneakers (3.3), group spawning (4.9; Kruskal-

territoriality, sneaking, and grouping tactics (KANOH)

Wallis test, $H = 75.97$, $p < 0.001$). In the 61 pair spawnings with sneakers, the average number of sneakers was 2.2 ± 1.4 SD.

In the present study, to estimate the fertilization success of each mating tactic and male individual, I collected the using of alternative mating tactics by marked males at each mussel site ($n = 13$). A total of 58 males and 30 females were captured with the nets. They were then marked for identification by cutting a small part off their fins, and released back into the pond. The spawning sites were scanned for multiple males, and the numbers of fish, other than the territorial male, were recorded. After observations of spawning activity, I pulled each cylindrical net and captured the fish which had participated in mating. I then measured their standard body length (to the nearest 0.1 mm) and recorded the brightness of their nuptial coloration. Reproductive behaviors were also recorded by an 8 mm video camera from the bank and were observed by using a mask and snorkel while kneeling near each mussel site.

Spawning patterns and male mating tactics

Spawning behavior was divided into three patterns: (a) pair spawning; (b) pair spawning with sneakers; and (c) group spawning (Kanoh, 1997, 2000).

(a) Pair spawning: Pair spawning involves only the one male and the one female. Thus, all males employed only the territorial tactic in pair spawning. A territorial male defends an area around a mussel, leads a mature female towards the mussel. The female arrives at the mussel and deposits her eggs through her long ovipositor into the exhalant siphon of the mussel. After the female oviposits, the male performs ejaculation movements, which are almost always accompanied by ejecting sperm, over the inhalant siphon of the mussel (Nagata, 1976; Kanoh, 1996).

(b) Pair spawning with sneakers: Pair spawning with sneakers includes

territoriality, sneaking, and grouping tactics (KANOH)

one or more sneaking males intruding into the territory of a courting pair and performing ejaculation movements before and/or after the pair spawns (Wiepkema, 1961; Kanoh, 1996, 2000). I have defined the term 'sneaking tactic' as meaning to intrude into the territory and eject sperm. Sneaking tactics are further categorized into two types. A 'type 1 sneaking' is intrusion behavior of a medium-sized male which forms a temporary territory and intrudes into neighboring established territories, while a 'type 2 sneaking' is used by a competitively subordinate small male which seldom forms a territory (Kanoh, 1997, 2000).

(c) Group spawning: Group spawning occurs when a territory is broken down by the intruding behavior of many sneaker males. Grouping tactic of males does not use courtship movements. In group spawning ejaculation movements by males and egg-layings by females take place continuously. Pair spawning with sneakers is distinguished from group spawning by whether courtship movements are shown. If the spawning starts out as territorial plus sneakers and then moves to group spawning, I classified the tactics of the males by whether courtship movements were shown at the spawning time of female. In short, it is thought, if these courting behaviors of territorial males were not observed, the sneakers could no longer sneak, as it was, and that therefore all males were using the grouping tactic (Kanoh, 2000).

Fertilization success

Isozyme electrophoresis

Isozyme analysis was conducted on tissue from adult fins and larvae by standard horizontal starch-gel electrophoresis and staining techniques, as described in Shaw & Prasad (1970). APM-citrate buffer at pH 6.0 was modified from Clayton & Tratiak (1972). Lactate dehydrogenase (LDH) and

territoriality, sneaking, and grouping tactics (KANOH)

6-phosphogluconate dehydrogenase (6PGD) loci are polymorphic (Nagata et al., 1996; Kanoh, 1996, 1997, 2000). They appear to be encoded by two alleles (1dh-a and 1dh-b; 6 pgd-d and 6pgd-e). The frequencies of the alleles and tests of Hardy-Weinberg were conducted for the study pond in 1988, 1989, and 1994. Chi-square tests of observed versus expected frequencies were consistent with the alleles in Hardy-Weinberg equilibrium ($df=1$, all $p>0.1$) and provided no generational shifts in allele frequencies between observation years ($df=2$, all $p>0.1$). Accordingly, there was no evidence of non-random mating from either of the two enzymes.

Density-dependent fertilization success in tanks

In 1988, 1990 and 1994 (from May to July), experiments on density dependent fertilization success were conducted indoors in small tanks (60 × 30 × 40 cm). All the fish were collected from the same pond used for the field observations. In each tank, the water was maintained at 20–25 °C, and filtered through glass wool. The fish were fed Tetramin.

A total of 12 territorial males, 20 sneakers and 17 females were used in the experiments. Encounters between a territorial male and from one to four sneakers were arranged for either one or two days in small tanks. For each experiment, the sneakers and the territorial male had different isozymes (LDH and/or 6PGD). I introduced the males and a female in a tank containing one mussel in which no eggs had been deposited. The process was repeated with different groupings of the territorial and sneaker males. After each of the observations, the paternity of the males was judged by using isozyme analysis. A small part of the fin of each fish was cut off to identify it and for isozyme analysis. The eggs were taken out of the mussel and incubated at 20 ± 1 °C in a Petri-dish for about 20 days. Whole-body extracts of the larvae except for yolk, were made for isozyme analysis.

territoriality, sneaking, and grouping tactics (KANOH)

Differences between the fertilization success of the sneakers and that of the territorial males were determined using the G-statistic (replicated goodness-of-fit test; Sokal & Rohlf 1995).

Although type 1 sneakers sometimes fought, they always lost in my experiments. They sometimes performed leading and courtship movements but were always chased away by the territorial male and could not spawn. They were sometimes successful at sneaking. Type 2 sneakers never fought, and instead always adopted the sneaking tactic. The mating tactics of the individual males depended on relative size and thus the tactics were fixed during an experiment of one group trial in a tank. However, the tactics of an individual sometimes varied among the trials of different groups according to his relative size therein.

RESULTS

Male density and sex ratios

The average number of males was 23.2 (± 9.5 SD, $n=6$: number of counted days) and the average number of territories was 7.73 (± 1.42 SD, $n=11$: number of counted days) on a study area over one breeding period. In this study, the average male density (number of males per territory) within the study area was approximately 3.0 (the average number of males/the average number of territories = $23.7/7.73$).

The pond sex ratios (males : females) were 1.2 : 1 (total number of individuals = 246, 27 June) and the average operational sex ratio (males/females) was 3.3 (± 1.0 SD, $n=6$) on the study area during the active breeding season.

Ratio of mating tactics

The males switched mating tactics according to their body size relative to

opponents at the spawning site. The standard body length of the territorial males (37.8 mm \pm 2.6 SD, n=55) was significantly greater than that of the sneaking males (34.5 mm \pm 2.1 SD, n=84; Mann-Whitney U-test, $z=6.80$, $p<0.001$). There was no significant difference in body size between sneaking and grouping males (34.9 mm \pm 2.5 SD, n=64; $z=0.819$, $p>0.1$). The body length of marked type 1 sneaking males (36.2 \pm 0.8 mm, n=12) was greater than that of the females (33.7 mm \pm 3.0 SD, n=47; $z=3.295$, $p=0.001$), but there was no significant difference in body size between the females and small type 2 sneakers (34.2 mm \pm 1.9 SD, n=8; $z=1.181$, $p>0.2$).

Although the distributions of body size for territorial and sneaking tactics were of approximately normal distribution (X^2 -test, territorial: $X^2=3.348$, $df=4$, $p>0.5$, sneaking: $X^2=4.66$, $df=5$, $p>0.3$), the body size of males which had taken part in group spawnings did not indicate a normal distribution ($X^2=13.77$, $df=5$, $p<0.05$).

The relationships between standard body length and the mating tactics adopted in 30 males, which were marked and recaptured at the spawning site, are shown in Figure 1. Use of the sneaking tactic was negatively correlated with body length (Kendall's rank correlation, $\tau = -0.648$, $z = -4.46$, $p<0.001$; Figure 1-b) while that of the territorial tactic was positively correlated with body length ($\tau = 0.706$, $z = 5.03$, $p<0.001$; Figure 1-a). There was, however, no significant correlation between use of the grouping tactic and male body length ($\tau = -0.212$, $z = -1.43$, $p>0.1$; Figure 1-c). The exclusive use of the sneaking tactic was shown in males with less than a 33 mm body length, while the body length of males using only the territorial tactic was above 38 mm. The switchpoint between the sneaking and territorial tactic is an approximately 36mm body length. The body size of males which participated in group spawnings ranged from 32-39 mm (n=16). Thus, use of mating tactics depends on body length. The males' length of

territoriality, sneaking, and grouping tactics (KANOH)

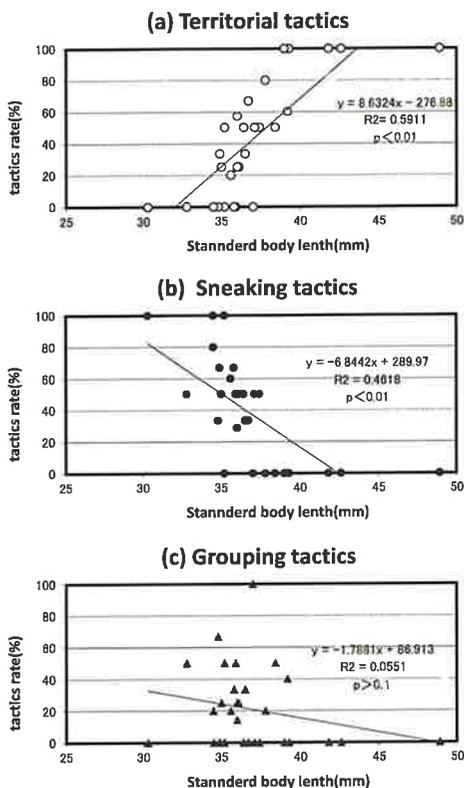


Figure 1

Relationships between the adopting ratio of each mating tactic and the standard body length on the marked malts ($n=30$).

visit to the observed spawning site did not correlate significantly with their body size ($\tau = 0.047$, $z = 0.357$, $p > 0.1$).

Density-dependent fertilization success

In the one-to-one encounters, fertilization success of territorial males (average 68%; ranging 28.6–100%, $n=11$) was significantly higher than

territoriality, sneaking, and grouping tactics (KANOH)

Table 1-a: Fertilization success of males in the tanks based on isozyme analyses.

No of exp.	Male identification			Male phenotype		Female phenotype		Offspring phenotype		Number of offspring	Fertilization success (%)	G-test results
	no.	size (mm)	tactics (type)	LDH	GP6D	LDH	GP6D	LDH	GP6D			
[1-to-1 encounters]												
1	3♂	36.1	T	EE		DD		DE		10	100	13.862 **
	9♂	38.6	S-1	DD		DD		DD			0	
2	31♂	55.3	T	EE		EE		EE		8	100	11.09 **
	32♂	42.2	S-1	DD		DD		DE			0	
3	13♂	46	T	BB		AA		AB		12	92.3	10.97 **
	7♂	37.4	S-1	AA		AA		AA			1	
4	21♂	50.3	T	DD		EE		DE		12	75	4.183 *
	22♂	35.3	S-2	EE		EE		EE			4	
5	31♂	55.3	T	EE		EE		EE		10	71.4	2.656 ns
	32♂	42.2	S-1	DD		DD		DE			4	
6	2♂	38.5	T	EE		DD		DE		10	66.7	1.698 ns
	4♂	31.2	S-2	DD		DD		DD			5	
7	1♂	44.8	T	DD		DE		DD		8	57.1	1.310 ns
	5♂	34.1	S-2	EE		EE		EE (DE)			6	
8	11♂	45.3	T	BB		AA		AB		14	53.8	0.153 ns
	12♂	35.2	S-2	AA		AA		AA			12	
9	2♂	38.5	T	BB		AA		AB		3	50	0 ns
	6♂	32	S-2	AA		AA		AA			3	
10	21♂	50.3	T	DD		EE		DE		4	36.4	0.828 ns
	22♂	35.3	S-2	EE		EE		EE			7	
11	11♂	45.3	T	BB		AA		AB		6	28.6	3.984 *
	12♂	35.2	S-2	AA		AA		AA			15	
pooled T: S= 97: 57 pooled G											10.509 *	

A sign and a figure in parentheses show phenotype and the number of offspring of indeterminate paternity out of 20 offspring fertilized.
 Mating tactics: T: territorial, S-1: fighting and streaking, S-2: sneaking
 G-test: *: p<0.05. **: p<0.01

that of sneakers (G test; pooled G = 10.509 df = 1, heterogeneity G = 40.225 df = 10, both p < 0.01, Table 1-a). In the one-to-two encounters, there was no significant difference for the fertilization success per individual male between territorial (32.9%) and sneaker (33.6%; G test; pooled G = 0.552 df = 1, heterogeneity G = 4.333 df = 4, both p > 0.1, Table 1-b). As the number of sneakers increased, the fertilization success of the territorial males decreased (Figure 2-a). Although the fertilization success per individual territorial male was negatively correlated with density of males (Kendall's rank correlation, $\tau = -0.71$, $z = 4.38$, $p < 0.001$; Figure 2-a), the absolute fertilization success per individual sneaker did not correlate directly with

territoriality, sneaking, and grouping tactics (KANOH)

Table 1-b: Fertilization success of males in the tanks based on isozyme analyses.

No of exp.	Male identification			Male phenotype		Female phenotype		Offspring phenotype		Number of offspring	Fertilization success (%)	G-test results
	no.	size (mm)	tactics (type)	LDH	6PGD	LDH	6PGD	LDH	6PGD			
[1-to-2 encounters] Expected ratio is 1:2												
12	G2♂	44.3	T	AA	EE	AA	DD	AA	DE	9	52.9	0.469 ns
	L15♂	41	S-1	BB	DE			AB	DD, DE			
	H2♂	36.2	S-2	AA	DD			AA	DD			
13	E1♂	41.1	T	AA	EE	AA	DD	AA	DE	11	40.7	2.754 ns
	E2♂	36.6	S-1	BB	EE			AB	DE			
	S1♂	35.2	S-2	AA	DD			AA	DD			
14	T3♂	49.4	T	AA	DD	BB	EE	AB	DE	8	36.4	0.090 ns
	S3♂	39.3	S-1	BB	EE			BB	EE			
	S4♂	35.3	S-2	BB	EE			BB	EE			
15	D2♂	41.3	T	BB	EE	AA	DD	AB	DE	2	25	0.262 ns
	S2♂	34.6	S-1	AA	DD			AA	DD			
	D4♂	31.2	S-2	AB	DD			AA, AB	DD			
16	G2♂	44.3	T	AA	EE	AA	DD	AA	DE	3	20	1.310 ns
	L15♂	41	S-1	BB	DE			AB	DD, DE			
	H2♂	36.2	S-2	AA	DD			AA	DD			
pooled T: S= 33: 56 pooled G											0.552 ns	
[1-to-3 encounters] Expected ratio is 1:3												
17	K1♂	52.5	T		DD	DD		DD		5	21.7	0.134 ns
	K3♂	44.2	S-1	EE				DE				
	K4♂	39.4	S-1	EE				DE				
	K5♂	37.6	S-2	EE				DE				
18	L2♂	54	T	DD		DD		DD		2	15.4	0.711 ns
	K3♂	44.2	S-1	EE				DE				
	K4♂	39.4	S-1	EE				DE				
	K5♂	37.6	S-2	EE				DE				
pooled T: S= 7: 29 pooled G											0.626 ns	
[1-to-4 encounters] Expected ratio is 1:4												
19	D2♂	38.5	T	BB	EE	AA	DD	AB	DE	3	14.3	0.464 ns
	G3♂	36.1	S-1	AA	EE			AA	DE			
	O1♂	44.9	S-1	AB	DD			AA, AB	DD			
	D9♂	37.8	S-1	AB	DD			AA, AB	DD			
	S2♂	33	S-2	AA	DD			AA	DD			
20	L2♂	54	T	DD		DD		DD		2	8	2.764 ns
	K2♂	39.3	S-1	EE				DE				
	K3♂	44.2	S-1	EE				DE				
	K4♂	39.6	S-1	EE				DE				
	K5♂	37.6	S-2	EE				DE				
pooled T: S= 5: 41 pooled G											2.763 ns	

#: it is determined based on behavioral data that every offspring of the phenotype AA DD was fertilized by sneaker S2♂ in the experiment 19.
 Mating tactics: T: territorial, S-1: fighting and streaking, S-2: sneaking
 G-test: ns: non-significance

density of males ($\tau = -0.269$, $z = -1.66$, $p > 0.1$; Figure 2-c). When the number of sneaker males increased, the fertilization success was lower only in the territorial males, because time-budget data of the territorial males indicated a trade-off between chasing and courtship behavior, while the sneaking males were able to intrude and eject sperm easily (Kano,

territoriality, sneaking, and grouping tactics (KANOH)

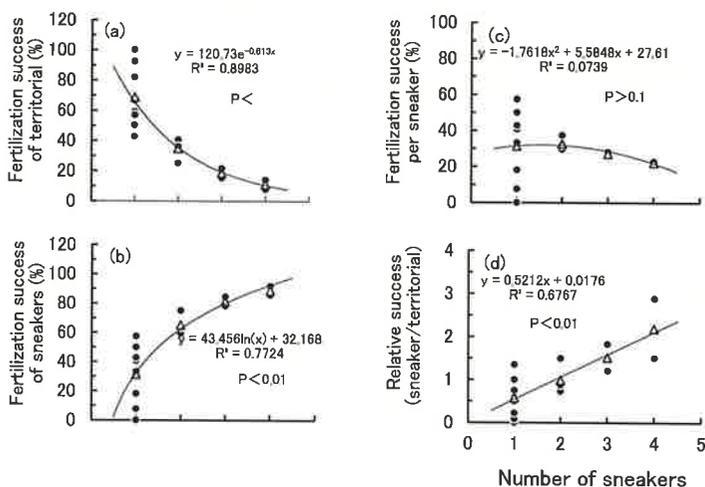


Figure 2

Relationship between the fertilization success of males (a: territorial males; b: sneakers; c: sneakers on an individual basis) and the number of parasitic males. And relationship between the success rate on an individual basis (d: sneaker/territorial male) and the number of sneaker males. Open triangle = the average of percentage.

1996, 1997, 2000). Thus, the relative success rate of sneakers to territorial males (success of sneaker/that of territorial male on an individual basis) was positively correlated with density of males ($\tau = 0.453$, $z = 2.79$, $p < 0.01$; Figure 2-d). The relative fertilization success per individual became approximately equivalent at a ratio of 1 : 2 territorial males to sneaker males. Moreover, on an individual basis, the relative fertilization success of sneaking males was higher than that of territorial males when the local population density became four or more males. Under such conditions, one would expect the territory to break down and group spawning to take place, because the relative fertilization success of the territorial male decreases as local population density increases.

In the one-to-one encounters, there was no significant difference in

fertilization success between type 2 sneakers and territorial males (G test; pooled $G=0.2294$ $df=1$, $p>0.1$, heterogeneity $G=11,927$ $df=6$, $p>0.05$; Table 1-a). And the fertilization success of type 2 sneakers significantly exceeded that of type 1 sneakers (Mann-Whitney U test, $U=1$, $p=0.006$; Table 1-a). Also in the one-to-two encounters, the fertilization success of type 2 sneakers was significantly greater than that of type 1 sneakers (G test; pooled $G=16.450$ $df=1$, heterogeneity $G=10.793$ $df=2$, both $p<0.01$). Accordingly, there was negative size dependent advantage in fertilization success for the sneaking tactics.

Estimating the success of each mating tactics

The reproductive success of the mating tactics can be estimated in three different ways.

First, I had already estimated the success rate per spawning in each tactic, using pair spawning equivalents, that is, the fertilization success per spawning of each male is divided by the total number of males that have participated in the spawning (Warner et al., 1975), through behavioral data from field observations in 1989 (Kanoh 1997, 2000). In the field observations the spawning rates were: pair spawning (36%), pair spawning with sneakers (46%) and group spawning (18%). In each spawning pattern, the local average number of operational males were: pair spawning (1.0), pair spawnings with sneakers (3.2), and group spawning (9.0). The females spawned approximately only one egg per egg-laying into the mussels (0.95 ± 0.3 SD, $n=19$; number of mussels). There was no significant difference for body size of territorial male between pair spawning and pair spawning with sneakers (in pair spawning: $38.8 \text{ mm} \pm 4.2$ SD, $n=11$; in pair spawning with sneakers: $38.8 \text{ mm} \pm 4.5$ SD, $n=15$; Mann-Whitney U-test, $U=78$, $p>0.1$). Accordingly, the average reproductive success per spawning for each tactic was:

territoriality, sneaking, and grouping tactics (KANOH)

$$\text{territoriality} = (0.36 \times 1 + 0.46 \times (1/3.2)) / (0.36 + 0.46) = 0.61,$$

$$\text{sneaking} = 1/3.2 = 0.31, \text{ and}$$

$$\text{grouping} = 1/9 = 0.11,$$

and thus the success rate of the tactics were not equal.

Another method is by performing isozyme analysis of fish in tanks. The average number of sneaking males in pair spawnings with sneakers was 2.2 in field observations (Kano, 1997). The fertilization success of each tactic can be calculated from the approximate curve of the relationship between fertilization success and the number of parasitic males (Figure 2).

The approximate curve for territorial males is:

$$y = 111.64e - 0.5919x$$

when $x = 2.2$, $y = 30.4$ (Figure 2-a).

Therefore, the average success of the territorial tactic is: $(0.36 \times 1 + 0.46 \times 0.30) / (0.36 + 0.46) = 0.61$. While, the approximate curve for sneaking males on an individual basis is:

$$y = -1.147x^2 + 1.830x + 32.92$$

when $x = 2.2$, $y = 31.4$ (Figure 2-c).

Thus, the average success of the sneaking tactic is 0.31. Finally, the success

Table 2 Size-dependence in mating success on an individual basis

	Large territorial	Medium-sized territorial sneaking 1 grouping	Small sneaking 2 grouping	Kruskal-Wallis test H
Body length (mm)	40.2 ± 3.7 (n=10)	36.2 ± 0.8 (n=12)	34.2 ± 1.9 (n=8)	19.691 p < 0.001
Relative mating success	2.0 ± 1.4 (n=10)	1.5 ± 0.8 (n=12)	1.3 ± 0.6 (n=8)	2.423 p > 0.2

Mean ± SD are shown with number of samples in parentheses. Differences were tested by Kruskal-Wallis test.

territoriality, sneaking, and grouping tactics (KANOH)

rate of the grouping tactic is 0.11 (1/9.0).

Accordingly, the average mating success per spawning of territorial, sneaking and grouping tactics is 0.61, 0.31 and 0.11, respectively. The estimate by isozyme analysis supported the behavioral estimate using pair spawning equivalents. However, both ways of estimating disregarded the negative size dependence in mating success for the sneaking tactic.

Success of individual males

First, success of individual males was estimated from behavioral data by using pair spawning equivalents. I estimated the mating success per spawning of 30 marked males by multiplying the ratio of mating tactics used by each individual by success (0.61 : 0.31 : 0.11) per spawning in each tactic. The switchpoint of alternative reproductive tactics was an approximately 35 mm body length. Total success rate per spawning correlated with standard body length for each individual (Kendall's rank correlation, $\tau = 0.6328$, $z = 4.911$, $p < 0.001$). The relative mating success of each individual was calculated by multiplying the estimated success per spawning by the number of observed spawnings for each individual. In the 30 marked individuals, there was a positive relationship between mating success of individuals and standard body length ($\tau = 0.2907$, $z = 2.256$, $p < 0.05$).

The switchpoint between alternative reproductive tactics was approximately 36 mm. The total success rate per spawning for each individual is shown in Figure 3-d. The relative mating success of each individual was calculated by multiplying his estimated success per spawning by the number of observed spawnings he made. In the 30 marked individuals, a significant correlation was not indicated between mating success of individuals and standard body length (Kendall's rank correlation test, $\tau = 0.2072$, $z = 1.588$, $p > 0.1$; Figure 3-e).

territoriality, sneaking, and grouping tactics (KANOH)

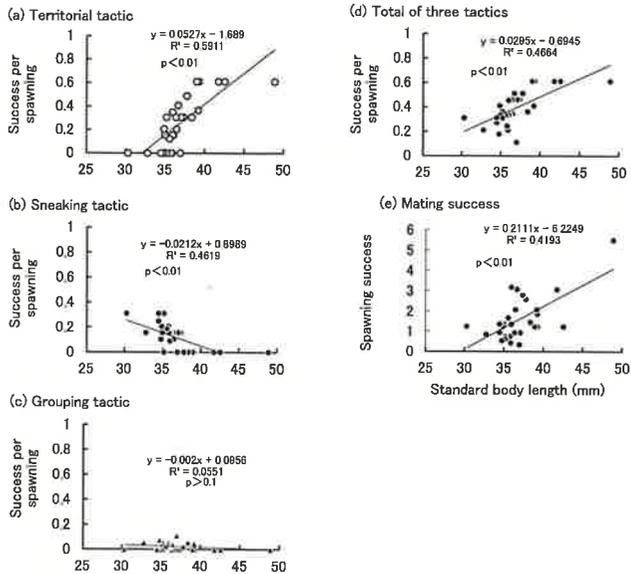


Figure 3

Relationship between mating success per spawning in tactics (a: territorial; b: sneaking; c: grouping; d: total) and standard body length on the marked males ($n=30$) based on isozyme analysis. And (e) relationship between relative mating success and standard body length on the marked males ($n=30$).

DISCUSSION

Reproductive strategy

In this study, male rose bitterlings employed mating tactics depending on the relative size and number of encountered opponents. Marked individuals undoubtedly employed plural mating tactics depending on the social situations. Thus, each individual is genetically monomorphic for deciding the adopting tactics. In the present population, the average reproductive success of alternative mating tactics was not equal, and the switchpoint was approximately 36 mm of standard body length. The success of both territorial and sneaking tactics at the switchpoint was equal. Accordingly,

it is suggested that the reproductive strategy of the rose bitterling could be taken to be a conditional strategy.

However, in fact, there exist both pair spawnings, in which size-dependent selection leads to mating success of alternative tactics, and group spawnings, in which size-dependent selection scarcely operates in the rose bitterling. Therefore, it is suggested that the reproductive success of each tactic depends not only on relative size but also local population density.

Recent findings suggest that almost all alternative reproductive phenotypes are due to alternative tactics within a conditional strategy (Eadie and Fryxell, 1992; Karino, 1993; Brockman, 1994; Emlen, 1994; Taborsky, 1994, 2008). A complete theory for the evolution of alternative tactics has yet to be developed. Gross (1996) insists on the need for new theoretical modeling to combine frequency- and status-dependent selection and solve for their joint equilibrium. In Gross's model, when the fitnesses of alternative tactics are functions of both the frequency of the tactic and the status of the individual, the switchpoint that evolves must balance these two often opposing selection pressures.

In the rose bitterling, if territories were always maintained by territorial males, a switchpoint between the alternative tactics would usually exist. However, group spawning exists and the switchpoint may disappear in this form of spawning. Since local male population density influenced spawning patterns, one has to consider the special effects of local male density on success for alternative mating tactics of this species.

Several examples of switchpoint adjustment to ecological events, including local density and/or operational sex ratio, exist (Eadie and Fryxell, 1992; Radwan, 1993; Carroll and Corneli, 1995; Lucas and Howard, 1995; Gross, 1996). In the acarid mite, *Caloglyphus berlesesi*, which has fighter and non-fighter male phenotypes, not only does density influence the potential

success of the alternative tactics, but the individual choice of tactic is sensitive to the density as well as to body size (Radwan, 1993). Eadie and Fryxell (1992) considered combining frequency -and density- dependent selection, and developed a simple model to explore the alternative female breeding tactics in a cavity-nesting bird, the Barrow's goldeneye. Their results showed that there was a density threshold above which frequency dependence plays a prominent role. In short, success of alternative tactics is equal at a specific density threshold, but not equal at other densities.

In this study, the average number of males per territory within the study area was 3.0 (the average number of males/the average number of territories=23.7/7.73). This average number corresponded with local male density (3 males) when the success of territorial and sneaking tactics were approximately equal in the tanks. This coincidence involved the fact that the average number of males per territory within the study area was affected by the reproductive success of the males. And it is suggested that an ESS switchpoint of alternative mating tactics is a 36 mm body length with an above-3.0 male density in this study population. Accordingly, despite a conditional strategy, the average success of alternative male tactics might be adjusted to be equal above a male density threshold wherein the average number of males within a territory was approximately 3.0.

In other populations of the rose bitterling, it has been estimated that the life span for most male individuals dose not exceed two years (Solomon et al., 1985). In this study, the males' length of visit to the observed spawning site did not correlate significantly with their body size. If most males participate in mating during only one breeding period, small type 2 sneakers and large territorial males would be equivalent in life-time fitness in the study population (KanoH, 1996). Presented data and the mentioned reports supported a new model, that is, modifying Gross's model in effect of

density on interaction between frequency -and status- dependent selection for alternative reproductive male tactics in the rose bitterling. However, at the present time, I can't determine the life-time fitness of the rose bitterling from my empirical data.

On the other hand, as the average operational sex ratio is 3.3 in this study area, it can be calculated that approximately one-third of the females in a pond will visit a spawning site ($1/(\text{operational sex ratio of spawning site}/\text{operational sex ratio of whole pond}) = 1/(3.3/1.2) \approx 1/3$). In short, this average may depend on the spawning cycle of female rose bitterlings.

Further, to explain the occurrence of the grouping tactic, we must compare the cost-benefits in local and non-local situations. Locally, the grouping tactic can be a way to make the best of a bad situation, but from a universal point of view, the success of the grouping tactic for individual males will be relatively low. Why does such a difference of success occur between local and non-local situations? The difference may be related to finite information among individuals. In the future, it is necessary to study the interaction of males and females of the rose bitterling.

ACKNOWLEDGEMENTS

I would like to thank Y. Nagata and M. Imafuku for critical comments and stimulating discussion on this work. I am also grateful to M. R. Gross, M. Hori, S. Mori, M. Kon and K. Maekawa for critical reading, advice and comments on this manuscript, and to J. L. Yohay, L. Ethier, and S. Kanoh for editing assistance. I am indebted to coworkers at Osaka Kyoiku University, O. Saitoh and members of the Biology club of Seifu High School, T. Endoh, Y. Atarashi, and Y. Takawashi, for their assistance in carrying out the field study.

REFERENCES

- Blanchfield, P. J., Ridgway, M. S., and Wilson, C. C. 2003. Breeding success of male brook trout (*Salvelinus fontinalis*) in the wild. *Molecular Ecology* 12, 02417–2428.
- Brockman HJ, Conlson T, Potts W, 1994. Sperm competition in horseshoe crabs (*Limulus polyhemus*). *Behav Ecol Sociobiol* 35: 153–160.
- Carroll SP, Corneli PS, 1995. Divergence in male mating tactics between two populations of the soapberry bug: Genetic change and the evolution of a plastic reaction norm in a variable social environment. *Behav Ecol* 6: 46–56.
- Chan T-Y, Ribbink AJR, 1990. Alternative reproductive behaviour in fishes with particular reference to *Lepomis macrochira* and *Pseudocrenilabrus philander*. *Env Biol Fish* 28: 249–256.
- Clayton JW, Tretiak DN, 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J Fish Res Bd Can* 29: 1169–1172.
- Cooke DF, 1990. Differences in courtship, mating and post-copulatory behaviour between male morphs of the dung beetle *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae). *Anim behav* 40: 428–436.
- Danforth BN, 1991. The morphology and behavior of dimorphic males in *Perdita portalis* (Hymenoptera: Andrenidae): evidence for male mimicry. *Behav Ecol Sociobiol* 29: 235–247.
- Eadie JM, Fryxell JM, 1992. Density dependence, frequency dependence, and alternative nesting strategies in goldeneyes. *Am Nat* 140: 621–641.
- Emlen DJ, 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc R Soc London Ser B* 256: 131–136.
- Field J, 1992. Intraspecific parasitism as an alternative reproductive tactic in nest-building wasps and bees. *Biol Rev* 67: 79–126.
- Gross MR, 1984. Sunfish, salmon and the evolution of alternative reproductive strategies and tactics in fishes. In: *Fish reproduction: strategies and tactics* (Potts G, Wootten R, eds). pp. 55–75. London: Academic Press.
- Gross MR, 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol Evol* 1: 92–98.
- Hutchings JA, Myers RA, 1988. Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia* 75 (2): 169–174.
- Kanoh Y, 1996. Pre-oviposition ejaculation in externally fertilizing fish: how sneaker male bitterling contrive to mate. *Ethology* 102: 883–899.
- Kanoh Y, 1997. Reproductive behavioral ecology in male rose bitterlings: territoriality, sneaking and grouping tactics. Shirakawa Sakyo-ku, Kyoto 606–8502. Kyoto University (Ph D dissertation)
- Kanoh Y, 2000. Reproductive success associated with territoriality, sneaking, and grouping in male rose bitterling, *Rhodeus ocellatus* (Pisces: Cyprinidae) *Environmental Biology of Fishes* 57: 143–154.

- Karino K, & Nakazono A, 1993. Reproductive behavior of the territorial herbivore *Stegastes nigricans* (Pisces: Pomacentridae) in relation to colony formation. *J Ethol* 11: 99-110.
- Lank DB et al., 1995. Genetic polymorphism for alternative mating behaviour in lekking male ruff, *Philomachus pugnax*, *Nature* 378: 59-62.
- Lucas J, Howard RD, 1995. On alternative reproductive tactics in anurans: dynamic games with density and frequency-dependence. *Am Nat* 146: 365-397.
- Maynard Smith J, 1982. *Evolution and the Theory of Games*. Cambridge: University Press.
- Mank, J. E. and Avise, J. C. 2006. Comparative phylogenetic analysis of male alternative reproductive tactics in ray-finned fishes. *Evolution* 60: 1311-1316.
- Nagata Y, 1976. Reproductive behaviour of a bitterling, *Rhodeus ocellatus* (Kner). *Physiol Ecol Japan* 17: 85-90 (in Japanese).
- Nagata Y, 1985. The effect of social organization of the bitterling, *Rhodeus ocellatus* (Kner) and the condition of a bivalve, *Anodonta woodiana* Lea in a small pond on the number of fish eggs being laid in a bivalve. *Mem Osaka Kyoiku Univ Ser* 34: 9-26 (in Japanese).
- Nagata Y, Tetsukawa T, Kobayashi T, Numachi K, 1996. Genetic markers distinguishing between the two subspecies of the rosy bitterling, *Rhodeus ocellatus* (Cyprinidae). *Ichthyol Res* 43 (2): 117-124.
- Nakamura M, 1969. Cyprinid Fish of Japan. *Res Inst Nat Resour Spec Publ* 4: 1-455 (in Japanese).
- Philipp DP, Gross MR, 1994. Genetic evidence for cuckoldry in bluegill *Lepomis macrochirus*. *Mol Ecol* 3: 563-569.
- Radwan J, 1993. The adaptive significance of male polymorphism in the acarid mite *Caloglyphus berlesesi*. *Behav. Ecol Sociobiol* 33: 201-208.
- Reynolds JD, Gross MR, Coombs MJ, 1993. Environmental conditions and male morphology determine alternative mating behaviours in Trinidadian guppies. *Anim behav* 45: 145-152.
- Rico C, Kuhnlein U, Fitzgerald GJ, 1992. Male reproductive tactics in the three stickleback - an evaluation by DNA fingerprinting. *Mol Ecol* 1: 79-87.
- Ryan MJ, Pease CM, Morris MR, 1992. A genetic polymorphism in the swordtail *Xiphiphorus nigrensis* testing the prediction of equal fitness. *Am Nat* 139: 21-31.
- Shaw CR, Prasad R, 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochem Genet* 4: 297-320.
- Shirai K, 1962. Correlation between the growth of the ovipositor and ovarian condition in the bitterling, *Rhodeus ocellatus*. *Bull Fac Fish Hokkaido Univ* 13: 137-157.
- Shuster SM, Wade ML, 1991. Equal mating success among male reproductive strategies in a marine isopod. *Nature* 350: 608-610.
- Solomon G, Matsushita K, Shimizu M, Nose Y, 1985. Age and growth of rose bitterling in the Shin Tone river. *Bull Japan Soc Sci Fish* 51: 55-62.
- Sokal RR, Rohlf FJ, 1995. *Biometry*. New York: W H Freeman.
- Taborsky M, 1994. Sneakers, satellites, and helpers: parasitic and cooperative in fish reproduction. *Adv Study behav* 23: 1-100.

territoriality, sneaking, and grouping tactics (KANOH)

- Taborsky M, 2008. Alternative reproductive tactics in fish. *Alternative reproductive tactics* (Rui F. Oliveira, Michael Taborsky, H. Jane Brockmann eds). 251–299. Cambridge University Press.
- Warner RR, Robertson DR, Leigh EGJ, 1975. Sex change and sexual selection. *Science* 190: 633–638.
- Warner RR, Lejeune P, 1985. Sex change limited by paternal care: A test using four Mediterranean labrid fishes, genus *Symphodus*. *Mar Biol* 87: 89–99.
- Wiepkema PR, 1961. An ethological analysis of the reproductive behaviour of the bitterling (*Rhodeus amarus* Bloch). *Arch Neerl Zool* 14: 103–199.