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RELATIVE IMPORTANCE OF DIRECT AND INDIRECT INTERACTION AMONG INDIVIDUAL SNAILS

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SUMMARY

Individual organisms interact directly through behavior, and indirectly through resource consumption and environment modification. The effects of different kinds of interactions on individual growth and reproduction will differ. Freshwater snails may interact directly for food resources and indirectly through substances dissolved in water. I separated the effects of the direct behavioral interaction and indirect interaction through waters using laboratory experiments with freshwater snails *Physa acuta*. Behavioral direct interaction have negative effects on the growth, but indirect interaction through water environments has positive effect on the growth. The importance of distinction of different kinds of interactions were discussed.

KEYWORDS: *Physa*, interaction, competition, freshwater snails, the effects of direct and indirect interaction on growth.

INTORDUCTION

Individual organisms interact in various ways. Individuals compete for food or space through direct behavioral interaction. When a group of individuals consumes the same resources but they do not interact directly with each other, individual fitness is affected by the amount of available resources, which is determined by the total amount of resources utilized by the group of individuals. Some organisms interact through non-resource substances, such as pheromones and metabolic substances, emitted into the environment by organisms. These various interactions may occur at the same time, and the fitness of the individuals will be influenced by the combined effects of different interactions.

So far models of interaction such as competitive interaction have been formulated only either as behavioral effects or density-dependent effects of resource depletion. For instance, behavioral ecology focuses on the effects of behavioral interaction on individual fitness (e.g. Krebs and Davis, 1991). On the other hand, population ecology focuses on the density-dependent effects on reproductive rates and mortalities of a population, often assuming that the effects of density is homogeneous for all the individuals (Lotoka, 1932; May, 1977; Roughgarden, 1979; criticism see Lomnicki, 1988). Most of the models for population change do not explicitly separate the effects of direct and indirect competition.

Animals will have simultaneous both effects of behavioral interaction and the effect of resource depletion and environments that are changed by the animals. In addition, members of groups within which individuals interact directly and those interact indirectly may differ since individuals, for instance, interact behaviorally with neighboring individual, while individuals may interact for nutrient resources at larger scale. Thus it is important to recognize different interactions and the relative roles of the effects of these interaction.

Pulmonate freshwater snails may compete for periphyton (Osenberg 1989), since grazing by snails reduces the amount of periphyton (Bronmark et al., 1991; Brown 1982; McCormick and Stevenson, 1991; McMahon, 1983). Kawata and Inaba (1992) showed that when four individuals of *Physa acuta* were placed in an experimental box with a small sized food patch, that there are significant differences in the number of "stays" on the food patch among individual snails. This suggests that individuals compete for food patches. In addition to competitive interaction, snails interact through non-food substances dissolved in water. For instance in *Biomphalaria glabrata*, media conditioned by snails contained factors that enhanced the growth of conspecific snails (Thomas et al., 1975; Thomas, 1982; Thomas and Aram, 1974; Thomas and Behjamin, 1974). Thomas and Behjamin (1974) found that *Biomphalaria glabrata* grow more rapidly in water conditioned by higher densities of conspecific snails than in water conditioned by lower densities. Thus freshwater snails may be suitable animals for observing the effects of direct and indirect interaction through water-dissolved substances.

The purpose of this paper is to separate the effects of direct and indirect interaction among conspecific individuals, and to identify the relative roles of these effects in laboratory populations of the freshwater snail, *Physa acuta*.

Materials and Methods

Laboratory Experiments

The *Physa acuta* used in the experiments are F_1 offspring whose mothers were collected from Asabata marsh in Shizuoka City, Japan in 1991. Egg capsules laid by collected snails were isolated in 200 ml plastic beakers until they hatched. Ten days after hatching, juvenile snails were kept individually in polystyrene bottles filled with 15 ml of reconstituted water (pH=7.4, $[Ca^{++}]=80$ ppm; Lam and Calow 1988), which is prepared as follows: Twenty-five ml of each of the following four solutions were mixed and the total volume was made up to 1 liter with distilled water: (1) 11.76 g of $CaCl_2 \cdot 2H_2O$/ 1 liter distilled water, (2) 4.93 g of $MgSO_4 \cdot 7H_2O$/ 1 liter distilled water, (3) 2.59 g of $NaHCO_3$/ 1 liter distilled water and (4) 0.23 g of KCl/ 1 liter distilled water. The snails were fed with 0.5 cm $\times$ 0.5 cm squares of lettuce.

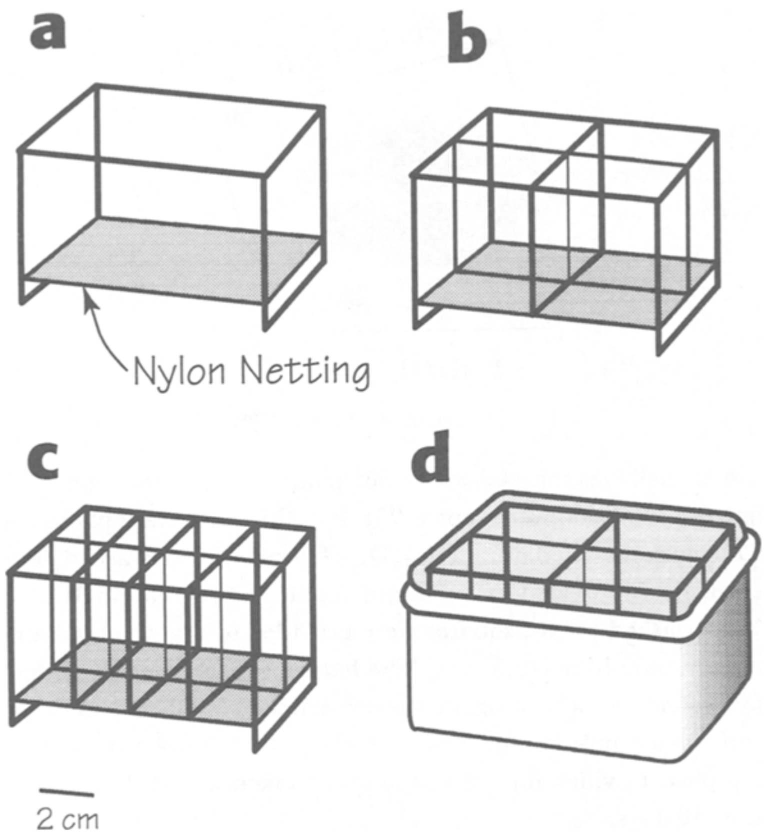

Fig. 1. Experimental boxes. Experimental boxes are divided by acrylic plates into one (a), four (b), and eight (c) compartments. The bottoms of the boxes are made from nylon netting through which water can flow but juvenile snails cannot pass. The boxes are placed in the plastic cases with 100 ml of reconstituted water.

Experimental boxes were divided by acrylic plates into one, four and eight compartments (Fig. 1a, 1b and 1c). The box bottoms were made from nylon netting through which water can flow but juvenile snails cannot pass (Fig. 2). The experimental boxes were placed in plastic cases with 100 ml of reconstituted water (Fig. 1d). Five experimental conditions were designed: (1D) Only one individual was placed in one of four compartments of a box (Fig. 1b); (4D) one individual was placed in each of four compartments of a box (Fig. 1b) and thus four individuals in a box cannot interact directly with each other but interact through water; (4UD) Four individuals were placed in a non-divided box (Fig. 1a) and thus individuals can directly interact; (8D) an individual was placed in each of eight compartments of a box (Fig. 1c) and thus there are eight individuals without direct interaction in a box; (8UD) Eight individuals were placed in a non-divided box (Fig. 1a).

Juvenile snails (10-day-old) from the same egg capsules were randomly chosen and allocated to two or three of the above five experimental conditions. 409 individuals from 50 egg capsules taken from 25 mothers were used. For 4UD and

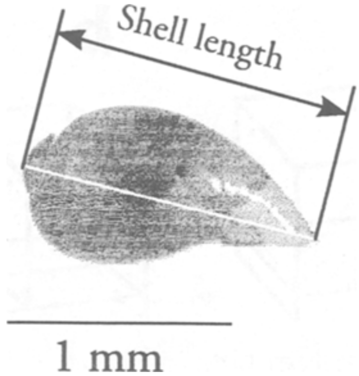

Fig. 2. Shell length of 10-day-old *Physa acuta*.

8UD, each snail shell was marked with color paints for identification. The snails in each experimental condition were kept at 27±0.2°C in a constant photo period of 12 h light and 12 h dark for 10 days. For 1D, 4D and 8D, a washed lettuce square (0.5 cm×0.5 cm) was provided for each individual. For 4UD and 8UD, 1 cm×1cm and 1 cm×2cm washed lettuce squares were provided so that the total amount of the lettuce per snail was 0.5 cm×0.5 cm. The lettuce squares were replaced with fresh lettuce 5 days after the beginning of the experiments. Shell length (Fig. 2) was measured with image processing software (Image 1.41, public domain software for Macintosh) applied to video images of the snails taken as PICT. Shell growth was measured after 10 days.

Statistical Analysis

The average growth in each experimental treatment was estimated using all the data that were obtained from the number of individuals and the numbers of egg capsules shown in Table 1. The difference in growth between different experimental conditions was compared using two-way ANOVA, since there was significant variation in growth among egg capsules (Kawata and Ishigami 1992). Thus the growth comparisons were made only between experimental treatments utilizing the same egg capsules. Under rigorous statistical testing, simultaneous or multiple comparison should be conducted to test heterogeneity of means among the treatments (i.e. 1D, 4D, 4UD, 8D and 8UD) before the comparisons between two treatments. In this experiment, however, simultaneous multiple comparison could not be conducted since there was an insufficient number of individuals from single egg capsules to allocate into all the treatments. Thus only pair-wise comparisons were conducted, and significance levels were adjusted by using the sequential Bonferroni method to reduce the type-I error rate (Rice 1989). Four comparisons (1D vs. 4D, 4D vs. 4UD, 4D vs. 8D and 8D vs. 8UD) were conducted. The growth data were transformed with Cox-Box transformation prior to the analysis ($\lambda=0.42122$, Sokal and Rohlf, 1981). After transformation, the homogeneity of variance is met among treatments (log

Table 1. The average growth in different experimental box.

	1D	4D	4UD	8D	8UD
Average growth	0.23	0.34	0.17	0.29	0.14
Standard error	0.021	0.020	0.026	0.016	0.16
No. egg capsules†	24	34	16	15	8
No. of individuals	73	134	60	108	57

† Egg capsules for 1D were obtained from 20 mothers. The numbers of individuals from the same egg capsules are as follows; 4 individuals = 10 cases; 3 individuals = 8 cases; two individuals = 3 cases; one individual = 3 cases. Egg capsules were obtained from 21 mothers for 4D. In 10 cases one individual died during the experiment. Egg capsules for 4UD were obtained from 12 mothers. In 4 cases one individual was dead during the experiments. Egg capsules for 8D were obtained from 8 mothers. In 8 cases one individual was dead and in two cases two individuals were dead during the experiments. Egg capsules for 8UD were obtained from 5 mothers. In 2 cases one individual died and in 2 cases two individuals were dead during the experiments.

anova, $F_{4,42}=0.77$, $P>0.05$) and among egg capsules ($F_{3,78}=0.91$, $P>0.05$).

Results

Table 1 shows the average growth for different experimental condition. Table 2 shows the resulting ANOVA table for comparison between different experimental

Table 2. Two-way ANOVA table for comparison between the treatments. Data were transformed with Cox-Box transformation. Significance levels, a, were adjusted by using sequential Bonferroni test (Rice 1989).

Source:	df	Sum of Squares:	Mean Square:	Fs:	P value:	Adjusted P value
a Comparison 1D and 4D*						
1D vs. 4D	1	0.979	0.979	5.41	0.022	.044
Among egg capsules	20	13.717	0.686	3.791	0.0001	.0002
interaction	20	1.695	0.085	0.468	0.9729	.9672
Error	104	18.817	0.181			
b Comparison 4D and 4UD						
4D vs. 4UD	1	4.679	4.679	31.52	0.0001	.0003
Among egg capsules	11	13.219	1.202	8.095	0.0001	.0003
interaction	11	5.783	0.526	3.542	0.0007	.0021
Error	63	9.352	0.148			
c Comparison 4D and 8D						
4D vs. 8D	1	0.375	0.375	2.637	0.1088	.1088
Among egg capsules	7	1.665	0.238	1.672	0.1297	.1297
interaction	7	2.633	0.376	2.644	0.1297	.1297
Error	72	10.243	0.142			
d Comparison 8D and 8UD						
8D vs. 8UD	1	4.887	4.887	40.601	0.0001	.004
Among egg capsules	7	4.283	0.612	5.082	0.0001	.0004
interaction	7	4.186	0.598	4.967	0.0001	.0004
Error	100	12.038	0.12			

conditions. The growth of 4D was significantly greater than that of 1D ($P = .02$) and 4UD ($P < .001$, Table 2 a and b). The growth in 4D was greater than 8D, although the difference was not significant (Table 2c). The growth of 8D was significantly greater than 8UD ($P < .001$, Table 2d). In all of the comparisons there is significant variation among egg capsules. The effects of interaction factors were significant except in comparison between 1D and 4D (Table 2).

Discussion

In the present study, I create experimental situations in which individuals grow with different interaction; (1) an individual do not interact with any other individuals (1D), (2) individuals can interact through water but they cannot interact directly (4D and 8D) and (3) individual can interact directly and indirectly (4UD and 8UD). The growth of 4D and 8D was greater than that of 1D and the difference between 4D and 1D was significant. This indicates that interaction through water has positive effect on the growth. The growth of 4UD and 8UD was smaller than those of 1D, 4D and 8D, which indicates that direct interaction for food have negative effect on the growth and the effects of direct interaction is greater than those of indirect interaction.

In all comparisons, there were significant differences in growth among egg capsules. Using two-level nested ANOVA for the growth of individuals, preliminary analysis was conducted to examine whether the difference in growth among egg capsules is due to added variance among mothers, among experimental cases, or among egg capsules from the same mother 1D. There are significant differences among egg capsules from the same mother ($P < .01$, $F_{10\,35} = 3.144$) but there are no differences among mothers ($P > .05$, $F_{5,10} = .364$). Although this analysis requires a larger sampling size, the result indicates that the differences among egg capsules are not due to differences among mothers (e.g. maternal effects and genetic differences), but due to differences in egg capsules, such as the number and size of eggs in the capsules. Furthermore, this result shows that differences among egg capsules are not merely due to difference among experimental boxes.

The effect of indirect interaction may include the effects of indirect competition (i.e. Exploitation of a resource by one individual that reduces the availability of that resource to others, Ricklefs, 1990) and interaction through water-born substances. Several studies showed that snails interact through non-food-substances dissolved in water, for instance in *Biomphalaria glabrata*, media conditioned by snails contained factors that enhanced the growth of conspecific snails. Thomas and Behjamin (1974) found that *Biomphalaria glabrata* grows more rapidly in water conditioned by higher rather than lower densities of conspecific snails. High densities cause a shortage of calcium that retards the growth of snails (McMahon, 1983; Thomas, et al., 1975; Thomas, 1982, Thomas and Aram, 1974, Thomas and Behjamin, 1974). Similarly, in the present experiments, growth may be accelerated by water-born substances in 4D and 8D, but the slightly smaller growth results in 8D indicate the effects of depletion of resources such as calcium.

Several aspects of ecological significance of the positive effects may be considered. First, if individual snails have higher fitness at moderate densities (the Allee effect) especially in new habitats in which snails immigrated, the positive effects is advantageous to the individual snails (Thomas and Behjamin, 1974). Second, the effects has no adaptive significance, but it may be a side effect of other physiological mechanisms.

Kawata and Ishigami (1992) found no effect of water conditioned by adult *Physa acuta* on juvenile snails. However, the present study showed the positive effects on the growth of juvenile snails through water in which other juvenile snails exit. The different results may be because water conditioned by unrelated adults have no effects on the growth. The further studies are needed to explain the difference in these results.

In examples of direct interaction, competitive interaction among individuals affects the growth of snails. Kawata and Inaba (1992) showed that when the size of food patches is small, there are significant difference in the frequency of access to food patches. Unfortunately, the present studies show only the average effects of direct interaction. If we can identify individual properties affecting competition, we can estimate the relative effects of individual properties and group properties (e.g. densities and the frequency of individual properties) by using contextual analysis (Damuth and Heisler, 1988; Heisler and Damuth, 1987).

The purpose of this study is to analyze the relative effects of different kinds of interaction in a model system. As the effects of interaction in the field may be more complicated, the model system may not reflect real conditions in the fields. *Physa acuta* usually eat periphytic algae and detritus. In addition, this experiment was conducted in very small closed boxes. However, the purpose of this study is not to estimate the degree of interaction in the fields but to separate the effect of direct and indirect interaction among individuals in a model situation. The present study indicates that the effect of direct behavioral interaction and indirect interaction through altering substances dissolved in water were different, and the relative importance of the effects will be estimated when the supposed factors affecting interactions are controlled. The kinds of interaction are sometimes identified by whether the effects of interactions are positive or negative (Abrams, 1987; Odum, 1971). For instance, competitive interactions results in negative effects among individuals. However, the effect of interaction should be divided into the component effects resulting from different kinds of interaction.

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巻貝個体の間でみられる直接的相互作用と間接的相互作用の相対的重要性

河 田 雅 圭

生物個体は，行動を通じて直接的に相互作用するとともに，資源の消費や環境の変化を通じて間接的に相互作用をしている．これらの相互作用が個体の成長や繁殖に及ぼす影響は，相互作用の種類によって異なっていると思われる．淡水性巻貝は餌資源をめぐって直接的に相互作用をし，さらに水溶性の物質を介して間接的に相互作用をしていると思われる．私は，淡水性巻貝，*Phya acuta*，の実験集団をもちい，直接的相互作用と水を介しての間接的相互作用の効果を区別，分離した．行動による直接的相互作用は成長に対し負の影響を与えるが，水環境を通じての間接的相互作用は成長に対し，正の影響を与えることが分かった．さらに，異なる種類の相互作用を区別する重要性について論議した．