

Wall-making Behavior as a Proximate Mechanism to
Generate Variation in Larval Competition in
Callosobruchus maculatus (Coleoptera: Bruchidae)

Hiroyuki Mano, Yukihiro Toquenaga

Division of Integrative Environmental Sciences, University of Tsukuba
Tsukuba City, Japan

Running Head:

Proximate Mechanism of Larval Competition

Corresponding Author:

Hiroyuki Mano

Correspondence:

Mano H

Division of Integrative Environmental Sciences, University of Tsukuba
1-1-1, Ten-Nou-Dai, Tsukuba City, Ibaraki 305-8572, Japan

Tel. / fax:

+81-298-53-7491

e-mail:

mano@pe.ies.life.tsukuba.ac.jp

Key words:

Bruchidae, competition strategy, contest competition, exploitation,
interference, scramble competition, stored pest

Abstract

Variation from contest to scramble in larval competition types was observed among laboratory lines derived from a geographic strain of *Callosobruchus maculatus*. In contest competition, only one adult can emerge from a small bean because the successful larva monopolizes resources. In scramble competition, however, multiple adults can emerge from the bean because larvae share resources. To explain the variation in competition types, we used six lines of the geographic strain to test the hypothesis that the larval competition type is determined by the larval behavior of building walls, which prevent larvae from interfering with each other, allowing multiple adults to emerge from a single bean. We also investigated the proportions of wall-making in contest-scramble hybrid lines to test whether the formation of a wall structure was genetically determined. Results support our hypothesis that wall-making behavior determines the type of larval competition within a geographic strain, and that the behavior is genetically determined. Scramble-type lines exhibited higher frequencies of wall-making than contest-type lines when two larvae of the same line infested a bean. Larval competition type and the tendency towards wall formation in contest-scramble hybrid lines ranged intermediate of parental lines. We concluded that the variation in larval competition type is determined by the variation in larval wall-making behavior among laboratory lines derived from the geographic strain. We will discuss the evolution of scramble-type larvae in *C. maculatus* based on our results.

Introduction

Resource competition has evolutionary and ecological effects on competing individuals. For example, competition affects population dynamics and the evolution of life history traits (Godfray 1987; Lomnicki 1988; Ives 1989). The effects of resource competition differ in various types of competition. Two extreme types of competition, contest and scramble, were characterized by resource monopolization and resource sharing based on Nicholson's dichotomy (Nicholson 1954; Lomnicki 1988). In pure contest competition, individual resource intake and the number of survivors remain constant irrespective of the increase of the number of competitors because of resource monopolization by dominant competitors. In pure scramble competition, resource sharing decreases individual resource intake as the number of competitors increases. The abrupt changes from complete survival to no survival arise when resources for survival become no longer enough. Investigating mechanisms producing variations in competition type would help us understand consequences of competition.

How competitors partition limited resources is often determined by individual traits such as aggressive behavior towards conspecifics or any behavioral, physiological or morphological traits that regulate aggressiveness (Toquenaga and Fujii 1990; Boivin and van Baaren 2000). The evolution of these traits can change the type of competition, and thus cause variations in competition type both among related species and within a species. Such variations are observed in the larval competition of bean weevils and

parasitoid wasps (e.g. Toquenaga 1990; Brodeur and Boivin 2004).

Evolutionary causes and conditions for evolutionary changes of larval competition type have been examined to understand the variations in the larval competition type (Smith and Lessells 1985; Godfray 1987).

The conditions for evolutionary change of competition type can depend on what types of individual traits determine the type of competition. In parasitoid wasps, for example, the conditions for scramble-type larvae to evolve in a population of contest-type larvae differ between two following behavioral mechanisms that determine the larval competition type: the presence or absence of interference behavior, and the difference in the degree of mobility (Pexton and Mayhew 2001; Brodeur and Boivin 2004). When the competition type was determined by whether larvae exhibited interference behavior, scramble-type larvae do not fight and always lose in the presence of contest-type larvae. This proximal mechanism provides stringent conditions for the evolution of scramble-type larvae in a contest-type population (Godfray 1987; Rosenheim 1993). In contrast, when the competition type is determined by whether larvae are mobile or immobile with fighting ability, scramble-type larvae can retain the ability to defend themselves against contest-type larvae (Boivin and van Baaren 2000). The proximal mechanism based on larval mobility relaxes the strict conditions for the spread of scramble-type larvae (Pexton et al. 2003). Investigating the proximal mechanisms behind larval competition may provide an insight into conditions for evolutionary change of competition type.

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) exhibits the

genetic variation of larval competition from contest to scramble among geographic strains (Toquenaga and Fujii 1991; Takano et al. 2001). Multiple larvae in a host bean cause larval competition. In contest competition, only one adult can emerge when multiple larvae exist in the same small bean. In scramble competition, several adults with small bodies relative to those without competition can emerge from a bean with multiple larvae. Previous experimental studies have demonstrated that bean size is an important selection pressure for larvae that exhibit each type of larval competition in *C. maculatus* (Tuda 1998; Takano et al. 2001; Messina 2004). Small beans favor larvae that present contest-type competition. In contrast, large beans favor larvae that exhibit scramble-type competition. The condition for evolutionary changes of larval competition type owing to bean size, however, remain poorly understood because the proximal mechanisms that produce the variation in larval competition of *Callosobruchus maculatus* have not been identified.

Two behavioral mechanisms that determine larval competition type have been proposed to explain the variation in *C. maculatus*. First, interference behavior may determine competition type (Smith and Lessells 1985; Smith 1990; Tuda and Iwasa 1998). Second, the larval propensity for feeding on the center of a bean may determine the frequency that larvae encounter each other in the bean, and thus larval competition type (Toquenaga 1993; Guedes et al. 2003). Contest-type larvae frequently interfere with each other because they tend to feed on the center of a bean. In contrast, scramble-type larvae encounter each other less frequently because they tend to feed on the

periphery of a bean, as shown in the related species *C. phaseoli* (Ike 1984).

Nevertheless, the behavioral mechanisms described above have not been supported in previous studies. *C. maculatus* larvae have interference ability irrespective of larval competition type (Toquenaga 1993). Thanthianga and Mitchell (1987) provided evidence that contest-type larvae tend to feed on the center of a bean. However, there is no clear evidence in previous experimental studies that scramble-type larvae feed on the periphery of a bean. In larval competition between contest- and scramble-type geographic strains, co-emergence of adults of both strains was proportionately low in a large bean (Messina 1991). The result indicates that scramble-type larvae may have no tendency of feeding on the periphery of a bean. Previous theoretical studies have predicted conditions for the evolutionary change of larval competition type owing to bean size in *C. maculatus*, based on interference behavior (Smith and Lessells 1985), or both interference behavior and feeding tendency (Smith 1990; Toquenaga et al. 1994; Tuda and Iwasa 1998). The theoretical models based on unrealistic behavioral mechanisms may have inaccurately predicted the conditions for evolutionary change of larval competition type in *C. maculatus*. Investigating the proximal mechanisms behind larval competition may provide an insight into the variation in the larval competition of *C. maculatus*.

The variation in larval competition type of *C. maculatus* could be caused by the difference in larval behavior to avoid interference after an encounter. *C. maculatus* larvae retain interference ability irrespective of their competition type (Toquenaga 1993). Moreover, there is no clear evidence

that scramble-type larvae avoid interference competition by feeding on the periphery of a bean before they encounter each other. Mitchell (1990) reported larval behavior to avoid interference in the IITA strain from Nigeria:

When larval burrows intersect, the larvae retreat to the rear of their burrows. They gather up fass, push it to the intersection and build a wall between the burrows. Beans opened after the intersected burrows are closed usually show larvae burrowing side by side in the same direction. This cooperative use of space in a bean allows more larvae to survive than would be possible if burrows were cut in a haphazard way.

Larval wall-making behavior could explain variations in the type of larval competition. Based on the behavioral mechanism described by Mitchell (1990), we propose the hypothesis that this wall-making behavior determines larval competition type. This hypothesis predicts the negative relationship between the proportion of wall-making and the rate of contest-type competition among populations that differ in the type of larval competition. Previous studies have shown that artificial selection can influence competition type in *C. maculatus* (e.g., Takano et al. 2001). Therefore, if the wall-making behavior is a proximate mechanism to determine the larval-competition type, it is likely genetically determined.

In this paper, we used laboratory lines derived from a scramble-type geographic strain (hQ strain), in which variations in competition type have been observed, to test our hypothesis. The variation among the laboratory

lines were caused by evolutionary changes, in the form of larval competition shifting from scramble- to contest-type through a host shift from large *V. angularis* bean to small *V. radiata* bean (Tuda 1998). In a previous study, we found that larvae of a mutant line of the hQ strain exhibited extreme contest-type competition (Takano et al. 2001). Moreover, another line of the hQ strain exhibited scramble-type competition through wall-making behavior (Mano and Toquenaga in preparation). Therefore, using laboratory lines of the hQ strain is appropriate for testing our hypothesis. We compared the frequencies of wall making inside a bean with two hatched eggs of the same line among six lines exhibiting different types of larval competition to examine whether the proportion of wall-making increased with a decreasing rate of contest-type competition. We also investigated the proportions of wall-making in contest-scramble hybrid lines to test whether wall-making behavior is genetically determined.

Materials and Methods

Life history of *C. maculatus*

C. maculatus is a major pest of cultivated legume seeds (Southgate 1979; Jackai and Daoust 1986). After hatching on the surface of bean pods or of stored dry beans, the larvae complete their entire development and pupation within beans; adults emerge from the beans.

Experimental lines

We used the hQ strain in the present study. The base line of the hQ strain of *C. maculatus* was established in Japan in 1987 from infested *Vicia faba* (L.) (Fabales: Fabaceae) imported from New Zealand (M. Shimada, personal communication). In the present experiments, we used six lines (MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2), which were derived from a single base line of the hQ strain (Fig. 1). These lines were maintained in different ways, and consequently varied in larval competition type. Fig. 1

The MS1-3 and MS1-5 lines were established from a base line of the hQ strain that was transferred from the University of Tokyo to our laboratory at the University of Tsukuba in 1996. The MS1-3 line was established with over 100 adults, and was maintained on *Vigna angularis* (Willdenow) Ohwi et Ohashi beans until 2002. Since then, it has been maintained on medium *V. radiata* (L.) Wilczek beans (larger than No. 6 mesh but smaller than No. 5 mesh, $3.36 \text{ mm} < \text{aperture} < 4.00 \text{ mm}$). The MS1-5 line was established in 1996 by allowing one mated female to lay eggs on *V. angularis* beans. It has

since been maintained on *V. angularis* beans.

The MS1-6 line was established with an experiment on artificial selection for contest-type larvae. In the selection experiment, mated females were allowed to lay eggs on large *V. radiata* beans (larger than No. 5 mesh, aperture > 4.00 mm). Beans with two hatched eggs were placed into the wells of 48-well plates (Nuncclon, Roskilde, Denmark). Adults that emerged singly from large *V. radiata* beans with two hatched eggs were collected and then introduced into a Petri dish (90 mm in diameter, 20 mm in height) containing a layer of large *V. radiata* beans in order to produce beans with two hatched-eggs for the next generation. In March 1997, the first generation was derived from three pairs that had been produced after two generations of inbreeding between full sibs. This procedure was continued for seven generations in order to conduct a strong selection for contest-type larvae. This line has been maintained on large *V. radiata* beans since November 1997 when the selection process ended.

The T1-2, T2-1, and T3-2 lines were derived from experimental lines established by M. Tuda (Tuda 1998). The base line of these lines was established in 1992 and maintained on *V. angularis* beans, as described by Tuda (Tuda 1998). The T1-2 line was set up with less than 30 females from a line that was established with less than 40 adults. It has been maintained on *V. angularis* beans since 1997. The T2-1 line was obtained from one of two experimental lines, which were selected and maintained on medium *V. radiata* beans by Tuda (1998). It was set up with less than 40 adults and has been maintained on *V. radiata* beans since 1997. The T3-2 line was derived

from the other of the two experimental lines. It was established with a black-body adult. The genetic mechanism of the black color is not Mendelian. At first only a small proportion of the offspring of black parents were black. So we consecutively selected black individuals by crossing black parents over several generations, and fixed the black morph in a line. After fixation, we maintained the line with medium *V. radiata* beans. Previous studies have shown that the black line exhibits extremely contest-like competition (the hQ black strain in Takano et al. 2001).

For the present study, in all experiments females that emerged from stock cultures were used for oviposition within 48 hours. Large *V. radiata* beans (79.0 ± 1.2 mg [mean \pm SE], $n = 62$) were used for larval resource in all experiments. The beans were large enough for two larvae to complete their development if the two larvae were free from interference with each other. Thus, it would be much less likely that all of two larvae die as a consequence of shortage of resources. We maintained the culture of stocks and conducted all experiments at 30°C, 50 \pm 5% r.h. and 24 L- 0 D. Stock cultures in Petri dishes with a layer of beans were established every third or fourth day for approximately six months before the start of our experiments. Generations were renewed every 25- 30 days. We reared MS1-5 and T1-2 stock cultures on medium *V. radiata* beans (60.6 ± 0.71 mg, $n = 56$), MS1-6 stock cultures on large *V. radiata* beans, and T2-1 and T3-2 stock cultures on *V. angularis* beans (203.2 ± 7.1 mg, $n = 30$). *V. angularis* beans are much larger than large and medium *V. radiata* beans.

Larval competition type

We first examined larval competition types in the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines. We also examined the competition types of four hybrid lines: MS1-5 male \times MS1-6 female (F1), MS1-5 female \times MS1-6 male (F1R), MS1-5 male \times T3-2 female (F1), and MS1-5 female \times T3-2 male (F1R). To examine the larval competition type of each line, the patterns of adult emergences from beans with one hatched egg and those with two hatched eggs were investigated in each line. The data of beans with two eggs was used to calculate the observed proportion of the number of emerged adults. We cannot simply identify the type of larval competition by investigating only the pattern of adult emergences from beans with two hatched eggs because the adult emergence pattern depends on both the type of larval competition and the natural mortality independent of the larval competition. We should consider pre-competition natural mortality for examining larval competition type. Therefore, the pattern of adult emergence from beans with one hatched egg was used to estimate pre-competition natural mortality.

We collected beans bearing one and two hatched eggs from each of the six lines and the four hybrid lines. For the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines, over 30 stock females from each line were allowed to lay eggs, for less than two hours, on a layer of beans in a ventilated glass dish (120 mm in diameter, 30 mm in height). Twenty-five days after oviposition, over 20 virgin adults of each sex from each parental line, for each reciprocal cross

were obtained from isolated beans with one hatched egg. Virgin females were allowed to mate with males from the opposite line in Petri dishes for 24 hours. We allowed mated females to lay eggs on a layer of beans in a ventilated glass dish for less than two hours. Seven days after oviposition, beans with one and two hatched eggs from each line were collected and placed individually into 48-well plates. On the 60th day after oviposition, the number and sex of adults that emerged from each bean was recorded.

States inside beans

The states inside beans with two hatched eggs were investigated to examine the frequency with which a wall structure was formed in a bean where two larvae had encountered each other in ten treatments. Six treatments for the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines examined states inside beans with two larvae from the same line. Four treatments (two lines from the reciprocal crosses between the MS1-5 \times MS1-6 lines and two from the reciprocal crosses between the MS1-5 \times T3-2 lines) examined states inside beans with two hybrid larvae derived from the same reciprocal cross.

Beans with two hatched eggs from each treatment were collected as they had been in examining the type of larval competition. Preliminary observations showed that larvae started to pupate by the 18th day after oviposition in the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines. We, therefore, dissected beans with two hatched eggs on the 18th day after oviposition, and checked the state of larval competition.

When one individual survived in a bean, we investigated whether the

deaths of the other larvae were caused by interference. The fate of dead larvae was easily determined, as the surviving individual crushes the loser in an interference interaction. When larvae died within a bean for reasons other than interference interaction, the data was eliminated from the analysis. After the elimination, the beans with two hatched eggs were divided into three groups, based on the number of larvae and the formation of walls:

Group 1: Two surviving individuals that did not encounter each other

Group 2: One surviving individual without wall formations

Group 3: Two surviving individuals with a wall structure

In groups 2 and 3, two larvae encountered each other within a bean. We observed that all beans where two individuals encountered each other and survived had a wall structure.

Statistical analysis

To examine the larval competition type of each line independently of pre-competition natural mortality, we estimated a C-value based on the patterns of adult emergences from beans with one hatched egg and those with two hatched eggs (Takano et al. 2001). C-value is defined as the proportion of larvae that exhibit interference behavior in a bean with two hatched eggs to larvae within a population. C-value ranges from 0 to 1. A C-value of 0 indicates that all larvae in a population exhibit scramble-type behavior. In contrast, a C-value of 1 indicates that all larvae in a population exhibit contest-type behavior.

The following assumptions were made to estimate C-value: (1) a bean is large enough to sustain two larvae; (2) when both larvae are scramble-type, two adults emerge; (3) if one larva is contest-type, only one adult emerges, as the contest-type larva always kills the other; and (4) if both larvae are contest-type, the larvae will attack each other until one survivor eventually emerges. Further, it was assumed that natural mortality occurs before competition begins, and that males and females have the same pre-competition mortality: μ . Thus, a single bean with two hatched eggs has six possible patterns of emergence: none (N), one male (M), one female (F), two males (MM), two females (FF), and one male and one female (MF). The proportion of males is m , and the C-values of males and females are p and q , respectively. The expected frequencies of the six emergence patterns can be expressed as follows:

$$N = \mu^2 \tag{1}$$

$$M = (1 - \mu)^2 mp(2 - q - mp + mq) + 2\mu(1 - \mu)m \tag{2}$$

$$F = (1 - \mu)^2(1 - m)q(2 - q - mp + mq) + 2\mu(1 - \mu)(1 - m) \tag{3}$$

$$MM = (1 - \mu)^2 m^2(1 - p)^2 \tag{4}$$

$$FF = (1 - \mu)^2(1 - m)^2(1 - q)^2 \tag{5}$$

$$MF = 2(1 - \mu)^2 m(1 - m)(1 - p)(1 - q). \tag{6}$$

Note that $N + M + F + MM + FF + MF = 1$. There are four models for C-value estimation:

1. Estimating μ , m , p , and q , respectively.

2. Estimating μ , m , and p , assuming $p = q$.
3. Estimating m , p , and q , using fixed μ estimated from the proportion of adults that emerged from beans with a single hatched egg.
4. Estimating m and p , assuming $p = q$ with fixed μ estimated from the proportion of adults that emerged from beans with a single hatched egg.

The parameters of each model were estimated using a maximum likelihood method. Akaike's Information Criterion (AIC) was used to compare each model. We adopted the model with the lowest AIC score as the most parsimonious. AIC's and parameters were calculated with *stlga*, which is a general purpose genetic algorithm for obtaining AIC and parameter values for specified alternative models. The program is written in C++ and is available from YT.

To examine differences in the proportion of beans where two larvae encounter each other, pairwise comparisons of the frequencies of group 1 and the sum of groups 2 and 3 were performed for the six treatments of the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines using Fisher's exact tests. We also used pair-wise comparisons of Fisher's exact tests to investigate the differences between the MS1-5, MS1-6 and two reciprocal hybrid lines, and those between the MS1-5, T3-2 and two reciprocal hybrid lines. To control type-I errors, we applied sequential Bonferroni corrections ($\alpha = 0.05$) to the results from each set of pair-wise comparisons (Sokal and Rohlf 1995). Then, we examined differences in the proportion of beans with a wall structure in comparison to beans where two larvae encountered each other. Fisher's exact

tests with sequential Bonferroni corrections ($\alpha = 0.05$) were conducted for pair-wise comparisons of frequencies of group 2 and group 3 between the six treatments of the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines. We also used pair-wise comparisons of Fisher's exact tests to investigate the differences between the MS1-5, MS1-6 and two reciprocal hybrid lines, and those between the MS1-5, T3-2 and two reciprocal hybrid lines. To examine the association between larval competition type and the proportion of beans with two survivors separated by a wall structure, we used a Spearman rank correlation test across six treatments of the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines. The average of male and female C-values weighted by the proportion of males was used as an index of larval competition type. All statistical analyses other than calculating C-values were performed using R 2.3.0 (R Development Core Team 2006).

Results

Larval competition type

Estimated m and μ parameters for adult emergences are shown in Table 1. Table 1
The proportion of males, m , among the ten experimental lines ranged from 1
0.442 to 0.552. Estimated rate of mortality, μ , indicates that a small
proportion of individuals in each line die before adult emergence. C-values
for both sexes varied among the ten lines (Fig. 2). The MS1-5 and T3-2 lines Fig. 2
exhibited the lowest and highest C-values, respectively, for both sexes (Fig.
2a). This indicates that the proportion of larvae exhibiting interference
behavior in a population varies among the ten lines. The four hybrid lines
exhibited intermediate C-values, falling between those of parental lines. This
indicates that the frequency of larvae exhibiting interference behavior in a
population is genetically determined. The patterns of C-values in the two
crosses appeared to be linear, although the C-values of hybrid lines were
lower than the mean values of the parents (Fig. 2b, c). Females exhibited
higher C-value than males in the MS1-5, T1-2, and four hybrid lines.

States inside beans

Figure 3 shows the proportions of beans where two larvae encountered each other by the 18 day after oviposition in ten treatments. Two larvae Fig. 3
encountered each other in a high percentage (86.5 % - 100 %) of beans in all
treatments. Two survivors that had not encountered each other had started
to pupate in seven treatments other than those of the MS1-3, MS1-6 and

T2-1 lines. One of two survivors that had not encountered each other was a fourth-instar larva in 0.022 % of 91 beans in the MS1-3 line, and 0.014 % of 147 beans in the MS1-6 line. In the T2-1 line, pairs of fourth-stage larvae were observed in 0.042 % of 95 beans. In the MS1-3, MS1-6, and T2-1 lines, fourth-instar larvae were observed in a very low proportion of beans where two survivors had not encountered one another.

The MS1-5 and T1-2 lines differed significantly from the MS1-6 and T3-2 lines in the frequency with which two larvae encountered each other (Fig. 3a). These results indicate that the MS1-5 and T1-2 lines had a lower frequency of two-larvae encounters than did the MS1-6 and T3-2 lines. A significantly lower frequency of two-larvae encounters was observed in the two reciprocal MS1-5 and MS1-6 hybrid lines than in the MS1-6 line (Fig. 3b). A significantly lower frequency of two-larvae encounters was also observed in the two reciprocal MS1-5 and T3-2 hybrid lines than in the T3-2 line (Fig. 3c).

Figure 4a shows the proportions, for the six pure lines, of beans with one individual survivor and with two individual survivors separated by a wall structure to beans where two larvae encountered each other. The bar graphs correspond to C-values, in descending order (Fig. 2a). The multiple comparisons showed that the six lines could be classified into three groups: (1) The MS1-5 and T1-2 lines; (2) The MS1-3, MS1-6, and T2-1 lines; (3) The T3-2 line. Across six treatments of the MS1-3, MS1-5, MS1-6, T1-2, T2-1, and T3-2 lines, we detected a strong, negative correlation between the weighted average C-value and the proportion of beans with two individuals separated by a wall structure (Spearman rank correlation: $r_s = -0.976$, P

< 0.001). This indicates that proportions of larvae exhibiting interference behavior decrease as proportions of beans with two surviving individuals separated by a wall structure are likely to increase.

Figures 4b, c show the proportions of one individual survivor and two individual survivors separated by a wall structure in beans where two larvae encountered each other in the MS1-5 and MS1-6, and MS1-5 and T3-2 cross lines. The frequencies of two surviving individuals separated by a wall structure in hybrid lines were intermediate, falling between those of parental lines. No significant differences in the frequency of two surviving individuals separated by a wall structure were detected between the two reciprocal hybrid lines in either of the two crosses. The frequencies of the two reciprocal MS1-5 and MS1-6 hybrid lines appeared to be larger than frequencies of the two reciprocal MS1-5 and T3-2 hybrid lines.

Discussion

Our results support the hypothesis that the larval behavior of making walls determines the type of larval competition within the hQ strain of *C. maculatus*. The variation in larval competition type within the hQ strain was observed in terms of C-value, which indicates the frequency of larvae exhibiting interference behavior in a population (Fig. 2). Two reciprocal crosses showed that the larval competition type is genetically determined (Fig. 2a, b), as shown in previous studies (Takano et al. 2001). Our results support the two predictions in our hypothesis. The frequency of the formation of a wall structure increases with decreasing C-values (Figs. 2a and 4a). The proportions of wall-making behavior in the hybrid lines had intermediate values, falling between those of parental lines (Fig. 4b, c). These results indicate that the formation of a wall structure is genetically determined. Therefore, the variation in larval competition type can be determined by the variation in larval wall-making behavior among laboratory lines derived from the hQ strain.

Our results show significant differences in the frequency of larval encounters (Fig. 3). The MS1-5 and T1-2 lines had significantly lower frequencies of two-larvae encounters than did the MS1-6 and T3-2 lines (Fig. 3a). This indicates that low frequencies of larval encounters decreased interference, and thus increased the frequency of two-adult emergences in the MS1-5 and T1-2 lines. In contrast, high frequencies of larval encounters might not increase interference. The MS1-3, MS1-6, T2-1 and T3-2 lines did

not differ in the frequency of larval encounters although they exhibited different C-values. This indicates that the variation in the frequency of larval encounters provides a less explanation for at least one aspect of the variation in competition type within the hQ strain than does the variation in the frequency that either one or both of two larvae form walls after an encounter. Therefore, the mechanism that determines the frequency of larval encounter may be a secondary mechanism causing variation in the larval competition type of the hQ strain.

Evolutionary change of larval competition type in the hQ strain

Tuda (1998) demonstrated that evolutionary changes, in the form of larval competition moving from scramble- to contest-type, were caused by a host shift from *V. angularis* bean to *V. radiata* bean. However, the proximal mechanism behind larval competition was not proposed in that study. The present study clearly demonstrates that the larval behavior of making a wall structure determined the form of larval competition within the hQ strain.

Therefore, we suggest that *V. radiata* beans be selected for larvae in order to reduce the frequency of wall formation when larval borrows intersect, and to encourage contest-type lines in the hQ strain. *V. radiata* beans are much smaller than *V. angularis* beans, and their size would create selection pressure for contest-type larvae, as shown in previous studies (Takano et al. 2001).

Genetic back-ground of wall-making behavior

Our results indicate that the tendency to make a wall structure was genetically determined (Fig. 4a, b). The study by Takano et al. (2001) suggested that larval competition type in the hQ strain is determined mainly by additive genetic inheritance. However, in this study, we were not able to precisely specify what genetic effects influenced the larval behavior of wall-making. In the reciprocal crosses between the MS1-5 and MS1-6 lines, the formation of a wall structure appeared to be a dominant behavior. In contrast, in the reciprocal crosses between the MS1-5 and T3-2 lines, it appeared to be recessive. Non-additive genetic effects such as epistatic and maternal effects as well as additive genetic effect may influence the tendency towards the formation of a wall structure by larvae. Further experiments are required in order to more closely investigate genetic effects on wall-making behavior.

In addition, in the present study, we were not able to provide a detailed genetic basis for larval wall-making behavior. Two hypotheses have been proposed: The first is that the likelihood of a larva constructing a wall may be genetically determined. Larvae may adopt a mixed strategy, where larval behavior is stochastically determined (Tuda and Iwasa 1998). This hypothesis predicts that when larvae encounter each other at any developmental stage, they will make walls based on a genetically determined probability. The second hypothesis is that a larva over either a given stage or a given size can create a wall structure. Larvae may adopt a pure conditional

strategy. The larval stage or size is identified as a developmental switch point, which may be genetically determined. When larvae that have reached the developmental switch point encounter each other in a bean, they can make walls. Although another of our studies seems to support the second hypothesis because the fourth-instar larvae from the MS1-5 line started to make walls on the 12th day after oviposition (Mano and Toquenaga in preparation), a focused study is necessary to understand the genetics behind the construction of a wall structure.

Variation in larval competition type between geographic strains

Variation in larval competition type is observed between geographic strains of *C. maculatus* (Takano et al. 2001). Our study appears to be the first to explain, in terms of larval behavior, variations in larval competition type of *C. maculatus*. The larval behavior of creating a wall may be one of the main traits determining larval competition type in *C. maculatus*. Mitchell (1990) briefly reported that the larvae of a scramble-type geographic strain other than the hQ strain (the IITA strain) exhibited wall-making behavior.

However, the behavior of making walls was not considered a proximate mechanism to determine competition type in *C. maculatus*. Variation in wall-making behavior can explain the variation of competition type in geographic strains of *C. maculatus*.

The evolution of scramble-type larvae

Scramble-type larvae are observed in most geographic strains of *C. maculatus* (Toquenaga 1993; Takano et al. 2001). Laboratory studies have shown that large sized beans such as *V. unguiculata* and large *V. radiata* beans are selection forces for scramble-type larvae (Takano et al. 2001, Messina 2004). Our results suggest that scramble-type larvae may benefit from making walls after encounters with other larvae in the large beans where two larvae can potentially develop, but the larval encounter often occurs. Although we cannot identify the mechanisms that provide the benefits to wall-making larvae in the present study, we propose one possibility, based on previous studies. Making walls may protect larvae from the cost of interference in the large beans. Messina (1991a; 2004) showed that the frequency of zero-adult emergence was relatively high when two contest-type larvae were in *V. angularis* and *V. unguiculata* beans. This may indicate the cost of a reduction in the success of emergence for a contest-type larva winning interference. This cost may increase the relative fitness of scramble-type larvae compared with contest-type larvae, and thereby lead to the selection of scramble-type larvae in large beans. To test this hypothesis, further study is required to investigate whether larval interference reduces the occurrence of successful emergence for winning larvae in large beans.

We conclude that variations in larval competition type within the hQ strain of *C. maculatus* is determined by the variation in the tendency towards wall-making behavior. Our study appears to be the first to explain

variations in larval competition type of *C. maculatus* in terms of larval behavior. Although scramble-type competition is observed in geographic strains of *C. maculatus*, the condition for the evolution of larvae exhibiting scramble-type competition behavior has been examined based on unrealistic assumptions of larval behavior. Examining the evolution of wall-making behavior will contribute to the understanding the conditions for the evolution of scramble-type larvae.

Acknowledgments

We thank Dr. M. Shimada and Dr. M. Tuda for providing information on the history of the hQ strain. We also thank anonymous reviewers for their valuable comments and suggestions. This work is dedicated to Dr. Utida.

References

- Boivin G, van Baaren J (2000) The role of larval aggression and mobility in the transition between solitary and gregarious development in parasitoid wasps. *Ecol Lett* 3:469-474
- Brodeur J, Boivin G (2004) Functional ecology of immature parasitoids. *Ann Rev Entomol* 49:27-49
- Colegrave N (1997) Can a patchy population structure affect the evolution of competition strategies? *Evolution* 51:483-492
- Godfray HCJ (1987) The evolution of clutch size in parasitic wasps. *Am Nat* 129:221-233
- Guedes RNC, Smith RH, Guedes NMP (2003) Host suitability, respiration rate and the outcome of larval competition in strains of the cowpea weevil, *Callosobruchus maculatus*. *Physiol Entomol* 28:298-305
- Ike T (1984) Experimental studies of interspecific competition between bruchid species and system stability in experimental ecosystem. Dissertation, University of Tsukuba
- Ives AR (1989) The optimal clutch size of insect when many females oviposit per patch. *Am Nat* 133:671-687
- Jackai LEN, and Daoust RA (1986) Insect pests of cowpeas. *Ann Rev Entomol* 31:95-119
- Lomnicki A (1988) Population ecology of individuals. Princeton

University Press, Princeton

- Messina FJ (1991) Competitive interactions between larvae from divergent strains of the cowpea weevil (Coleoptera: Bruchidae). *Environ Entomol* 20:1438-1443
- Messina FJ (2004) Predictable modification of body size and competitive ability following a host shift by a seed beetle. *Evolution* 58:2788-2797
- Mitchell R (1990) Behavioral ecology of *Callosobruchus maculatus*. In: Fujii K, Gatehouse AMR, Johnson, CD, Mitchell R, Yoshida T (eds) *Bruchids and legumes: Economics, ecology, and coevolution*. Kluwer, Dordrecht, The Netherlands, p 317
- Nicholson AJ (1954) An outline of the dynamics of animal population. *Austral J Zool* 2:9-65
- Pexton JJ, Mayhew PJ (2001) Immobility: the key to family harmony? *Trend Ecol Evol* 16:7-9
- Pexton JJ, Rankin DJ, Dytham C, Mayhew PJ (2003) Asymmetric larval mobility and the evolutionary transition from siblicide to nonsiblicidal behavior in parasitoid wasps. *Behav Ecol* 14:182-193
- R Development Core Team (2006) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> of subordinate document. Cited 24 April 2006
- Rosenheim JA (1993) Single sex broods and the evolution of nonsiblicidal parasitoid wasps. *Am Nat* 141: 90-104

- Smith RH (1990) Adaptation of *Callosobruchus* species to competition.
In: Fujii K, Gatehouse AMR, Johnson, CD, Mitchell R, Yoshida T
(eds) Bruchids and legumes: Economics, ecology, and coevolution.
Kluwer, Dordrecht, The Netherlands, p 351
- Smith RH, Lessells CM (1985) Oviposition, ovicide and larval
competition in granivorous insects. In: Sibly RM, Smith RH (eds)
Behavioral ecology. Blackwell Scientific, Oxford, p 423
- Sokal RR, Rohlf FJ (1995) Biometry. W. H. Freedman, New York
- Southgate BJ (1979) Biology of the Bruchidae. Ann Rev Entomol
24:449-473
- Takano M, Toquenaga Y, Fujii K (2001) Polymorphism of competition
type and its genetics in *Callosobruchus maculatus* (Coleoptera:
Bruchidae). Popul Ecol 43:265-273
- Thanthianga C, Mitchell R (1987) Vibrations mediate prudent resource
exploitation by competing larvae of the bruchid bean weevil
Callosobruchus maculatus. Entomol Exp Appl 44:15-21
- Toquenaga Y (1990) The mechanisms of contest and scramble
competition in Bruchid species. In: Fujii K, Gatehouse AMR,
Johnson, CD, Mitchell R, Yoshida T (eds) Bruchids and legumes:
Economics, ecology, and coevolution. Kluwer, Dordrecht, The
Netherlands, p 341
- Toquenaga Y (1993) Contest and scramble competitions in
Callosobruchus maculatus (Coleoptera: Bruchidae): II. Larval

- competition and interference mechanisms. *Res Popul Ecol* 35:57-68
- Toquenaga Y, Fujii K (1990) Contest and scramble competition in two bruchid species, *Callosobruchus analis* and *C. phaseoli* (Coleoptera: Bruchidae): I. Larval competition curves and interference mechanisms. *Res Popul Ecol* 32:349-363
- Toquenaga Y, Fujii K (1991) Contest and scramble competition in *Callosobruchus maculatus* (Coleoptera: Bruchidae): I. Larval competition curves and resource sharing patterns. *Res Popul Ecol* 33:199-211
- Toquenaga Y, Ichinose M, Hoshino T et al (1994) Contest and scramble competitions in an artificial world: Genetic analysis with genetic algorithms. In: Langton CG (ed) *Artificial Life III*. Addison Wesley and Reading, MA, p 177
- Tuda M (1998) Evolutionary character changes and population responses in an insect host-parasitoid experimental system. *Res Popul Ecol* 40:293-299
- Tuda M, Iwasa Y (1998) Evolution of contest competition and its effect on host-parasitoid dynamics. *Evol Ecol* 12:855-870

Figure legends

Fig. 1 The phylogeny of the hQ strain. Branch length depicts coarse evolutionary time, with the years indicated. Solid and dotted lines indicate lines maintained on *V. angularis* and *V. radiata* beans, respectively. The bold solid line shows the selection process for contest competition. Gray regions indicate that lines were maintained in the laboratory at the University of Tsukuba. For further details, see the Materials and Methods section.

Fig. 2 Estimated C-values for the six pure lines (a), the patterns of C-values for the crosses between the MS1-5 and MS1-6 lines (b), and in the cross between the MS1-5 and T3-2 lines (c). Filled and open bars indicate female and male C-values, respectively. F1 and F1R indicate two reciprocal hybrid lines (see Larval competition type in Materials and Methods section).

Results for the MS1-5, MS1-6, and T3-2 lines in (a) are the same as shown in (b) and (c).

Fig. 3 Proportions of beans with two survivor that did not encounter each other (open regions; Group 1, see Statistical analysis section) and beans where two larvae encountered each other (filled regions; Groups 2 and 3) by the 18th day after oviposition in the six pure lines (a), the patterns of the proportions in the crosses between the MS1-5 and MS1-6 lines (b), and in the crosses between the MS1-5 and T3-2 lines (c). In each graph, frequencies with the same lowercase letter above the bars do not differ significantly from each other at significant levels adjusted by the sequential Bonferroni

criterion. Numbers in parentheses below the line names indicate numbers of sample beans. F1 and F1R indicate two reciprocal hybrid lines (see Larval competition type in Materials and Methods section). Results for the MS1-5, MS1-6, and T3-2 lines in (a) are the same as shown in (b) and (c).

Fig. 4 Proportions of beans with one survivor and one killed individual (open regions; Group 2, see Statistical analysis section) and beans with two larvae separated by walls (filled regions; Group 3) by the 18th day after oviposition in the six pure lines (a), the patterns of the proportions in the crosses between MS1-5 and MS1-6 lines (b), and in the crosses between the MS1-5 and T3-2 lines (c). In each graph, frequencies with the same lowercase letter above the bars do not differ significantly from each other at significant levels adjusted by the sequential Bonferroni criterion. F1 and F1R indicate two reciprocal hybrid lines (see Larval competition type in Materials and Methods section). Results for the MS1-5, MS1-6, and T3-2 lines in (a) are the same as shown in (b) and (c).

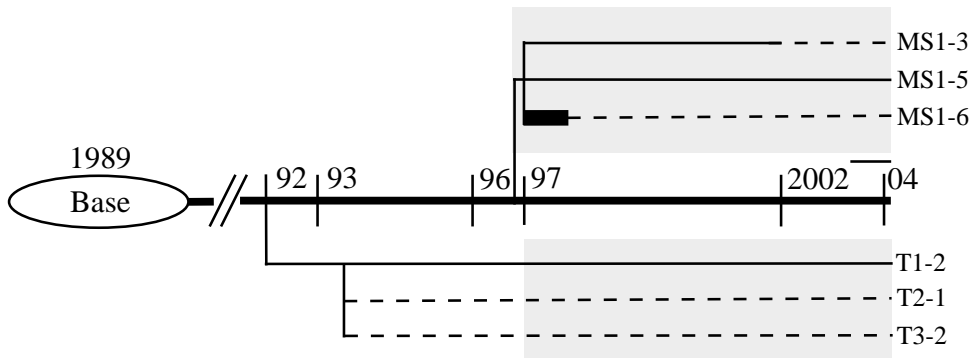
Table 1. Sample sizes and model numbers for parameter estimation and estimated parameters of male proportion (m) and natural mortality (μ).

Line	Sample size		Model ^c	Estimated parameters	
	n_1^a	n_2^b		m	μ
MS1-3	120	163	2	0.470	1.53×10^{-5}
MS1-5	104	161	3	0.552	0.08
MS1-6	96	164	2	0.457	1.53×10^{-5}
T1-2	96	226	3	0.466	0.01
T2-1	144	90	2	0.535	1.53×10^{-5}
T3-2	143	162	4	0.442	0.03
Hybrid line (female \times male)					
MS1-5 \times MS1-6	96	96	3	0.522	0.04
MS1-6 \times MS1-5	96	94	3	0.487	0.03
MS1-5 \times T3-2	144	362	3	0.460	0.01
T3-2 \times MS1-5	144	284	3	0.484	0.03

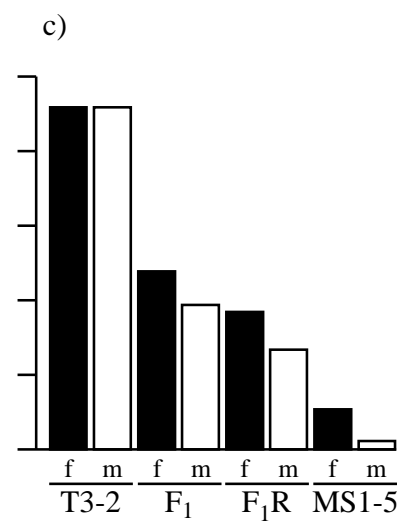
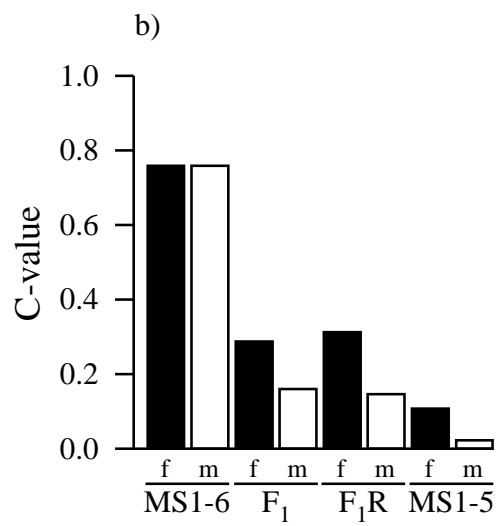
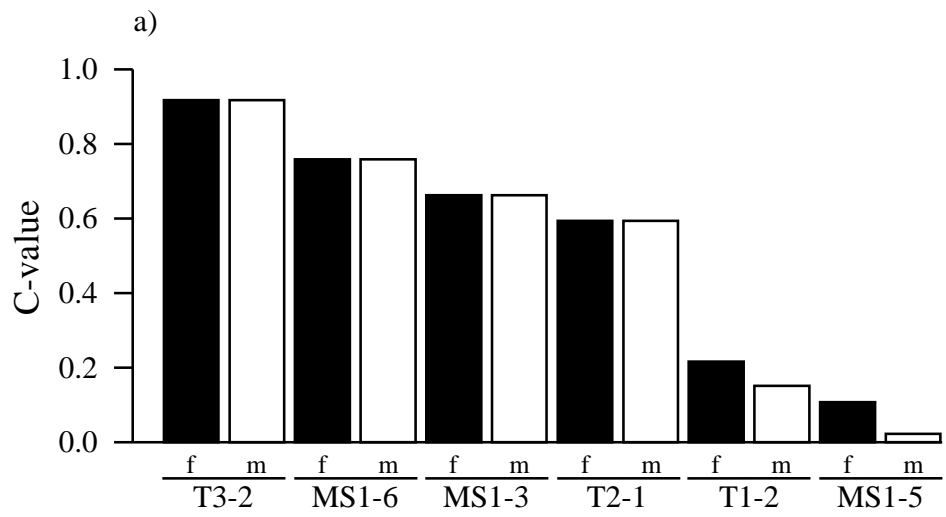
^a n_1 indicates the number of beans with one hatched egg.

^b n_2 indicates the number of beans with two hatched eggs.

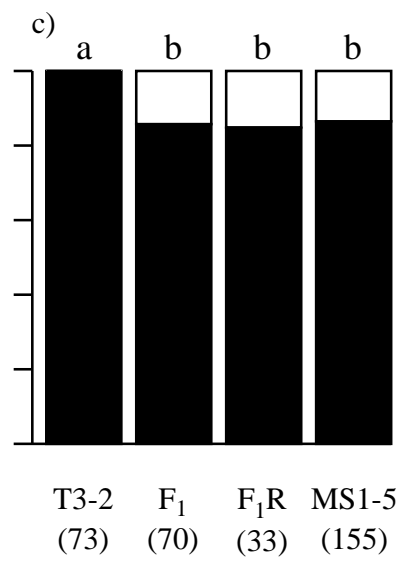
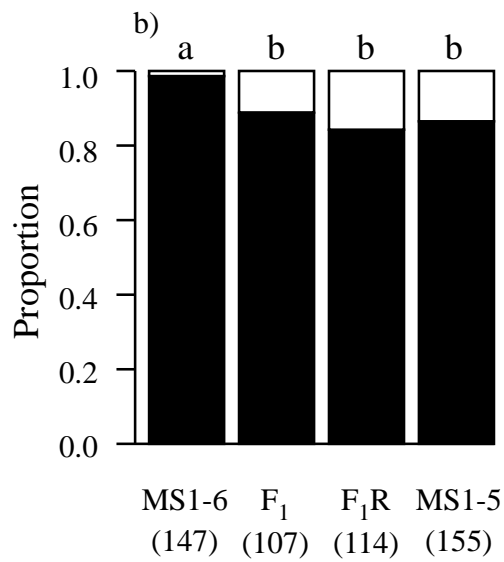
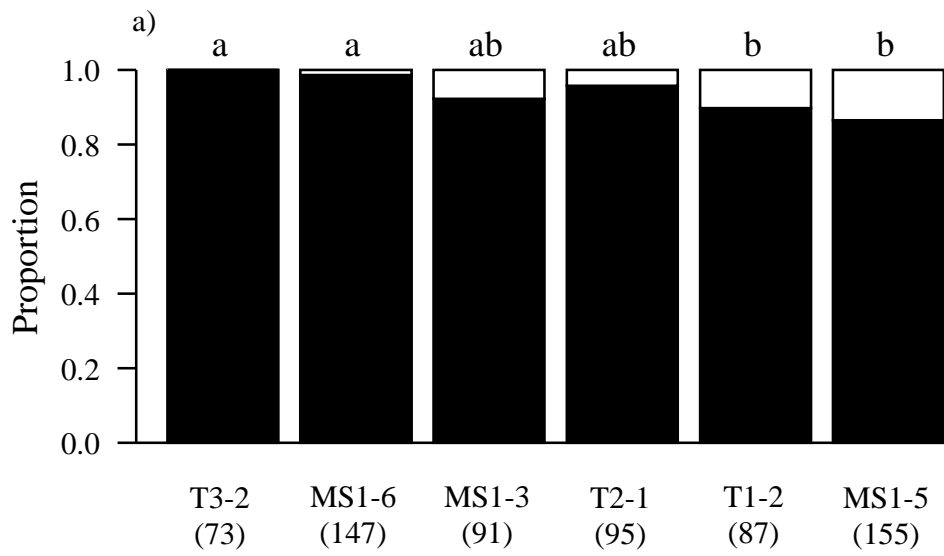
^c Model indicates the number of model with the lowest AIC score (see Statistical analysis in Materials and Methods section).



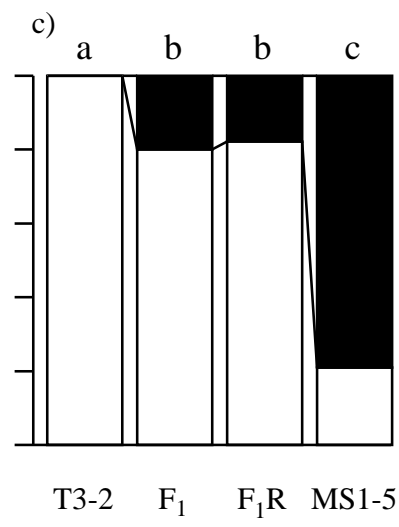
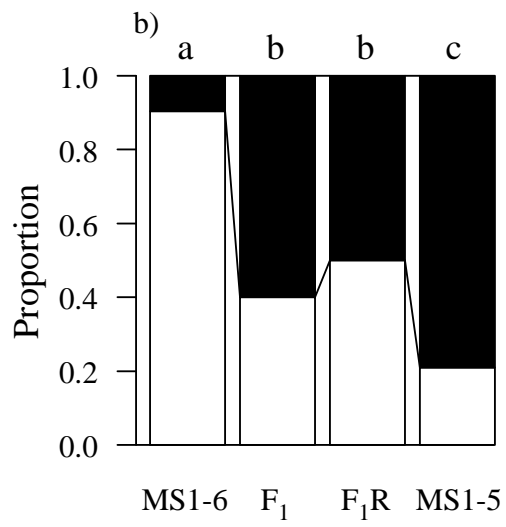
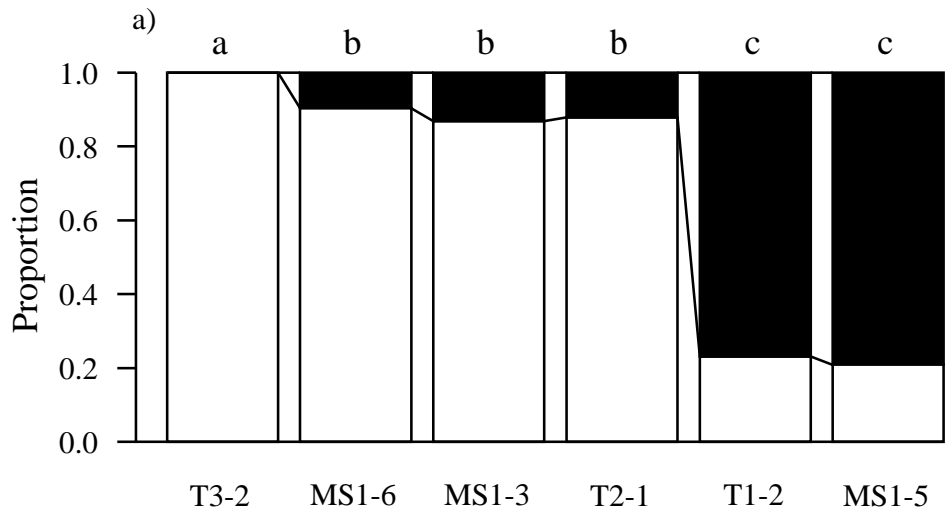
Mano, H., Toquenaga, Y.: Figure 1



Mano H and Toquenaga Y: Figure 2



Mano H and Toquenaga Y: Figure 3



Mano H and Toquenaga Y: Figure 4