Quantitative genetic analysis of fitness-related traits in the soldier-producing aphid, *Pseudoregma bambucicola* (Homoptera: Aphididae): Genetic variation and costs of soldier production

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Abstract

Genetic bases of several traits of the soldier-producing aphid *Pseudoregma bambucicola* (larval period, reproductive age, adult body size, colony fitness, and proportions of adults, alates and soldiers) were examined by quantitative genetic analyses with particular reference to colony-level stabilizing selection on soldier proportion. Five aphid clones were grown on *Bambusa multiplex* shoots that were clonal but of different ages. The age of the shoot was regarded as an environmental factor affecting aphid fitness. Mixed model ANOVA detected no significant effects of genotype and genotype-environment interaction on colony fitness, whereas the fitness was higher on younger hosts. This suggests that no single clone exceeds other clones in fitness in all environments. While a significantly larger amount of genotypic variance was detected in body size and adult proportion, the soldier proportion exhibited less genotypic variance. This result suggests that the soldier proportion is intimately associated with fitness and is under strict stabilizing selection. A negative genetic correlation was detected between soldier proportion and colony growth. This also suggests that stabilizing selection has operated on soldier proportion so as to maintain eusociality in *P. bambucicola* that lacks kin recognition.

Key words: Soldier-producing costs, eusociality, *Pseudoregma bambucicola*, quantitative genetics, colony-level stabilizing selection

INTRODUCTION

Understanding the factors responsible for the evolution and maintenance of eusociality in animals has been a central theme in evolutionary biology. Aphids are ideal study organisms for addressing this problem, not only because their caste systems are diverse, but also because their colonial structures are simpler than those of other eusocial animals, because of clonal reproduction (Aoki, 1987; Itô, 1989; Stern and Foster, 1996). Thus they provide promising opportunities for studying the origin and maintenance of eusociality.

The discovery of sterile soldier castes in aphids (Aoki, 1977) has confirmed a prediction from kin selection theory that proposes that altruistic behaviour is likely to evolve in clonal organisms (Hamilton, 1964; Dawkins, 1979).

Actually, altruistic soldier castes, or defending morphs, have independently evolved at least nine or more times in Aphidoidea (Stern and Foster, 1996), exhibiting extensive behavioural and morphological diversity of soldier types (e.g., Aoki, 1977, 1987; Aoki and Kurosu, 1986; Moran, 1993; for reviews see Itô, 1989; Stern and Foster, 1996).

Despite this advantage of social evolution in aphids, no evidence of kin discrimination has so far been found in any soldier-producing aphids in mating (Foster and Benton, 1992) or in fighting or defence (Foster, 1990; Aoki et al., 1991; Sakata and Itô, 1991; Carlin et al., 1994; also see Stern and Foster, 1996, 1997). These facts suggest that if a population of this eusocial group is once infested by a “selfish” mutant which produces fewer or no soldiers, it would rapidly increase within the population at the

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expense of soldier-producers, resulting in the extinction of eusociality (Hamilton, 1987; Stern and Foster, 1996; also see Williams, 1966). Thus, it is likely that some other mechanisms besides kin selection are responsible for the maintenance of eusociality in aphids.

The aphid *Pseudoregma bambucicola* (Takahashi), which produces a sterile soldier caste, exhibits no kin discrimination behaviour (Sakata and Itô, 1991; Shibao, MS). A previous study (Shibao and Tsuji, MS) has predicted that stabilizing selection on the proportion of soldiers within a colony is a plausible mechanism for the maintenance of eusociality in this species (inter-colonial selection hypothesis). Under this selection, colony fitness should be highest at an intermediate level of soldier proportion. This selection will eliminate colonies with extremely biased caste ratio, possibly owing to a shortage of defenders or reproducers. Therefore, a colony with selfish mutants is more likely to go extinct due to the invasion of natural enemies.

To test this hypothesis, I present here a quantitative genetic study of soldier production in *P. bambucicola*. In the previous study no attention was paid to the genetic bases of soldier-related traits in *P. bambucicola*. An understanding of the genetic architecture of soldier production is important not only to clarify the evolutionary response to predators but also to predict the evolutionary direction of soldier-related traits. Thus, a quantitative genetic approach is essential to evaluate the past histories of natural selection acting on *P. bambucicola*.

Genetic variation in fitness-related traits is lower than in other traits (Stearns, 1992), because any form of continuous selection eliminates genetic variability (Falconer, 1989). Genetic diversity can be maintained within a population when the population is not panmictic and when no single clone exceeds other clones in fitness in all environments, i.e. in the presence of genotype-environment ($G \times E$) interaction (Service and Lenski, 1982). Therefore, if there is evidence for no $G \times E$ interaction in fitness, then lower levels of genetic variance in the soldier proportion would be explained by the inter-colonial selection hypothesis.

On the other hand, the caste ratio theory predicts that production of soldiers enhances defensive ability, but inevitably entails lower growth rates of the colony due to allocation of resources into non-reproductive individuals (e.g., Akimoto, 1996; Stern and Foster, 1996; for Hymenoptera, Oster and Wilson, 1978). Thus, colony defence should be costly, leading to reduced colony growth in the absence of predators. If there is genetic variation in the amount of costs among clones, evolutionary trade-off can be expected between colony defence and colony growth. This trade-off will make an intermediate level of soldier proportion evolutionarily stable, and thus would corroborate the possibility of colony-level stabilizing selection.

In this paper, therefore, I focus on the following three questions: (1) is there any significant $G \times E$ interaction in fitness itself?; (2) is there any significant genotypic variation in soldier proportion?; and (3) do investments in soldiers have a negative genetic correlation with colony growth? Through the quantitative genetic analyses of aphids, I will emphasize the importance of colony-level selection, which would serve as a mechanism for maintaining eusociality in animals without kin discriminative behaviour.

MATERIALS AND METHODS

Study animals. The aphid, *Pseudoregma bambucicola* (Takahashi), is widely distributed in South-eastern Asia (Blackman and Eastop, 1984) including southern Japan (Takahashi, 1958) and Taiwan (Takahashi, 1923). This species is a eusocial aphid with sterile soldiers that defend their colony against predators (Ôhara, 1985a, b; Aoki, 1987; Moffett, 1989; Schütze and Maschwitz, 1991; Sakata and Itô, 1991; Sakata et al., 1991; Morimoto and Shibao, 1993; Tanaka and Itô, 1995; Shibao, 1998). *P. bambucicola* generally grows and reproduces on *Styrax suberifolia* (primary host) and *Bambusa* spp. (secondary host). However, in Japan, since the primary host plant is absent, it passes through parthenogenetic generations on a secondary host, *Bambusa multiplex* (Lou' Raeusch), throughout the year. New shoots of *B. multiplex* appear from late July to November, and

aphids mainly colonize these young growing shoots (Shibao personal observation). Soldiers and alates are produced from October to November (Sunose et al., 1991; Itô et al., 1995). Since the alates of *P. bambucicola* play no significant role in its life cycle but are produced by phylogenetic inertia in Japan (Sunose et al., 1991; Shibao, 1997), Japanese populations of this aphid may have been obligately parthenogenetic for hundreds of years, with the sexual phase entirely eliminated from its life cycle.

Methods. In late April 1995, aphid strains (genotypes) of *P. bambucicola* were collected from five *Bambusa multiplex* stands shown in Fig. 1 and were reared in the field of Kagoshima University. One reproductive female was collected from each stand and the five females were separately reared on shoots of a single bamboo clone in the green garden. Colonies initiated by these females were reared in breeding cages for about 90 days, i.e. for at least three generations, to eliminate the maternal effects on the traits of her offspring.

In early August 1995, 10 reproductive females were collected from each cage, and two of them were put on each of five bamboo shoots that were aged 10, 20, 30, 40 and 50 days after sprouting. On each shoot, therefore, 10 females were reared separately in breeding cages. When each female had produced five progeny, she was removed from her cage, and the five nymphs

were subsequently observed to clarify their larval period and reproductive age. Because the nymphs were immobile and differed in growth rates, they were individually identified by tracing their daily location and body size. Twenty-four hours after the start of reproduction, each aphid was collected and then slide-mounted to measure the following 14 characters under a dissecting microscope: frontal horn length, length of ultimate rostral segment, fore femur length, fore tibia length, fore tarsus length, mid femur length, mid tibia length, mid tarsus length, hind femur length, hind tibia length, hind tarsus length, body length, maximum pronotum width and length of 3rd–4th antennal segments (Fig. 2). As a general size indicator of each adult, the PC1 score in the principal component analysis (PCA) was used (Table 1). In this analysis, variation in PC1 explained 68.7% of overall variation.

Twenty four hours after the beginning of larviposition, females produced some nymphs, which were subsequently allowed to establish a colony in each cage. In early November 1995, when soldier production peaked in most colonies, colony size and proportions of soldiers, alates and apterous adults were recorded in each cage without disturbing them. Colony fitness was defined as the final number of adults within a cage at the end of the season.

After the number of adult aphids was count-

Fig. 1. Study sites: localities in southern Kyushu.

ed in early November (A_n), the colonies were reared in the cages until early December, when adult numbers were counted again (A_d) to examine the genetic correlation between the soldier proportion and colony growth rate (A_d/A_n).

The following eight traits were available for quantitative genetic analysis: for individual level: larval period, reproductive age and adult body size (PC1) for 250 individuals (5 genotypes $\times$ 5 environments $\times$ 2 cages $\times$ 5 progenies); for colony level: colony fitness, proportions of soldiers, alates and adults, and colony growth rate for 50 colonies (5 genotypes $\times$ 5 environments $\times$ 2 cages). These data were analyzed by a three-way mixed ANOVA in which "genotype" and "environment" were fully crossed and the third factor "cage" was nested within each combination of genotype and environment (Table 2). Genotype and cage were treated as random effects, whereas environment was treated as a fixed effect. When colony traits were analyzed, the third factor constituted residual error in a two-way mixed ANOVA (Table 2) and the colony traits other than fitness were subject to ANCOVA with colony size as a covariate.

Variance components (VC%) were estimated by ANOVA using the residuals of regressions on colony size. Additionally, for the soldier proportion, the variance component was estimated by the liability-threshold model, because soldier production can be regarded as an all-or-none trait (Falconer, 1989).

Heritabilities in a broad sense are given by the proportion of genotype effects (V_G) to total phenotypic variance (V_P). In calculation of genetic correlation (r_G), the average value of each genotype was calculated for each trait and the Pearson product-moment correlations between eight traits were obtained, while phenotypic correlations (r_P) were calculated based on the values of all individuals. Environmental correlations (r_E) were calculated from the following formula: $r_P = h_X h_Y r_G + e_X e_Y r_E$ where h_X and h_Y denote square roots of broad-sense heritabilities for trait X and Y , and e_X and e_Y square roots of $1 - h_X^2$ and $1 - h_Y^2$, respectively (Falconer, 1989). Since some aphids died during development, designs became unbalanced, and thus standard errors for variance components were not obtained.

Fig. 2. Measurements of morphological characters in adult *P. bambucicola*.

Table 1. Principal component analysis (PCA) for 14 body size characters of adults of *P. bambucicola*. First three principal components are shown ($N=178$).

Character	Mean $\pm$ SD	Component loadings		
		PC1	PC2	PC3
Frontal horn length	0.079 $\pm$ 0.012	0.497	-0.294	-0.701
Length of ultimate rostral segment	0.089 $\pm$ 0.008	0.647	0.013	-0.165
Fore femur length	0.464 $\pm$ 0.042	0.957	0.048	0.038
Fore tibia length	0.608 $\pm$ 0.067	0.969	0.032	-0.049
Fore tarsus length	0.147 $\pm$ 0.015	0.817	0.038	-0.096
Mid femur length	0.474 $\pm$ 0.044	0.968	0.041	0.056
Mid tibia length	0.667 $\pm$ 0.076	0.970	0.048	0.001
Mid tarsus length	0.155 $\pm$ 0.015	0.869	0.116	0.109
Hind femur length	0.565 $\pm$ 0.057	0.961	0.035	0.034
Hind tibia length	0.909 $\pm$ 0.103	0.968	0.036	-0.005
Hind tarsus length	0.166 $\pm$ 0.017	0.849	0.068	-0.047
Body length	2.418 $\pm$ 0.241	0.584	-0.140	0.531
Maximum pronotum width	0.710 $\pm$ 0.448	0.353	-0.882	0.164
Length of 3rd-4th antennal segment	0.179 $\pm$ 0.016	0.867	0.126	0.047
Cumulative % of total variance explained		68.719	75.357	81.511

Table 2. Degrees of freedom (df) and expected mean squares (MS) in the quantitative genetic analysis by ANOVA models. In *F*-test, numerator-MS/denominator-MS is shown.

Source	df	Expected MS	MS no.	<i>F</i> -test
(a) Three-way analysis of variance with cross-nested design				
Genotype (G)	$g-1$	$\sigma^2 + n\sigma_C^2 + nce\sigma_G^2$	MS1	MS1/MS4
Environment (E)	$e-1$	$\sigma^2 + n\sigma_C^2 + nc\sigma_{GE}^2 + ncgK_E^2$	MS2	MS2/MS3
G $\times$ E	$(g-1)(e-1)$	$\sigma^2 + n\sigma_C^2 + nc\sigma_{GE}^2$	MS3	MS3/MS4
Cage within G and E	$ge(c-1)$	$\sigma^2 + n\sigma_C^2$	MS4	MS4/MS5
Within cage (error)	$gec(n-1)$	σ^2	MS5	
Total	$gecn-1$			
(b) Two-way analysis of variance				
Genotype (G)	$g-1$	$\sigma^2 + ce\sigma_G^2$	MS1	MS1/MS4
Environment (E)	$e-1$	$\sigma^2 + c\sigma_{GE}^2 + cgK_E^2$	MS2	MS2/MS3
G $\times$ E	$(g-1)(e-1)$	$\sigma^2 + c\sigma_{GE}^2$	MS3	MS3/MS4
Cage within G and E (error)	$ge(c-1)$	σ^2	MS4	
Total	$gec-1$			

Genotypes treated as random effect ($g=5$); Environments as fixed effect ($e=5$); Cages as random effect nested within G and E ($c=2$); model (a) performed on individual measurements (i.e. larval period, reproductive age, and adult body size [PC1], $n=5$ per cage), model (b) on colonial measurements (i.e. fitness, proportions of apterous adults, alates and soldiers).

Statistics. Statistical analyses were performed using the SYSTAT statistical package (Wilkinson, 1992) unless otherwise stated. In parametric statistical tests, the proportional data were transformed to arcsine square-root and other data were transformed into natural logarithm to satisfy normality and constant variance (Sokal and Rohlf, 1995).

RESULTS

One-way ANOVA showed that aphid strains differed significantly in larval period ($p=0.001$), adult body size ($p<0.001$), soldier proportion ($p=0.044$) and adult proportion ($p=0.047$), illustrating the potential for quantitative genetic analyses of soldier-related traits

Table 3. Mean values and SDs of eight traits measured for five strains of *P. bambucicola*. Sample size is shown in parentheses.

Variable	Aphid strain					Total	<i>F</i> -value	<i>p</i>
	Miyanojo	Miyazaki	Fukiage	Ikeda Lake	Kaminokawa			
Individual level								
Larval period (days)	11.10±1.21 (29)	10.09±1.48 (43)	11.12±1.98 (33)	11.38±1.39 (39)	11.07±1.24 (43)	10.93±1.53 (187)	5.132	0.001
Reproductive age (days)	12.83±1.20 (29)	12.02±1.73 (42)	12.73±2.31 (33)	13.00±1.17 (36)	12.55±1.73 (42)	12.59±1.71 (182)	2.182	0.073
Adult body size (PC1)	-1.18±0.97 (28)	0.78±0.62 (42)	0.15±0.82 (32)	0.37±0.57 (36)	-0.44±0.82 (40)	0.00±1.00 (178)	34.267	<0.001
Colonial level								
Fitness (total no. adult aphids)	3.10±3.67 (10)	9.90±21.71 (10)	0.78±1.72 (9)	4.30±8.55 (10)	3.40±6.20 (10)	10.35±22.76 (49)	0.875	0.487
Colony size	39.14±42.00 (7)	124.14±200.22 (7)	9.50±7.78 (2)	51.00±73.96 (6)	25.33±43.57 (6)	57.86±110.98 (28)	0.617	0.673
Soldier proportion (%)	22.36±20.83 (7)	39.75±29.59 (7)	0±0 (2)	23.03±31.07 (6)	0.91±2.23 (6)	20.65±26.18 (28)	2.908	0.044
Alate proportion (%)	0.45±1.20 (7)	3.21±6.61 (7)	0±0 (2)	0.93±1.43 (6)	0.15±0.37 (6)	1.15±3.46 (28)	0.672	0.618
Adult proportion (%)	27.43±34.30 (7)	22.52±11.65 (7)	41.67±11.79 (2)	14.76±13.51 (6)	62.93±41.18 (6)	32.11±30.97 (28)	2.856	0.047

Fig. 3. Reaction norms of larval period (A), reproductive age (B) and adult body size (PC1) (C). Ten colonies (5 clones, i.e. genotype, $\times$ 2 colonies) were reared on five shoots of different ages (environment), and, on each shoot, an average of two clonal colonies was calculated and designated as genotypic mean and an average of 10 colonies as environmental mean. The linear regression of each genotype was obtained by the least-squares method (MJ: Miyanojo; MZ: Miyazaki; FA: Fukiage; IL: Ikeda Lake; KK: Kaminokawa). Mean response of the genotypes is indicated by the dashed line.

(Table 3).

When environmental conditions were adjusted statistically, a significant genotypic variance was detected only in adult body size, with 44.3% of the total phenotypic variance being explained by genetic variance (Table 4). There

was no evidence for significant genotypic variance in larval period or reproductive age, while a significant $G \times E$ interaction was detected in reproductive age. This was consistent with Fig. 3B where the reaction norms of the reproductive age were crossed. For all three traits,

Table 4. ANOVAs for the effects of genotype (strain), environment (host age) and cage on larval period, reproductive age and adult body size (PC1)

Source	df	MS	F-value	p	VC%
Larval period					
Genotype (G)	4	0.071	1.949	0.133	4.8
Environment (E)	4	0.081	1.361	0.291	3.0
G × E	16	0.060	1.635	0.131	16.3
Cages within G and E	25	0.037	6.113	0.000	44.0
Within cage (error)	137	0.006			31.9
Total	186				100.0
R^2		0.778			
Reproductive age					
Genotype (G)	4	0.034	1.501	0.232	1.6
Environment (E)	4	0.129	1.932	0.154	9.1
G × E	16	0.067	2.965	0.007	32.3
Cages within G and E	25	0.023	4.021	0.000	25.0
Within cage (error)	132	0.006			32.1
Total	181				100.0
R^2		0.786			
Adult body size (PC1)					
Genotype (G)	4	18.804	14.404	0.000	44.3
Environment (E)	4	1.197	0.591	0.674	0.0
G × E	16	2.026	1.552	0.158	9.1
Cage within G and E	25	1.305	6.277	0.000	27.8
Within cage (error)	128	0.208			18.8
Total	177				100.0
R^2		0.850			

Table 5. ANOVAs for the effects of genotype (strain) and environment (host age) on fitness and proportions of soldiers, alates and adults

Source	df	MS	F-value	p	VC%	df	MS	F-value	p	VC%
Fitness (total no. adults)						Adult proportion				
Colony size	—	—	—	—	—	1	0.001	0.021	0.891	—
Genotype (G)	4	0.934	0.594	0.650	0.0	3	2.533	13.621	0.008	81.6
Environment (E)	4	3.001	11.471	0.000	15.1	4	0.157	1.271	0.335	0.9
G × E	16	0.262	0.166	1.000	0.0	12	0.124	3.155	0.107	11.1
Error	24	1.573			84.9	5	0.039			6.4
Total	48				100.0	25				100.0
R^2		0.384					0.964			
Alate proportion						Soldier proportion				
Colony size	1	0.035	6.930	0.046	—	1	0.085	0.763	0.422	—
Genotype (G)	3	0.002	0.487	0.706	0.0	3	0.130	1.173	0.407	0.0
Environment (E)	4	0.002	0.283	0.883	0.0	4	0.045	0.540	0.709	3.3
G × E	12	0.008	1.551	0.329	32.4	12	0.083	0.748	0.687	0.0
Error	5	0.005			67.6	5	0.111			96.7
Total	25				100.0	25				100.0
R^2		0.908					0.851			

there were highly significant cage effects (25–44% of total variance) within G and E. The cage effects may be attributed to within-host environmental heterogeneity, including maternal environmental effects that are due to environmental heterogeneities in maternal stocks.

Table 5 shows the results of quantitative genetic analyses for colony fitness and proportions of soldiers, alates and adults. In the Fukiage strain, 8 of 10 colonies became extinct before early November, 1995, so this strain was omitted from the quantitative genetic analysis. Colony fitness was significantly influenced by environment: it was higher on younger hosts. However, in colony fitness, the effects of genotype and $G \times E$ interaction were not significant, and the reaction norms were not crossed (Fig. 4A). With regard to adult proportion, a significant genotypic effect was found, with the variance component constituting

27.4% of the total phenotypic variance. In the alate proportion, the variance component of $G \times E$ made up 43.5%, although this was not statistically significant probably due to the elimination of the effects of the covariate, colony size. This suggests that the alates are produced in response to aphid density. With regard to soldier proportion, no variance was detected in genotype or $G \times E$, and the variance component of genotype accounted for 4.4% in the liability-threshold model. Although neither environment nor colony size contributed significantly to this trait in the mixed model ANOVA (Table 5), the overall test revealed a positive correlation between colony size and soldier proportion ($r=0.647$, $p<0.001$).

Genetic correlation showed a similar pattern to phenotypic correlations (Mantel-test, matrix correlation $r=0.459$, randomized one-tailed, $p=0.047$) (Table 6). However, the matrix

Fig. 4. Reaction norms of colony fitness (A), adult proportion (B), alate proportion (C) and soldier proportion (D). Ten colonies (5 clones, i.e. genotype, $\times 2$ colonies) were reared on each of five shoots of different ages (environment), and, on each shoot, the average of two clonal colonies was calculated and designated as genotypic mean and the average of 10 colonies as environmental mean. The linear regression of each genotype was obtained by the least-squares method (MJ: Miyanojo; MZ: Miyazaki; FA: Fukiage; IL: Ikeda Lake; KK: Kaminokawa). Mean response of the genotypes is indicated by the dashed line. The proportions of soldiers, alates and adults were adjusted by a covariate, colony size.

Table 6. Phenotypic, genetic and environmental correlations among seven variables

	LP	RA	PC1	W_{ijk}	PSO	PAL
Phenotypic correlation						
Larval period (LP)	—					
Reproductive age (RA)	0.761	—				
Adult body size (PC1)	-0.292	-0.311	—			
Fitness (W_{ijk})	-0.201	-0.012	0.002	—		
Soldier proportion (PSO)	-0.252	0.120	0.140	0.498	—	
Alate proportion (PAL)	-0.369	0.075	0.033	0.763	0.626	—
Adult proportion (PAD)	-0.039	-0.177	-0.254	-0.208	-0.449	-0.245
Genetic correlation						
Larval period (LP)	—					
Reproductive age (RA)	0.950	—				
Adult body size (PC1)	-0.513	-0.492	—			
Fitness (W_{ijk})	-0.601	-0.462	0.120	—		
Soldier proportion (PSO)	-0.577	-0.304	0.382	0.788	—	
Alate proportion (PAL)	-0.747	-0.590	0.861	0.795	0.787	—
Adult proportion (PAD)	0.066	-0.217	-0.381	-0.313	-0.812	-0.619
Environmental correlation						
Larval period (LP)	—					
Reproductive age (RA)	0.759	—				
Adult body size (PC1)	-0.287	-0.358	—			
Fitness (W_{ijk})	-0.207	-0.012	0.003	—		
Soldier proportion (PSO)	-0.237	0.131	0.128	0.506	—	
Alate proportion (PAL)	-0.381	0.076	0.044	0.763	0.637	—
Adult proportion (PAD)	-0.129	-0.351	-0.078	-0.485	-0.749	-0.572

Table 7. Phenotypic and genetic correlations between soldier proportion and colony growth rate

	PSO	CGR
Soldier proportion (PSO)	—	$r_p = -0.089$ ($n=16$)
Colony growth rate (CGR)	$r_G = -0.889$ ($n=4$)	—

correlation coefficient ($r=0.425$) between genotypic and environmental correlations was not statistically significant. This suggests that the genetic and environmental factors exert their influences through different physiological mechanisms on each trait.

Phenotypic and genetic correlations between the soldier proportion and colony growth rate were not statistically significant due to the small sample size (Table 7). However, the genetic correlation was highly negative ($r_G = -0.889$, $n=4$), while the phenotypic correlation was low ($r_p = -0.089$, $n=16$). This may indicate that

there is a genetic basis for trade-offs between colony defence and colony growth.

DISCUSSION

The vulnerability of caste systems to invasion of “selfish cheaters” is a fundamentally serious problem for eusocial aphids because they have no ability to assess kinship among themselves. This provides a special difficulty for the theory of kin selection in maintaining eusociality (Itô, 1989; Hamilton, 1987; Stern and Foster, 1996). Therefore, it is of great theoretical importance to know what forces in combination with kin selection have maintained the soldier caste in aphids despite cheaters.

A previous study (Shibao and Tsuji, MS) showed that stabilizing selection acted on the proportion of soldiers within a colony in *P. bambucicola*. This colony-level stabilizing selection is due to high predation pressures on aphid colonies. Under this selective regime, colonies dominated by selfish cheaters may eas-

ily become extinct. Since soldiers of *P. bambucicola* are able to kill or remove a range of natural predators such as *Eupeodes confrater* (Diptera: Syrphidae) and *Synonycha grandis* (Coleoptera: Coccinellidae), soldier production is highly effective in defending the colonies against attacks by such predators (Shibao, 1998). Therefore, stabilizing selection could be the main evolutionary factor promoting the maintenance of eusociality in this species. However, this process is phenotypic (Endler, 1986), and knowledge of the genetic architecture is needed for a better understanding of the past histories of natural selection on *P. bambucicola*.

The present study revealed the genetic architecture of soldier production and fitness-related traits in *P. bambucicola*. The estimated genetic variation and $G \times E$ interactions are broad-sense measures of genetic variance, including additive, dominance and epistatic variance components (Falconer, 1989). However, such broad-sense measures are appropriate for predicting evolutionary responses to selection in obligatorily asexual organisms, because the entire genotype, rather than a gene, is transmitted from parents to offspring (Stratton, 1992).

Genetic diversity in clonal organisms can be maintained in a population unless the fitness of a single clone exceeds that of other clones in all environments (Service and Lenski, 1982). Some theoretical studies have shown that $G \times E$ interaction can maintain the genetic variation or retard its depletion (Gillespie and Turelli, 1989; but see Via and Lande, 1987). Therefore, it is important to clarify whether there is a significant effect of $G \times E$ interaction on fitness (Mousseau and Roff, 1987; Stratton, 1994). The present study indicated that there was no significant $G \times E$ interaction and, thus, that heterogeneous selective environments played no significant role in maintaining sufficient amounts of heritable genetic variation in fitness-related traits. Mixed model ANOVA detected no significant genotypic variance in colony fitness, suggesting that because of consistent selective pressures heritable variation in fitness is exhausted.

While a significantly large amount of genotypic variance was detected in the body size and

proportion of adult aphids, there was less genotypic variance in the soldier proportion. Considering that genetic variation in fitness-associated traits is generally lower than in other traits (Stearns, 1992), the estimated lower genotypic variance in the soldier proportion indicates that strong selection has long been acting on this trait. Therefore, the results of the present study agree with the prediction of the inter-colonial selection hypothesis, suggesting that stabilizing selection on the soldier proportion is the critical factor responsible for the maintenance of eusociality in *P. bambucicola*.

The present analysis also indicated a negative genetic correlation between the soldier proportion and colony growth rate ($r_G = -0.889$), genetic trade-offs between fitness traits. Since the genetic correlation among traits can increase or decrease the rates of evolutionary change and can affect the evolutionary trajectories of all traits (Lande, 1982), this correlation would constrain short-term responses to selection in the fitness-related traits (Rausher and Simms, 1989; Boulding and Hay, 1993; Han and Lincoln, 1994). Because of these genetic constraints, it is unlikely that this species will achieve both a higher soldier proportion and faster colony growth simultaneously. As a consequence, the soldier proportion will be kept at an intermediate level. Thus, the constraints may be critical in maintaining eusociality in *P. bambucicola*.

In summary, this study is the first report on the measurement of genotypic variance for soldier-related traits in eusocial aphids. The absence of adequate genotypic variance of the soldier proportion within a colony supports the intercolonial selection hypothesis in *P. bambucicola*. Since aphid colonies can be viewed as analogous to individuals of sexual organisms, in which soldiers correspond to non-reproductive somatic cells, this result suggests the importance of colony-level adaptation in *P. bambucicola*. At present, we have no data on the genetic structure of aphid colonies other than *Ceratovacuna nekoashi* (Fukatsu and Ishikawa, 1994). DNA fingerprinting techniques would enable us to estimate the relatedness among colony members and to examine the existence of socially parasitic clones, i.e. cheaters, in natural

populations of eusocial aphids including *P. bambucicola*. The present study points out that group-level natural selection may have played an important role in shaping elaborate caste systems and maintaining highly social behaviours in animals which do not show kin discrimination.

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