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INBREEDING DEPRESSION, INCREASED PHENOTYPIC VARIANCE, AND A
TRADE-OFF BETWEEN GONADS AND APPENDAGES IN SELFED PROGENY
OF THE APHID *PROCIPHILUS ORIENS*

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EFFECTS OF SELFING IN APHIDS

Abstract.---Trade-offs are potentially common among two or more traits whose development is dependent on the same resources. To detect genetic trade-offs, the techniques of quantitative genetics, pedigree analyses and selection experiments, have been used. The present study demonstrated genetically based trade-offs between gonads and appendages in hatched larvae of the aphid *Prociphilus oriens* by focusing on enlarged variance among the families of selfed progeny. The selfed and outbred families were compared in respect of the size of morphological traits, gonad volume, and hatch dates, as well as egg volume. Selfing not only increased the among-family variance component in all larval traits examined, but it also increased the mean size of all the morphological traits significantly. In contrast, gonad volume, a fitness component, was reduced with selfing. Calculation of the allometry (log-transformed regression) of larval traits to egg volume indicated that in the outbred group, morphological traits grew slowly relative to egg volume with slopes below 0.25, whereas gonads exhibited isometric growth. With selfing, most morphological traits had significantly steeper slopes, whereas the slope for gonads was greatly decreased. When the effect of egg volume was statistically removed from the means of selfed families, significant negative correlation was detected between the adjusted means of gonad volume and those of tibia length. This result suggests genetic trade-offs between gonad volume and tibia length. Thus, the evidence implies that at the loci governing the development of appendages, the dominant alleles function to canalize the development of tibiae into an optimal size, irrespective of egg volume. It is hypothesized that increased homozygosity of the deleterious recessive alleles reduced gonad volume through increasing the resource allocation to tibiae. The hypothesis of the gonad-appendage trade-off could be applied to explain the phenotypic evolution in some aphid species.

Key words.---Selfing, outbreeding, allometry, canalization, development, genetic variance, common principal component.

Trade-offs between traits constrain the direction of evolution, thereby constituting a fundamental element of phenotypic evolution (Roff 1992; Stearns 1992; Charnov 1993; Futuyma 1998). Although it is presumed that trade-offs are prevalent between numerous morphological and life-historical traits, detecting genetically based trade-offs is not easy, requiring pedigree analyses and selection experiments (Roff 1992, 2002). In particular, traits that are strongly related to fitness maintain a limited

amount of additive genetic variance (Mousseau and Roff 1987, Roff and Mousseau 1987, but see Møller and Swaddle 1997). This generally leads to large standard errors for estimated genetic correlation. Therefore, detecting significant trade-offs between fitness-related traits requires a larger number of families in pedigree analyses and a longer term in selection experiments (Roff 1992). The present study attempts to demonstrate commonly latent trade-offs using families of selfed progeny.

During embryogenesis in eggs, latent trade-offs are expected among the size of morphological traits or internal organs because embryogenesis entails the allocation of a limited amount of resources in eggs to several traits. A number of studies have presumed that natural selection has canalized the development of traits such that the final morphology is included in a narrow range of variation (Waddington 1960; Møller and Swaddle 1997; Gibson and Wagner 2000; Debat and David 2001; Hoffmann and Woods 2001). However, traits are often destabilized during development due to genetic or environmental factors, one of which is increased homozygosity due to inbreeding (Lerner 1954; Møller and Swaddle 1997; Fowler and Whitlock 1999; Reale and Roff 2003). When a population is subdivided with inbreeding into several family lines, variance in a trait increases among the lines (Wright 1951; Robertson 1952; Goodnight 1987; Falconer 1989; Lopez-Fanjul and Villaverde 1989), and the instability of the trait (FA) is expected to increase (Møller and Swaddle 1997; Reale and Roff 2003). During embryogenesis, because a trait grows interactively with the growth of other traits, a large change in a certain trait due to inbreeding would result in a coordinated change in the final size of other traits or organs via trade-offs (Nijhout and Wheeler 1996; Klingenberg and Nijhout 1998; Nijhout and Emlen 1998; Moczek and Nijhout 2004).

The present study first attempts to impose inbreeding upon a generation so as to increase phenotypic variance in hatched larvae of the next generation, and secondly attempts to detect negative correlations, i.e., trade-offs, among the size of traits in hatched larvae. It is expected that with inbreeding the relationship between traits changes depending on changes in mean trait values and the amounts of additive and nonadditive genetic variance (Roff and DeRose 2001). The merit of this method is that there is a potential to detect usually overlooked trade-offs from among many combinations of traits.

The traits of hatched larvae have several advantages in assessing the effect of inbreeding on the phenotype. First, the resources for embryogenesis are limited to the yolk the mother provided, so that the amount of resources can readily be estimated by measuring egg volume. Unless the amount of resources available for development is

estimated, it is difficult to detect expected trade-offs (Van Noordwijk and de Jong 1986). Secondly, because food supply is not necessary for eggs, there is likely to be little environmental variance due to artifacts under the same rearing conditions. Thirdly, it is likely that the phenotypic effects of inbreeding are prominent in hatched larvae. In contrast, the effect of inbreeding on adult phenotypes might be restricted because larvae with a large inbreeding effect cannot mature. Thus, it is expected that phenotypes of a wide range would provide information about typically latent relationships between traits.

The present study examines how the relationships between traits change with inbreeding, using the aphid *Prociphilus oriens* (Aphididae, Eriosomatinae); in particular, attention is paid to the effects of inbreeding on (1) the relationship between egg volume and traits of hatched larvae, and (2) the relationship between a reproductive trait (gonad volume) and morphological traits of hatched larvae. Aphids generally reproduce parthenogenetically from spring to autumn, and a single aphid clone produces males and mating females in autumn (Dixon 1985). Thus, crossing males and females of the same clone leads to intense inbreeding that is genetically equivalent to selfing (Komatsu and Akimoto 1995). In order to assess the effects of selfing precisely, one has to compare the traits of selfed progeny with those of outcrossed progeny, not with naturally produced progeny (Charlesworth and Charlesworth 1987). Thus, the present study uses three kinds of eggs prepared by selfing, interpopulation crosses, and mating in the field and compares the larval traits among the three groups to clarify the effects of inbreeding and outbreeding.

MATERIALS AND METHODS

The aphid

Prociphilus oriens Mordvilko alternates host plants seasonally between the primary host *Fraxinus mandshurica* and the secondary host *Abies sachalinensis*. In early October, a winged generation appears on *A. sachalinensis* and migrates to *F. mandshurica*. On the trunk, winged females parthenogenetically produce males and mating females. Each winged female contains four males and a varying number of females in its abdomen (Yamaguchi 1985). These sexuals are dwarfish, molt four times without feeding, and mature sexually in one week. The abdomen of each mating female contains only a single egg (Heie 1980), which is deposited in bark crevices about five days after copulation (Akimoto unpublished). The mating behavior and breeding system of *P. oriens* are almost the same as those of *Pemphigus spyrothecae*

described by Foster and Benton (1992). Eggs overwinter in the crevices of the trunk and hatch in late April before the bud burst of *F. mandshurica*.

Mating and hatching

Selfed and outbred families in 1998.--- In mid October of 1997, at a locality in Bibai, northern Japan, winged females were collected on the trunk while walking down to larviposition sites and were confined individually in a plastic container (9ml) with thin paper (kimwipe). A few days later, 61% of these winged females deposited males and females at 15°C in the dark. Half of the containers were kept intact. In all these containers, males and females of the same brood mated successfully, and eggs were deposited on the thin paper. Eggs were deposited in 72 containers, and eggs in each container constituted a family of selfed progeny.

To obtain outbred eggs, immediately after being deposited, males were removed from the rest of the containers, into which immature males collected at a different locality were transferred (five to seven males per container). Thus, outbred eggs were obtained by crossing males and females of different localities. Females of the Bibai population were used both in selfed families and in outbred families. The two localities, 13km from each other, were selected such that winged females could not migrate directly between them. There were no geographical barriers between the two localities, with continuous distribution of the primary and secondary host plants. Crossing resulted in 67 outbred families.

Wild population in 1999 and selfed families in 2000.--- Eggs were additionally prepared in 1998 and 1999 and were hatched the following spring. In mid October of 1998, approximately 100 eggs were collected randomly from the wild in Bibai and used for incubation (wild population 1999). To obtain further selfed families, selfed eggs were prepared using winged females collected in mid October of 1999 in Bibai. Fifty-five selfed families (2.4 eggs per family) were prepared and hatched in 2000 (selfed families 2000).

Egg hatching.---In each year, eggs from mating experiments or from the wild were individually transferred into a labeled plastic container lined with three sheets of filter paper moistened with distilled water. These eggs were initially maintained at 15 °C in an incubator, and then the temperature was decreased, mimicking the conditions in the field. In early December, these containers were placed outdoors, and they were kept buried in the snow for about four months, as in Akimoto and Narita (2002). In early April, all the eggs were dug out of the snow and transferred to an incubator kept at 4

°C. Post-chilling incubation was started on April 4 (in 1998) or on April 2 (in 1999 and 2000) under an alternating temperature regime (19 °C for 8h and 6 °C for 16h) in the dark. Preliminary experiments showed that alternating temperatures were a prerequisite for successful hatching of aphid eggs (Akimoto and Narita 2002).

Measurements

The size of all the eggs used in this experiments were measured one to three weeks after being deposited. Because the egg shape was considered ellipsoidal, egg volume was estimated as $(\pi ab^2)/6$ by measuring the long axis a and the short axis b . For measurement, the images of the eggs were captured into a computer via a microscope camera, and the length was measured with NIH Image ver. 1.62.

In spring of 1998, after the start of incubation, all the eggs of the selfed and outbred families were checked daily for hatching at 3:30 p.m. Newly hatched larvae were mounted on slides with Hoyer's mountant. The images of the slide-mounted larvae were captured into a computer via a microscope camera. The length of nine morphological traits (Table 1) was measured using NIH Image.

In families with four or more eggs, one egg was randomly chosen, and the hatched larvae were fixed in Carnoy's fixative for estimation of the gonad volume. The larvae kept in Carnoy's fixative were transferred into acetocarmine solution, in which they were stained for 24 h at room temperature. The stained larvae were dissected in a drop of 30 μ l acetocarmine on slides. Using very fine insect pins, the abdomen was cut open, and the whole gonad was removed and mounted using acetocarmine as a mountant. The image of the gonad was captured into a computer under the same light intensity. In hatched larvae, the gonad was composed of undifferentiated germaria (Blackman 1987). Because the germaria were intensely stained with acetocarmine, the area stained more intensely than a threshold was measured.

For the wild population 1999 and selfed families 2000, all the hatched larvae, 87 and 104, respectively, were fixed in Carnoy's fixative. The gonad volume of each larva was assessed as described above, as well as the length of seven morphological traits (Table 4).

Data analyses

Means and variance.---The means and variances of traits and hatchability were compared between the selfed and outbred families 1998. In the selfed and outbred groups, 2.3 and 2.5 larvae per family, respectively, were available for morphological

comparison (4.4 and 4.6 for the comparison of hatching time). A preliminary study indicated significant differences between families for all the traits, suggesting that the use of individual scores violates the assumption of independence. Hence, means were calculated in each family for all the traits except gonad volume and these means were used for computing the grand means.

A preliminary study showed that egg volume affected the size of all the larval traits. Thus, to precisely estimate the difference in means between the selfed and outbred groups, the effect of egg volume was statistically removed from each trait. Using ANCOVA, the least square means of the larval traits were calculated for each group. Percent change in the mean with selfing was computed as $100 \times (M_S - M_O) / M_O$, where M_S and M_O are the least square means for the selfed and outbred groups, respectively.

Variance was calculated using all hatched larvae, and the magnitude was compared between the selfed and outbred families. In this and later comparisons, to render the variance independent of the mean, the scores of all the traits were log-transformed (Falconer 1989). The variance component between families was estimated using the restricted maximum likelihood technique (the REML option in the SAS procedure varcomp, SAS 1990), and its percentage to the total variance was indicated.

In multiple comparisons of means, variances, or regression coefficients among several traits, the significance level P was adjusted using the sequential Bonferroni method (Rice 1989), and the adjusted P value is represented by $P(\text{adjusted})$.

Allometry.--- The allometry (log-log ordinary least squares regression) of larval traits to egg volume or body size was compared between the selfed and outbred families 1998 using ANCOVA. In addition, the allometric coefficients of the wild population 1999 were calculated, and they were compared with those of the outbred families 1998.

CPC analysis for larval morphology.---Common principal component (CPC) analysis was conducted to assess the differentiation in the covariance matrix of morphological traits between the selfed and outbred groups 1998, or other data sets (wild population 1999 and selfed group 2000). This analysis determines how many common principal components exist among covariance matrices (Flury 1984; Phillips and Arnold 1999; Tatsuta and Akimoto 2000). This study adopted the step-up approach (Phillips and Arnold 1999), which first tested whether or not a single common principal component (CPC(1)) existed among the PC1s of covariance matrices using the log-likelihood ratio test. If this hypothesis was not rejected, then the hypothesis that the covariance matrices share two common principal components (CPC(2)) for their

PC1s and PC2s was tested against the CPC(1) model. Subsequently, the hypothesis that a particular model is valid was tested against the next lowest model, using the CPC program developed by Phillips (2000).

Trade-off.---Analyses based on the selfed and outbred groups 1998 suggested that the growth of gonads was antagonistic to the growth of larval appendages (see Results). In order to test if there is a trade-off between these two trait groups and to determine which morphological trait contributes most negatively to the growth of gonads, multiple regression was conducted using the mean values of 52 selfed families (the selfed group 2000). The regression model treated log-transformed mean gonad volume as the response variable and log-transformed mean egg volume and seven larval traits as the independent variables. However, because the morphological traits were highly correlated with one another (on average, $r = 0.93$, $N = 21$), the use of these morphological traits caused multicollinearity. Thus, principle component analysis was first applied to the correlation matrix of the seven morphological traits, and three principal components were extracted. Multiple regression was conducted with the three PCs, as well as egg volume, being treated as the independent variables. The calculated partial regression coefficients for the three PCs were back-transformed into the coefficients for the seven original morphological traits (Philippi 1993). The same series of analyses was applied to the wild population 1999.

When a certain morphological trait was suggested to contribute negatively to the growth of gonads by the multiple regression, the correlation between the length of that trait and gonad volume was estimated using the family means. The 95% confidence interval for the correlation coefficient was also calculated. The estimate of genetic correlation based on the family means is an approximation, because the variance and covariance calculated from the family means include a fraction of the within-family variance component (Via 1985; Roff 2002). Nevertheless, it is difficult to statistically test the coefficient of genetic correlation estimated with any other method (Via 1985). All analyses were conducted using JMP Ver. 5 (SAS 2002), unless stated otherwise.

RESULTS

Means and variance

Outbred eggs hatched more successfully (93.8%, $N = 323$) than did selfed eggs (83.8%, $N = 371$), and this difference was significant (for selfed and outbred groups 1998, $\chi^2 = 17.7$, $df = 1$, $P < 0.0001$). However, the high hatchability in the selfed

group renders it meaningful to compare larval traits between the selfed and outbred groups.

No significant difference was found in egg volume between the selfed and outbred groups 1998. However, for all larval traits except gonad volume, the means for the selfed group were significantly larger than the means for the outbred group (Table 1). Only in gonad volume, the mean for the selfed group was smaller, though not significantly, than that for the outbred group. When ANCOVA was applied to log-transformed gonad volume using log-transformed egg volume as a covariate, significant difference was found in gonad volume between the selfed and outbred groups ($df = 1$, $F = 5.61$, $P = 0.021$, $P(\text{adjusted}) = 0.025$). Calculation of the least square means showed that when selfing was forced, the change in the mean was over 4% in the hind tibia and antennal segments 3 + 4, whereas it was approximately 1% in the whole body and ultimate rostral segment (Table 1). The means for the life-history traits (gonad volume and hatch date) changed more notably than did those of the morphological traits.

For all the larval traits, the variance in the selfed group was significantly larger than that in the outbred group (Table 2). An increase of more than 200% was found in the variance of the hind tibia, antennal segments 3 + 4, and hatch date with selfing. For every larval trait, the estimated among-family variance component for the selfed group was markedly larger than that for the outbred group. This result suggests that an increase in variance of the selfed group is mainly attributed to the increased among-family variance component. The within-family variance component in larval traits also increased with selfing; in hatch dates, for which larger sample size was available, among-family and within-family variance components in the selfed group were increased to approximately nine times and three times those in the outbred group, respectively.

Allometry

The allometric coefficients of all the morphological traits were below 0.25 in the outbred group 1998. The allometric coefficients for the selfed group were significantly larger than those for the outbred group in all the morphological traits but the ultimate rostral segment (ANCOVA, for the group $\times$ traits interactions of 8 traits, $P(\text{adjusted}) < 0.05$) (Fig. 1). In contrast, the allometric coefficient of gonad volume in the outbred group, 1.43, was not significantly different from unity ($df = 28$, $t = 0.69$, $P = 0.49$), whereas gonad volume in the selfed group had a coefficient not significantly different

from zero ($df = 35$, $t = 0.01$, $P = 0.99$). For the wild population 1999, the allometric coefficient of gonad volume was also not different from unity ($b = 1.076 \pm 0.154$ (SE), $df = 73$, $t = 0.49$, $P = 0.62$), while the coefficient in the selfed group 2000 was 0.439 ± 0.192 (for a coefficient of unity, $df = 48$, $t = 2.91$, $P = 0.005$; for a coefficient of zero, $t = 2.28$, $P = 0.027$).

The allometric coefficients of the morphological traits in the wild population 1999 were similar to those in the outbred group 1998 (Fig. 1), and there were no significant differences between the two groups (ANCOVA, for the group $\times$ traits interactions of all the traits, $P(\text{adjusted}) > 0.05$).

Calculation of the allometric coefficient to larval body size indicated that in the selfed group 1998, hind tibiae and antennal segments 3 +4 had coefficients greater than unity, suggesting an accelerated growth relative to body size (Fig. 2). In the tibiae and antennae, the allometric coefficients of the selfed group were significantly larger than those of the outbred group.

CPC analysis for larval morphology

CPC analysis revealed no common principal components between the covariance matrices of the selfed and outbred groups 1998 (Table 3). Similarly, no common principal components were found between the covariance matrices of the selfed group 1998 and wild population 1999, or between the covariance matrices of the outbred group 1998 and wild population 1999. These results, therefore, suggest a great differentiation among these three covariance matrices. However, two common principal components were detected for the two selfed groups 1998 and 2000, the PC1 and PC2 of which, in total, accounted for 96% of the total variance. This suggests that a similar covariance matrix resulted from selfing.

Trade-off

Principal component analysis for the selfed families 2000 showed that the PC1 was composed of positive loadings of nearly the same size, suggesting that the PC1 constituted the general size factor (Table 4). The PC2 allowed us to distinguish the tibiae and antennae from the femora. The PC3 distinguished the antennae relative to the tibiae. Similar results were obtained for the PC loadings of the wild population 1999 (Table 5).

Multiple regression for the selfed families 2000 indicated that egg volume and the PC3 had significantly positive effects on gonad volume (for $\log(\text{egg volume})$, $b =$

0.656, $t = 2.06$, $P = 0.046$; for PC3, $b = 0.165$, $t = 2.24$, $P = 0.030$), whereas the PC2 contributed negatively and significantly to gonad volume ($b = -0.163$, $t = -3.20$, $P = 0.003$). This suggests that there is a trade-off between gonad volume and the PC2. When the partial regression coefficients for the PCs were back-transformed into the coefficient for each trait, only fore-, mid- and hind-tibiae had negative coefficients (Table 4). Standardized regression coefficients showed that mid-tibiae had the most strongly negative effect on gonad volume. Correlation analysis based on family means also showed that when the effect of egg volume was removed from both gonad volume and tibiae, the correlation between the adjusted means for mid-tibiae and those for gonad volume was most strongly negative (Fig. 3); for mid-tibiae and gonad volume, $r = -0.41$ $[-0.62, -0.15]$, $N = 50$, $P = 0.003$; for fore-tibiae and gonad volume, $r = -0.30$ $[-0.53, -0.02]$, $N = 50$, $P = 0.035$; for hind-tibiae and gonad volume, $r = -0.36$ $[-0.58, -0.09]$, $N = 50$, $P = 0.010$.

In contrast, multiple regression for the wild population 1999 indicated that no variables exhibited significantly negative effects on gonad volume (for log(egg volume), $b = 0.755$, $t = 3.29$, $P < 0.0001$; for PC1, $b = 0.032$, $t = 2.29$, $P = 0.025$; for PC2, $b = 0.060$, $t = 1.13$, $P = 0.263$; for PC3, $b = -0.001$, $t = -0.01$, $P = 0.99$). Standardized regression coefficients were negative in fore-, mid- and hind-femora (Table 5). However, the correlation between the adjusted length of the femora and adjusted gonad volume was positive (for fore-femur and gonad volume, $r = 0.24$, $N = 75$, $P = 0.035$; for mid-femur and gonad volume, $r = 0.18$, $N = 75$, $P = 0.13$; for hind-femur and gonad volume, $r = 0.31$, $N = 75$, $P = 0.006$).

DISCUSSION

The present study, focusing on hatched larvae, evaluated the effect of inbreeding on the relationships among larval traits. Selfing resulted in the next generation with an inbreeding coefficient (F) of 0.5, or more if there was a history of inbreeding in the ancestors. The results of these experiments showed that this intense inbreeding had a large effect not only on the individual traits but also on the relationships between the traits. Almost all studies have shown that inbreeding reduces the mean size of morphological traits (Charlesworth and Charlesworth 1987; Falconer 1989; Lynch and Walsh 1998; Roff 1998; DeRose and Roff 1999), although a small fraction of inbred lines may increase in mean size (Pray and Goodnight 1995). In contrast, the present study indicated a conspicuous increase in all the morphological traits due to selfing. It

is necessary to examine whether or not this increase in the mean size is a characteristic specific to hatched larvae.

Given that the resources available for development are limited in eggs, increased size of some larval traits should have a negative effect on the growth of other traits. Multiple regression and correlation showed that increased tibia length contributed negatively to gonad volume. These analyses were based on family means from which the effect of egg volume was removed. The sizes of eggs in a family may be affected by common maternal effects. However, the variance in the adjusted family means, i.e., the mean trait size relative to egg volume, was considered to include genetic variance. Therefore, the evidence strongly suggests that the negative correlation between gonad volume and tibia length has a genetic basis.

The effect of selfing varied among traits. Egg volume was not affected by selfing at all. This is reasonable because egg development is completed in females prior to mating so that egg formation is completely under the control of the maternal genotype (Weigensberg et al. 1998). Of the larval traits, the length of the whole body and ultimate rostral segment was scarcely affected by selfing, whereas the length of the tibiae and antennae was greatly increased. It has been reported that inbreeding reduces the mean size of traits that form an important component of fitness, but leads to a little or no changes in traits that are not related to fitness (Falconer 1989; Roff 1998). In accordance with this generalization, a large change was found in the size of the tibiae that are closely connected to fitness via a trade-off with gonad volume.

A number of studies have indicated that traits that are closely connected to fitness are subject to directional selection, which also leads to directional dominance across the loci (Mather, 1973; Falconer 1989; Lynch and Walsh 1998; DeRose and Roff 1999). When inbreeding is forced, the means of traits that are under directional selection should shift toward the value of more recessive alleles. In contrast, when there is no directional dominance in a trait, the effects of dominance at individual loci could be canceled out with inbreeding, resulting in little change in the mean. Because life-history traits are generally subject to stronger directional selection, life-history traits are expected to show more intense inbreeding depression than do morphological traits (Roff 1998; DeRose and Roff 1999). This hypothesis was also supported in the present study, where gonad volume and hatch dates changed greatly in the means with selfing. Hence, it is hypothesized that a large change due to selfing in adaptively important traits could induce coordinated changes in other traits. Forcing selfing was effective in uncovering a trade-off between gonads and tibiae, that was not detected in

the wild population, nor has it been reported for any other insects. The detection of trade-offs based on enforced inbreeding differs from the techniques of quantitative genetics in that the former allows for the phenotypic effects of homozygous recessive alleles. However, this method is not useful if selfed lines have experienced small generations of inbreeding or have a small inbreeding coefficient, because the large within-line component of variance will mask the effect of the between-line component.

The allometric coefficient of a trait to egg volume is represented by $cov(x, y)/var(x)$, where x represents egg volume and y the value of the trait. Since $var(x)$ does not change with selfing, the increase in allometric coefficients is due to increased covariance between egg volume and the trait. This increase may result from a more sensitive response of the developmental pathway to the developmental environments, i.e., egg volume. Variance in trait size has been used as an index of canalization (Debat and David 2001; Hoffmann and Woods 2001; Reale and Roff 2003). However, since the amount of resources used for development can be estimated as egg volume, the allometric coefficient of a larval trait to egg volume could be a more appropriate index of canalization. Thus, a lower allometric coefficient for a larval trait implies a stronger environmental canalization (*sensu* Debat and David 2001) on its development.

The increases in allometric coefficients due to selfing could also be explained by increased homozygosity of deleterious recessive alleles. Thus, a trait that is subject to stronger canalizing selection should exhibit a larger increase in the allometric coefficient with selfing. Actually, when outbreeding was forced the growth of the tibiae and antennae was more strongly canalized relative to egg volume than that of other traits, whereas when selfing was forced the growth of the two traits was more accelerated than was that of other traits. In contrast, gonads grew proportionately relative to egg volume in the outbred group, while in the selfed group they grew more slowly. These facts allow us to estimate the function of adaptive and dominant alleles in the normal developmental process of *P. oriens*. Such alleles are expected to canalize the development of tibiae into an optimal size, irrespective of the egg volume, such that almost all the remaining resources in the eggs are invested in the gonads. This allocation of resources may be adaptive in environments where high mobility and activity are not required for hatched larvae. Increased homozygosity of deleterious recessive alleles with selfing appears to have reduced gonad volume through removing the developmental constraint on tibiae.

It is notable that the tibiae and femora of the same legs had different effects on the gonad volume. The gonad volume was most negatively affected by the PC2 of the

larval morphology, which distinguished the tibiae and antennal segments from the femora. This difference corresponded to the magnitude of changes in allometric coefficients due to selfing; i.e., the growth of the tibiae and antennae was more accelerated relative to egg volume due to selfing than that of the femora. This implies that the gonads competed for egg resources with the traits that changed conspicuously with selfing. Gonads and tibiae are functionally different and distant in the position. Similar examples are found in the butterfly *Precis coenia*, in which an allocation trade-off is detected between hind wings and legs (Klingenberg and Nijhout 1998), and in the horned scarab beetle *Onthophagus taurus*, which exhibits a trade-off between distant traits, male horns and genitalia (Moczek and Nijhout 2004).

Selfing caused a delay in egg hatching. A similar trend is reported in marine invertebrates, in which individuals that are more heterozygous have an enhanced growth rate (Koehn and Shumway 1982; Garton 1984; Garton et al. 1984). Theoretical studies have predicted that nonadditive gene actions, dominance or epistasis, lead to a notable increase in additive genetic variance during a few generations of population bottleneck (Robertson 1952; Goodnight 1987; Willis and Orr 1993). The three-fold increase in the within-family variance component in hatch dates is consistent with this prediction. The delay in hatching with selfing might imply that *P. oriens* eggs are subject to directional selection toward earlier hatching. In fact, in gall-forming aphids, first instars that hatch earlier are likely to secure a favorable galling site (Whitham 1978), encounter more rapidly developing leaves (Akimoto and Yamaguchi 1994), and utilize a longer season for growth and reproduction (Koyama et al. 2004).

Another finding of interest was the phenotypic divergence that occurred between the outbred group and the wild population. The outbred group was produced by crossing populations in habitats that were 13 km apart. *P. oriens* exhibits a highly female-biased sex ratio, which Yamaguchi (1985) ascribed to local mate competition. If the sexual generation does not have the potential to recognize kin, as is usual in aphids (Sakata and Ito 1991; Aoki and Kurosu 1992; Carlin et al. 1994; Shibao 1999), the breeding system where local mate competition occurs inevitably leads to inbreeding. Since inbreeding in local populations causes random fixation of alleles, different local populations will tend to be fixed for different alleles at several loci. This suggests that the wild population is homozygous at some loci governing larval morphology, while the outbred group is more heterozygous. Thus, it is likely that the divergence in the covariance matrix between the outbred group and the wild population reflects such difference in the genetic architecture.

The gonad-appendage trade-off found in this study has broad implications for the phenotypic evolution of aphids. Akimoto (1988) reported a similar trade-off for the gall-forming generation of the *Eriosoma yangi* group (Aphididae; Eriosomatinae), in which *E. y. yangi* Takahashi is a gall former with short appendages and high fecundity, while *E. y. parasiticum* Akimoto is a parasite of other species' galls, having long appendages and low fecundity. Furthermore, some aphid species of the Eriosomatinae and Hormaphidinae produce soldiers that defend colony members against insect predators at the sacrifice of themselves (Aoki 1987; Stern and Foster 1996). Soldiers are characterized by a peculiar morphology; well developed and highly sclerotized fore- and mid-legs used for grasping predators. In most species, the soldiers are first instars and they are sterile. Soldiers differ from fundatrices in that soldiers develop parthenogenetically from unfertilized eggs. However, if the gonad-appendage trade-off exists in soldier-producing aphids, adaptation to morphological armament would reduce gonad volume via the trade-off, incidentally leading to sterility. It is worth examining whether or not this trade-off constitutes a factor of phenotypic evolution in other aphid species.

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Table 1. Length of morphological traits, gonad volume, and hatch dates in first instars resulting from outbreeding and selfing (outbred and selfed families 1998). When there was a significant difference in the magnitude of variance between outbred and selfed families, Welch ANOVA was used for the comparison of the means, with the *t* values asterisked. Hatch dates are the number of days from the start of incubation. The length of body and other morphological traits are in mm. The *P* values were adjusted by the sequential Bonferroni method. **P*<0.05.

Traits	Mean			<i>t</i> -test		
	Outbreeding (SD)	Selfing (SD)	% change	df	<i>t</i>	<i>P</i>
Egg volume, mm ³	0.060 (0.0080)	0.063 (0.0087)	-	137	1.84	0.0674
Whole body (BOD)	1.333 (0.0536)	1.365 (0.0723)	+0.9	133	2.89	0.0045*
Ultimate-rostral segment (URS)	0.224 (0.0074)	0.230 (0.0107)	+1.5	119	3.53	0.0006*
Fore femur (FFEM)	0.230 (0.0096)	0.238 (0.0120)	+1.8	134	3.87	0.0002*
Fore tibia (FTIB)	0.209 (0.0088)	0.219 (0.0141)	+3.4	117	4.88*	<0.0001*
Mid femur (MFEM)	0.235 (0.0093)	0.243 (0.0128)	+2.3	134	4.42	<0.0001*
Mid tibia (MTIB)	0.232 (0.0090)	0.243 (0.0160)	+3.2	110	4.82*	<0.0001*
Hind femur (HFEM)	0.258 (0.0116)	0.270 (0.0161)	+3.2	134	5.00	<0.0001*
Hind tibia (HTIB)	0.286 (0.0115)	0.304 (0.0256)	+4.9	97	5.51*	<0.0001*
Antennal segments 3 + 4 (ANT34)	0.303 (0.0137)	0.322 (0.0327)	+4.5	92	4.41*	<0.0001*
Gonad volume, mm ²	0.684 (0.2877)	0.559 (0.3121)	-18.3	65	1.69	0.0954
Hatch date	14.6 (1.45)	15.8 (2.47)	+7.7	113	3.29*	0.0013*

Table 2. Comparison of variance between hatched larvae resulting from outbreeding and selfing (outbred and selfed families 1998). Sample size (*N*) and the percentage proportion of the among-family variance component to the total variance in parentheses. The *P* values were adjusted by the sequential Bonferroni method.

**P*<0.05

Traits	Variance ($\times 0.01$)			Comparison of variance		
	Outbreeding (<i>N</i>)	Selfing (<i>N</i>)	% change	df	<i>F</i>	<i>P</i>
Log(Egg volume)	2.17 (206)	2.33 (232)	+7	231,205	1.07	0.3009
Among families	1.69 (78.1%)	1.91 (82.1%)				
Within families	0.47	0.42				
Log(Whole body)	0.222 (164)	0.321 (159)	+45	158,163	1.45	0.0096*
Among families	0.123 (55.6%)	0.214 (66.6%)				
Within families	0.098	0.107				
Log(Ultimate-rostral segment)	0.147 (163)	0.265 (158)	+80	157,162	1.80	0.0001*
Among families	0.076 (51.8%)	0.161 (60.9%)				
Within families	0.071	0.104				
Log(Fore femur)	0.219 (175)	0.320 (171)	+46	170,174	1.46	0.0067*
Among families	0.134 (61.4%)	0.204 (64.0%)				
Within families	0.084	0.115				
Log(Fore tibia)	0.223 (174)	0.493 (171)	+121	170,173	2.21	<0.0001*
Among families	0.117 (52.3%)	0.351 (71.3%)				
Within families	0.107	0.141				
Log(Mid femur)	0.212 (192)	0.366 (192)	+72	191,191	1.72	<0.0001*
Among families	0.114 (53.5%)	0.225 (61.4%)				
Within families	0.099	0.141				
Log(Mid tibia)	0.222 (192)	0.534 (192)	+140	191,191	2.40	<0.0001*
Among families	0.082 (36.7%)	0.362 (67.8%)				
Within families	0.141	0.172				
Log(Hind femur)	0.256 (194)	0.478 (196)	+87	195,193	1.87	<0.0001*

Among families	0.151 (59.1%)	0.292 (61.1%)				
Within families	0.104	0.186				
Log(Hind tibia)	0.230 (194)	0.814 (196)	+253	195,193	3.53	<0.0001*
Among families	0.094 (40.6%)	0.589 (72.4%)				
Within families	0.137	0.225				
Log(Antennal segments 3 + 4)	0.253 (164)	1.040 (156)	+310	155,163	4.11	<0.0001*
Among families	0.138 (54.7%)	0.870 (83.7%)				
Within families	0.115	0.170				
Hatch date	146.5 (303)	776.1 (311)	+430	310,302	5.30	<0.0001*
Among families	58.6 (40.0%)	515.2 (66.4%)				
Within families	87.9	260.8				

Table 3. Common principal component analysis for covariance matrices based on morphological traits of hatched larvae resulting from selfing (selfed 98 and 2000) and outbreeding (outbred 98) and those from a wild population (Wild 99). A *P* value lower than 0.05 suggests rejection of the higher-level model.

Matrices compared	Likelihood ratio test				
	Higher level	Lower level	χ^2	df	<i>P</i>
Outbred98 – Selfed98	CPC(1)	unrelated	102.9	8	<0.0001
Selfed98 – Selfed2000	CPC(3)	CPC(2)	11.3	4	0.0232
	CPC(2)	CPC(1)	4.5	5	0.4753
	CPC(1)	unrelated	1.9	6	0.9287
Outbred98 – Wild99	CPC(1)	unrelated	14.2	6	0.0278
Selfed98 – Wild99	CPC(1)	unrelated	40.4	6	<0.0001

Table 4. Principal component loadings for seven morphological characters of the selfed larvae (selfed families 2000) and partial regression coefficients b on log-transformed gonad volume.

Traits	PC1	PC2	PC3	Standardized	
				b	$b (\times 0.01)$
Log (Fore femur)	0.377	-0.454	0.330	0.120	0.737
Log (Fore tibia)	0.382	0.172	-0.217	-0.073	-0.569
Log (Mid femur)	0.375	-0.543	0.054	0.089	0.556
Log (Mid tibia)	0.377	0.238	-0.628	-0.151	-1.208
Log (Hind Femur)	0.385	-0.212	-0.109	0.008	0.057
Log (Hind tibia)	0.383	0.260	-0.064	-0.062	-0.595
Log (Antennal segments 3+4)	0.368	0.547	0.657	0.011	0.120
Eigenvalue	6.594	0.218	0.092	-	-
% to total variance	94.20	3.11	1.32	-	-
Log (Egg volume)	-	-	-	0.656	8.489

Table 5. Principal component loadings for seven morphological characters of the larvae from the wild population 1999 and partial regression coefficients b on log-transformed gonad volume.

Traits	PC1	PC2	PC3	Standardized	
				b	$b (\times 0.01)$
Log (Fore femur)	0.372	-0.530	0.119	-0.020	-0.093
Log (Fore tibia)	0.374	0.234	-0.737	0.027	0.132
Log (Mid femur)	0.381	-0.359	0.087	-0.009	-0.038
Log (Mid tibia)	0.386	-0.003	-0.086	0.012	0.063
Log (Hind Femur)	0.386	-0.266	0.148	-0.003	-0.016
Log (Hind tibia)	0.380	0.366	-0.141	0.034	0.169
Log (Antennal segments 3+4)	0.366	0.575	0.621	0.046	0.243
Eigenvalue	6.364	0.236	0.137	-	-
% to total variance	90.92	3.36	1.96	-	-
Log (Egg volume)	-	-	-	0.755	12.15

Figure legends

Fig. 1. Allometric coefficients ($\pm$ SE) of larval traits on egg volume for the outbred and selfed families 1998 and for the wild population 1999. Asterisks represent significant differences in the coefficients between the outbred and selfed groups after sequential Bonferroni correction. Abbreviation for each trait is shown in Table 1.

Fig. 2. Allometric coefficients ($\pm$ SE) of larval morphological traits on body length for the outbred and selfed families 1998. Asterisks represent significant differences in the coefficients between the outbred and selfed groups after sequential Bonferroni correction.

Fig. 3. Negative correlation between log-transformed family means of mid-tibia length and those of gonad volume ($r = -0.41$, $P = 0.003$) in the selfed families 2000. The effect of log-transformed egg volume was removed from both parameters using regression analysis, and the residuals were used for this analysis.





