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Response to fish kairomone in *Daphnia galeata* life history traits relies on shift to earlier instar at maturation

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Abstract The aim of the present study was to clarify the mechanisms underlying the expression of phenotypic plasticity in *Daphnia* life history traits in response to fish kairomone and to explore how these mechanisms interact with genetic variation for life history traits. I studied the effect of fish kairomone on life history traits in 16 *Daphnia galeata* clones. Maturation occurred more often at the earlier instars in response to the fish treatment, resulting in earlier age and smaller size at maturity. The changes in age and size at maturity which occurred in the response to the fish treatment could be attributed to a large extent to this shift. In addition, fish kairomone resulted in smaller instar increments after the maturation process was initiated, but not during the juvenile period. Within groups of animals maturing at the same instar, larger size at birth resulted in larger size at maturity, but had no effect on age at maturity. However, larger size at birth brought about earlier maturity because animals which were larger at birth matured more often at the earlier instar. Fish treatment resulted in more, but smaller, offspring in the first clutch relative to the size of the mother. Genetic variation was found in all measured life history traits. Genetic variation in plasticities of life history traits was not detectable by standard methods, and was only shown when the above mechanisms of expression of life history traits were taken into account. This study illustrates the importance of a thorough understanding of the mechanisms underlying the expression of life history traits, in this case, sources and consequences of maturation in the multiple instars. Not only is this necessary to predict the long-term effect of the environment, but also to understand the response of life history traits and their plasticities to natural selection. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at http://dx.doi.org/10.1007/s00442-002-0901-0

Keywords Genotype × environment interaction · Induced defenses · Size-selective predation · Threshold size

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Introduction

The importance of phenotypic plasticity in life history traits has long been recognized (Bradshaw 1965). Recently both the conditions for the evolution of phenotypic plasticity (Van Tienderen 1991, 1997; Scheiner 1993; De Jong 1995; Scheiner and Callahan 1999) and its potential consequences (West-Eberhard 1989) have received increasing attention. The cases where phenotypic plasticity appears adaptive are of particular interest, for they suggest that plasticity has responded to selection. The existence of genetic variation for adaptive phenotypic plasticity indicates that there is potential for evolutionary change, and suggests that there might be certain costs to plasticity (DeWitt et al. 1998). Genetic variation for presumably adaptive plasticity is well documented for a variety of organisms [plants (Sultan and Bazzaz 1993a, b; Pigliucci and Schlichting 1996; Donohue and Schmitt 1999; Weinig 2000), insects (Gotthard et al. 1994; Blanckenhorn 1998; Fox et al. 1999; Thompson 1999; Roff and Bradford 2000), amphibians (Newman 1992, 1994; Semlitsch 1993; Reques and Tejedo 1997)], but the mechanisms that generate the plastic response are rarely understood. Studies investigating this issue, although scarce, have been illumi-

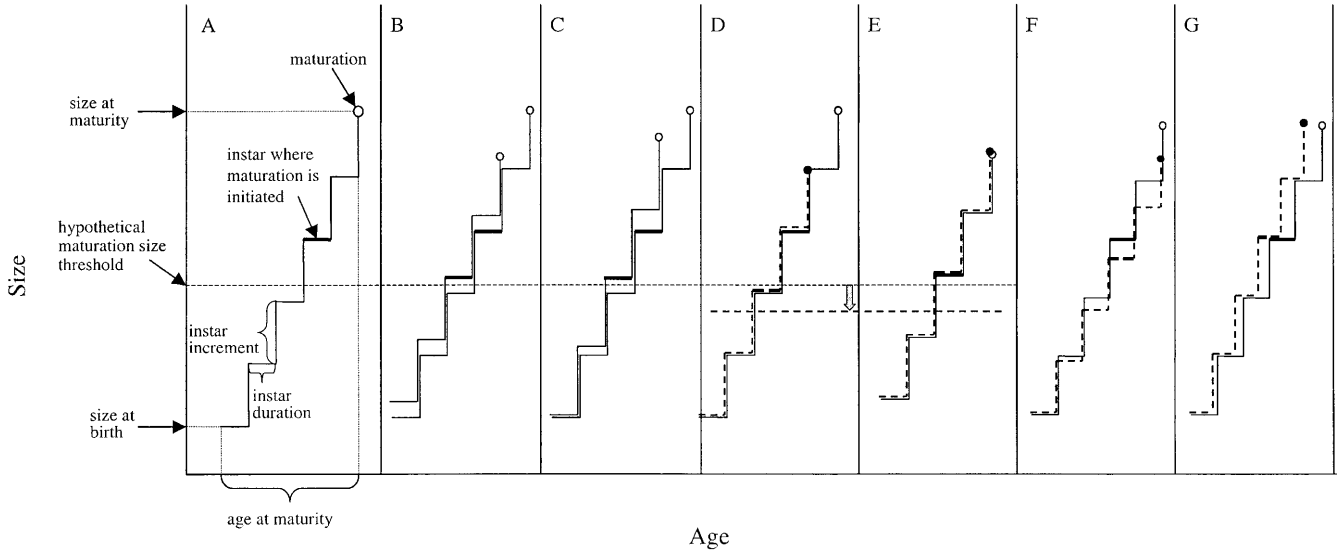

Fig. 1A–G Schematic representation of *Daphnia* life history and hypotheses about the mechanism generating phenotypic plasticity in size and age at maturity in response to fish cue. Life history in the presence of the fish cue is represented by *broken line*. **A** *Daphnia* grows in increments by molting. The moment when first eggs are deposited in the brood chamber is considered maturation. It always occurs at the beginning of the instar, very soon after molting. The process of maturation has to be initiated two instars before it is completed. There appears to be a size threshold (*horizontal broken line*) that has to be passed before maturation is initiated. Small differences in size at birth (**B**) or instar increment (**C**) may lead to large differences in age and size at maturity if the threshold is passed at the earlier instar. If fish cue lowers the threshold (*arrow*), an animal grown in the presence of fish cue (*thicker broken line*) could pass it in the earlier instar. Initiation of maturation and, consequently, maturation happens at an earlier instar. This leads to a large reduction in both size and age at maturity (**D**). However, if the size at birth happens to be larger (**E**), both individuals reach the threshold in the same instar, initiate maturation and mature at the same instar, and fish cue has no phenotypic effect. Irrespective of whether fish cue affects the threshold, and instar at maturation, it may affect instar increment. That would lead to reduced size at maturity, but not age at maturity (**F**). Similarly, if the fish cue effect were on instar duration, one would expect a reduction in age, but not size at maturity (**G**)

nating (Brakefield et al. 1996). Examining the mechanisms underlying phenotypic plasticity is essential for understanding its adaptive significance (Reznick 1990) and the limits to evolutionary change. Ideally, that should be done for organisms where the adaptive nature of plasticity could be evaluated through experimental evolution.

Daphnia has proven a useful model for studies of phenotypic plasticity in life history traits (Tollrian and Dodson 1999), and its small size, parthenogenetic reproduction, and short generation time make it particularly suitable for laboratory studies, including selection experiments. Because fish predation selects against larger individuals (Brooks and Dodson 1965), maturing earlier and at smaller size increases chances for successful reproduction when the predator is present. When the predator is absent, however, maturing at a larger body size is advantageous, for larger individuals have greater reproductive output. Lake-dwelling *Daphnia* species, such as *Daphnia galeata* Sars, nearly always co-occur with fish. Thus, size at maturity, a crucial determinant of fitness, has to be adjusted to the temporal fluctuations in predation pressure produced by large fluctuations in the populations of juvenile fish.

In *Daphnia*, growth is step-wise, and the instar where maturation occurs is not fixed (Fig. 1A). The eggs are deposited in the brood chamber at the beginning of the instar very soon after molting has taken place. This event is considered to represent completion of maturation, and the time when it occurs is defined as age at maturity, and the size of the instar when eggs are laid into the brood chamber as size at maturity. The initiation of maturation process, marked by visible ovary formation, occurs two instars before the maturation is achieved (Zaffagnini 1987). Thus maturation is a discreet event tied to step-wise growth (Ebert 1992, 1994, 1997). Analysis of phenotypic patterns of age and size at maturity suggests that there is no age threshold of maturation, but that there is a size threshold which has to be passed two instars before the maturation is achieved. Maturation at different instars could arise from small differences in size at birth or growth conditions (Fig. 1B, C), but it gives rise to dramatic consequences on life history because a difference of one instar at maturation corresponds to large differences in age and size at maturity. The continuous variation in size at birth and growth conditions is thus transformed into discreet variation among groups of animals maturing in different instars.

Several studies have shown that *D. galeata* matures earlier and at a smaller size in response to a waterborne chemical produced by fish (a kairomone) (Machácek 1991; Weber and Declerck 1997; Spaak et al. 2000). The fish cue provides reliable information about fish presence, and therefore increased risk for larger sized individuals, which strongly suggests that the observed response is adaptive. The question arises, how is this response generated in *Daphnia*? Preliminary observations and some indications from previous studies (Weider and

Pijanowska 1993; Machácek 1995) suggested that the mechanism of induction may consist in an increased chance of maturing at an earlier instar, which should result in earlier maturation at a smaller size (Fig. 1D). Although most studies do not report number of juvenile instars, earlier age and smaller size at maturity in response to fish cue are commonly found (Riessen 1999), which is consistent with that hypothesis. If the fish cue indeed increases probability of maturation at an earlier instar, it is plausible that all its other effects are a consequence of this shift. However, the kairomone may also affect the phenotype beside changes in instar at maturation. For example, the fish cue may decrease instar increments (Fig. 1F), which would lead to reduced size at maturity, also among animals maturing in the same instar. There is also a possibility that fish cue decreases instar duration (Fig. 1G). This would lead to earlier maturation but no reduction in size at maturity. In addition, size at birth may interact with the fish cue in determining how an individual reacts to fish kairomones (Fig. 1D, E). This is because larger neonates mature more often at an earlier instar, and instar at maturation has a major effect on age and size at maturity (Ebert 1991, 1994; Barata and Baird 1998).

Only a few studies on *Daphnia* have examined genetic variation in the plasticity of life history traits in response to fish cue. De Meester and Weider (1999) found substantial variation in plasticity in *D. galeata×hyalina* for all life history traits examined, while Spaak et al. (2000), in a study of the *D. longispina* species complex, did not find variation for plasticity in most traits. Boersma et al. (1999) found that populations of *D. magna* differ in plasticity, but it is unclear whether there was within population, among-genotype variation in plasticity.

My main goal here was to determine whether the reduction of size and age at maturity in response to fish cue arises because of increased probability of maturation at the earlier instar. I also examined other, (non-exclusive) possibility that effects of fish kairomone on age and size at maturity is also due to reduction of instar duration and increment. In addition, I investigated how the variation in size at birth modifies the response to fish kairomones. Finally, I studied genetic variation for plasticity in life history traits within a *D. galeata* population and explored how a specific *Daphnia* life history may affect its expression.

Materials and methods

The *D. galeata* clones used in this study were collected in the summer of 1998 in Lake Constance, Germany, and were kept in culture for about 2 years before the experiment started. Sixteen clones were used, with five or six replicates per clone, with total sample size of 162 animals. The replicates were lines grown for three generations in conditions identical to the experimental control. Each generation was initiated with one offspring per female. Thus the mothers, grandmothers, and great-grandmothers of experimental animals were treated identically. The experimental animals were offspring (less than 24 h old) from the second clutch. From each female, two siblings of similar size were taken. The first individual was selected randomly and its body size measured (in analysis referred to as "size at birth"). The second one was chosen to match the size of the first one. The experiment was started over a period of 3 days.

Animals were kept individually in 95 ml of modified AdaM medium (Klüttgen et al. 1994) (modified by using only 0.07 g l^{-1} SeO_2 solution and adding 20% water from a local well). The media were changed and food (3.5×10^4 cells ml^{-1} of green algae *Scendesmus*) was added daily. The water for the fish treatment was obtained by keeping two fish (*Leuciscus leuciscus* L., body length 7 cm) in a 10-l aquarium for 24 h; the control treatment received water from an identical aquarium but without fish. Both control and fish waters were filtered through a 0.2 µm filter before use. The aquaria were cleaned and refilled daily. The fish were fed frozen *Chironomid* larvae every second day in a separate aquarium.

The experiment was conducted at 16 °C and 16:8 h light/dark cycle. Every day the animals were checked for molting. Body size (measured from the top of the eye to the base of the tail spine) was measured for the first four instars. Size at maturity, defined as the body size of the instar where the eggs appeared in the brood chamber, was also measured. When the animals started maturing, they were checked every 12 h for the time of appearance of the first eggs in the brood chamber (age at maturity). The offspring from the first clutch were counted, and their body size was measured. Some animals died in the course of the experiment, and therefore, the degrees of freedom varied in the analysis of different traits.

Results

Instar at maturation

The effects of treatment and size at birth on instar at maturity were analyzed with binary exact logistic regression (Mehta and Patel 1995) using program LogXact (Cytel 1999). Clone was treated as a stratification variable. Most animals matured either at the 5th or the 6th instar, a few (seven) at the 7th and one at the 8th instar. I pooled data on animals maturing at the 6th, 7th and 8th instar into one group, "late instar", while animals that matured at the 5th instar are "early instar".

Daphnia in the fish treatment matured more often at the early instar than did control animals (Exact likelihood ratio=11.06, P=0.0003) (Fig. 2). Larger size at birth resulted in a significant increase in the probability of maturing at the earlier instar (Exact likelihood ratio=30.59, P<0.0001). Clone had a significant effect on instar at maturation (Monte Carlo estimate, Exact likelihood ratio= 61.97, P<0.0001). The model with size at birth and treatment as covariates and clone as stratification variable was highly significant (Likelihood ratio=50.49, df=2, P<0.0001, Deviance=50.26, df=76, P=0.99). The parameters for effects of size at birth and treatment were estimated with clone removed from the model, as estimation was not computationally feasible with the full model. However, the model which included only size at birth and treatment was still highly significant (Likelihood ratio= 39.17, df=3, P<0.0001, Deviance=12.63, df=17, P=0.76).

Overall treatment and clone effects

The traits analyzed as dependent variables included body size at birth, size at maturity, age at maturity, offspring

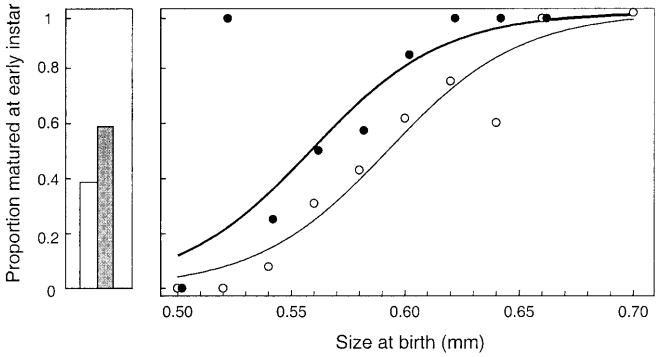

Fig. 2 The proportion of animals maturing at the earlier instar as a function of fish kairomone treatment and size at birth. *Open circles* and *empty bars* indicate control, *filled circles* and *shaded bars* indicate fish treatment. The *data points* are proportions calculated for size at birth classes. The *lines* are fitted by logistic regression; *thin line* represents control and *thick line* represents fish treatment. $P=1/[1+\exp(19.19–34.34\times\text{size-at-birth}+1.16\times\text{treat})]$ where P is probability of maturing at earlier instar; treat=1 for control, and treat=0 for fish treatment. These are maximum likelihood estimates

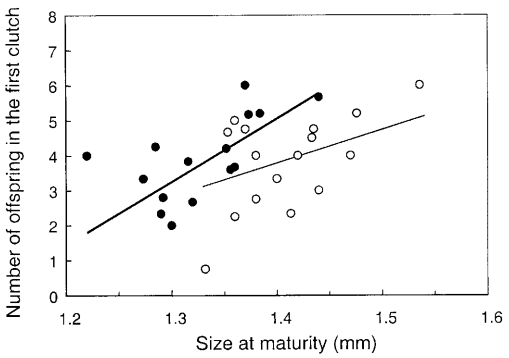

Fig. 3 Dependence of number of offspring produced in the first clutch on size at maturity. *Open circles* indicate control, *filled circles* indicate fish treatment. The *points* represent clonal means. Offspring=–20.21+(18.04×size-at-maturity)-(8.54×size-at-maturity×treat)+(10.7×treat). Treat=0 for fish and treat=1 for control. These are estimates from analysis of covariance

Table 1 The analysis of covariance for number of *Daphnia galeata* offspring in the first clutch. Clone and treatment are main effects, and size at maturity (*SAM*) is a covariate

Source	*df*	*MS*	*F*	*P*
Treatment	1	18.64	8.31	0.005
Clone	15	6.205	2.77	0.0014
SAM	1	67.93	30.29	0.0001
SAM × Treatment	1	9.839	4.39	0.039
Error	95	2.243		

number in the first clutch and offspring body size in the first clutch. Clonal variation in size at birth was analyzed with one way ANOVA as this trait was measured before the treatment was applied. For all other traits mixed model ANOVA (GLM, SAS 1993) was used with clone as a random and treatment as a fixed factor.

The overall effect of fish treatment conformed to the expectations. The animals in the fish treatment matured earlier than control animals (F=11.68, df=1,104, P=0.0009). Size at maturity (F=48,67, df=1,98, P=0.0001) and the offspring size from the first clutch (F=14.47, df=1,91, P=0.0003) were smaller in the fish treatment than in the control. The treatment had no effect on number of offspring in the first clutch (F=0.04, df=1,102, ns). However, when size at maturity was added to the model as a covariate, the positive effect of fish treatment on offspring number became apparent (Fig. 3, Table 1). There was significant clonal variation in all life history traits measured: size at birth (F=1.92, df=1,15, P=0.037), size at maturity (F=4.26, df=1,15, P=0.0001), age at maturity (F=11.68, df=1,15, P=0.0009), number (F=3.43, df=1,15, P=0.0001) and size (F=3.70, df=1,15, P=0.0001) of offspring from the first clutch. Although four univariate tests were performed for clone effects, the commonly used Bonferroni correction for multiple comparisons is not appropriate in this case, because the tests for various life history traits were not independent (Lynch and Walsh 1998, p 641). However, even if the correction were applied [here it would consist in dividing all the P-values by the number of performed tests (=4)], the results of all tests for clone effects would still be significant.

Variances were homogenous between treatments and among clones for all traits except offspring body size. Transformations did not improve this condition, and offspring size was excluded from more complex analyses. Welsch ANOVA (Sokal and Rohlf 1995), which takes into account heterogeneity of variances, confirmed that clones did differ in offspring size (F=2.97, df=15,28.07, P=0.006). For all traits, clone × treatment interactions were not significant with P-values larger than 0.2, and their removal from the model did not produce any qualitative changes in results. The effects of clone and treatment on survival (the number of animals surviving to first reproduction) were analyzed with logistic regression. Survival to first reproduction varied among clones (Monte Carlo estimate, Exact likelihood ratio=40.99, P=0.001) but was unaffected by treatment (Monte Carlo estimate, Exact likelihood ratio=0.80, P=0.48). The figure presenting means and standard errors of all analyzed traits for each of the clones is available in Electronic Supplementary Material.

Instar duration and increment

To determine whether the changes in instar duration and increment also contributed to overall changes in life history traits, it is necessary to look at animals from sibling pairs grown in the control and the fish treatment where both individuals from the pair matured at the same instar. Significant treatment effects on age or size at maturity for such individuals implies that, instar duration and increment were modified in response to the fish treatment, respectively. A total of 66 individuals belonged to the pairs where both siblings matured at the same instar. The

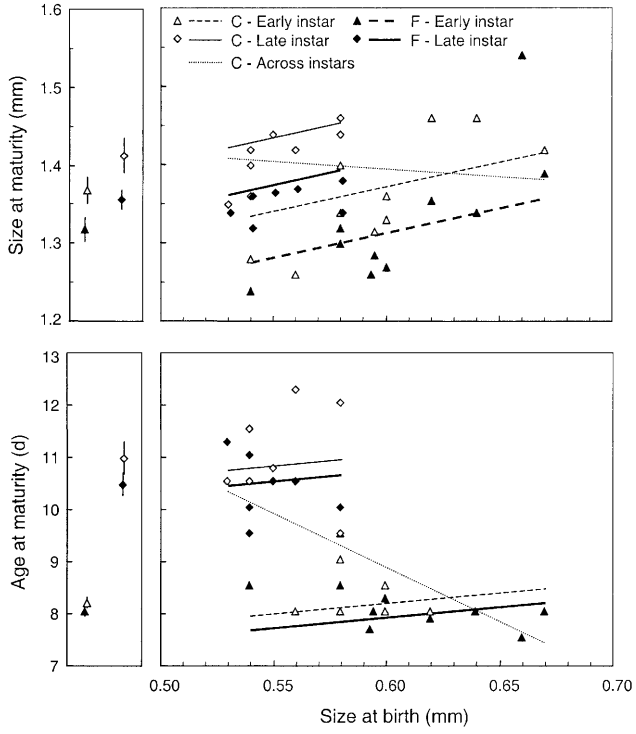

Fig. 4 Size and age at maturity as a function of treatment, instar at maturity, and size at birth. Only data on animals where both siblings, one grown in the control and the other in the fish treatment matured at the same instar, are included. *Data points* represent clonal means. On the *right panels* standard errors are given. Size at maturity= 1.395+(0.63×size-at-birth)-(0.094×instar)+(0.06×treat). Age at maturity=10.68+(4.05×size-at-birth)-(2.82×instar)+(0.28×treat). Instar=1 for early and instar=0 for late. Treat=0 for fish and treat=1 for control. These are estimates from analysis of covariance. *Dotted line* represents overall (across instar groups) dependence of size and age at maturity on size at birth. This is only shown for animals from the control treatment. Size at maturity=1.40-(0.19×size-at-birth). Age at maturity=9.22-(20.83×size-at-birth)

animals which matured at the fifth instar did so at a smaller size and earlier than those maturing at later instars (Fig. 4A). Within groups of animals which matured in the same instar, fish treatment resulted in maturation at smaller size than control (Table 2). The effect on age at maturity was not significant (Table 2), perhaps with only a tendency for earlier maturation (Fig. 4B).

If smaller increments in the presence of fish kairomone resulted from increased allocation to reproduction, fish cue should not have affected instar increments before the process of maturation was initiated. No allocation to reproduction should occur until two instars before maturation (see Fig. 1A). For the animals which mature at the earlier instar this is instar 3, and for those maturing later instar 4. To determine whether fish cue reduced instar increment before initiation of maturation, body size at instar 3 and 4 was compared for animals which matured in the earlier and later instar, respectively. Neither body size at instar 3 (F=1.59, df=1,25, ns) nor instar 4 (F=0.70, df=1,15, ns) was affected by the fish kairomone.

Table 2 The results of an ANOVA testing the effects of treatment, clone and instar at maturation on size and age at maturity in 16 *D. galeata* clones. Only the data on paired siblings, which both matured in the same instar are included. All the effects are tested over Error *MS*

Source	*df*	*MS*	*F*	*P*
Size at maturity				
Treatment	1	0.0562	24.65	0.0001
Clone	13	0.0128	5.63	0.0001
Instar	1	0.0263	11.52	0.0014
Error	49	0.0023		
Age at maturity				
Treatment	1	1.2959	2.99	0.09
Clone	13	0.6212	1.43	0.18
Instar	1	63.7400	146.98	0.0001
Error	50	0.4337		

Table 3 The analysis of covariance for size and age at maturity in 16 *D. galeata* clones. Clone and treatment are the main effects and size at birth is used as covariate. The results with and without the instar in the model are shown. Only the data on paired siblings, which both matured in the same instar are included. All effects are tested over error *MS*

Source	No instar in the model			Instar in the model		
	df	*F*	*P*	*df*	*F*	*P*
Size at maturity						
Treatment	1	20.74	0.0001	1	27.10	0.0001
Clone	13	4.26	0.0001	13	2.97	0.0001
Size at birth	1	0.97	0.33	1	5.80	0.02
Instar				1	17.15	0.0001
Error	49			48		
Age at maturity						
Treatment	1	1.58	0.21	1	2.96	0.09
Clone	13	2.47	0.01	13	1.53	0.14
Size at birth	1	28.81	0.0001	1	1.16	0.29
Instar				1	76.38	0.0001
Error	50			49		

Effects of size at birth

To investigate possible interactive effects of fish kairomone treatment and size at birth on age and size at maturity, these traits were analyzed with size at birth added to the model as a covariate. In addition, the analysis was repeated with instar at maturity also included in the model. Overall, size at birth had no effect on size at maturity, and its effect was revealed only when instar at maturity was added to the model: among animals which matured in the same instar, those which were larger at birth also matured at larger size (Fig. 4A, Table 3). In contrast, overall affect of size at birth on age at maturity was substantial; larger neonates matured earlier (Fig. 4B, Table 3). However, within groups of animals which matured in the same instar, size at birth had no effect on age at maturity. The interactions of size at birth with fish kairomone treatment were not important (P>0.2) and their removal from the models did not change the results shown in Table 3.

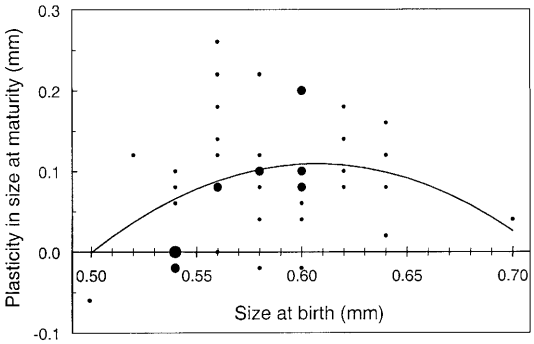

Fig. 5 The change in size at maturity (plasticity) in response to fish kairomones as the function of size at birth. The *points* represent single observations, which here are the differences between two individuals in a sibling pair. Multiple observations with the same values are represented by *points of larger size*. The *line fitted* is with the quadratic regression model. Plasticity=–3.43+(11.7×size-at-birth)-(9.6×size-at-birth^2)

Table 4 The results of an ANCOVA on plasticity in size at maturity in 16 *D. galeata* clones. Clone is the main effect, and size at birth and square of size at birth are covariates. Plasticity of size at maturity is the difference in size at maturity between paired siblings, one raised in control, the other in the fish treatment

Source	*df*	*MS*	*F*	*P*
Clone	15	0.00723	2.20	0.036
Size at birth	1	0.02711	8.23	0.008
Size at birth × size at birth	1	0.02621	7.96	0.009
Error	27	0.00329		

Analysis of plasticity

The differences between trait values in two environments can be analyzed as a trait itself. This type of analysis is more powerful in detecting genetic differences in plasticity (Scheiner et al. 1991; Ebert et al. 1993). For each pair, trait values for the individual in the fish treatment were subtracted from the corresponding trait values of its sibling in the control. These data were analyzed with one-way ANOVA with clone as the main factor. The same procedure was applied to data on instar at maturation. For example, if an individual in the fish treatment matured at instar 5, and its paired sibling in the control treatment at instar 6, the plasticity of instar at maturation for this pair is 1.

Clones did not differ in plasticity in any of the traits measured on a continuous scale (all *P*-values for clone effect were larger than 0.1). This confirmed the results of other analyses, where no clone × treatment interactions were significant. However, Fisher's Exact Test indicated that clones did differ in plasticity of instar at maturation (clone × instar plasticity P=0.009, sample size=49).

The results depicted in Fig. 3 suggested that size at birth has an effect on plasticity, and that this effect may not be linear, so that individuals of intermediate sizes at birth seemed to have produced the most plastic responses, at least with respect to instar at maturity. To investigate this, the effects of clone, size at birth and the square of size at birth on life history traits were analyzed. The data on 45 pairs were included here. Intermediate sizes at birth resulted in the greatest plasticity in size at maturity (Fig. 5). Clone effect was also significant (Table 4). Size at birth had no significant effect on plasticity of other traits.

Discussion

The mechanism of fish cue

The effect of fish cue on life history traits, consisting in reduced size and age at maturity, with no direct effect on clutch size, is consistent with earlier studies (see meta-analysis by Riessen 1999). The five most recently published studies (Sakwińska 1998; Boersma et al. 1999; De Meester and Weider 1999; Spaak et al. 2000; Stibor and Lampert 2000) confirm that pattern. As predicted, fish cue increased the probability of maturing at the earlier instar, which suggests that fish cue lowered the maturation threshold (Fig. 6). Animals that mature at the earlier instar are smaller and younger at maturity, and that is why shift in instar at maturation brought about a large reduction in age and size at maturity. Besides, size at maturity was reduced among the animals which matured in the same instar, but size at instar 3 was unaffected. This means that fish cue decreased instar increment but only after the onset of maturation had been initiated. Fish cue had no or little effect on instar duration, which means that it reduced age at maturity mostly because of its ´ effect on instar at maturation. Given specific range and little variation in size at birth, all animals may mature at the same instar irrespective of fish kairomone. This implies that whether any particular experiment detects an effect of fish cue on age at maturity depends on the range and variation in size at birth. The effect on size at maturity is bound to be more robust, because fish cue effect is apparent even within groups of animals which matured in the same instar. This finding is in agreement with earlier studies, where the effect on size at maturity is the most consistent one, while the effect on age at maturity has not always been found.

The fish kairomone effect on clutch size could be complex due to the often-found positive correlation between size of mother and number of offspring. In this study a large reduction in size at maturity in response to fish kairomones was found with no direct effect on first clutch size. This means that the relative clutch size increased, i.e. females of the same size produced more offspring in the fish treatment (Fig. 2). One might hypothesize that a decrease in size at maturity within instar at maturation was a consequence of higher allocation to reproduction found in animals exposed to fish treatment. Some studies have reported the significant increase in clutch size in response to fish kairomones; others did not find that effect, and only one (De Meester and Weider 1999) found a significant reduction, with meta-analysis

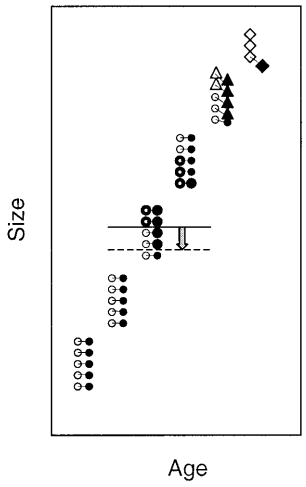

Fig. 6 Schematic representation and interpretation of the most important findings of this study. *Dots* represent the size at the beginning of each instar. Life history in the presence of the fish cue is represented by *filled symbols*. In the 3rd instar some animals initiated the maturation (*thicker dots*). Only those which are sufficiently large reach maturation threshold (*vertical line*). More animals in the fish treatment than in the control treatment reached the threshold, and initiated maturation, because the threshold was lowered in the fish treatment (*arrow* and *broken line*). As a consequence, more animals from the kairomone treatment reached maturity in the fifth instar (*triangles*). The remaining animals matured in the 6th instar (*diamonds*). In addition, animals from the fish treatment, which were identical at birth to their control counterparts, and matured at the same instar, were smaller at maturity, presumably because of higher allocation to reproduction. Thus reduction of size and age at maturity in the fish treatment resulted primarily from more frequent maturation at the earlier instar. In addition, a decrease in growth increments after the maturation had been initiated, brought about a reduction in size at maturity, even among animals which matured at the same instar

showing no overall effect of fish cue on this trait (Riessen 1999). However, whenever clutch size relative to the mother's size was considered (Weider and Pijanowska 1993; Stibor and Lüning 1994; Machácek 1995; Stibor and Machácek 1998), the effect of fish kairomone was to increase the clutch size. That could explain the inconsistencies among the studies. If the effect of fish kairomone on size at maturity were particularly strong, no or a negative effect on clutch size would have been found.

The effect of size at birth

This study confirmed the conclusion of earlier studies: for understanding the effects of size at birth on age and size at maturity, one has to take into account *Daphnia's* specific life history, in particular the phenomenon of maturation in multiple instars (Ebert 1991, 1994; Barata and Baird 1998). Larger neonates matured earlier because they were more likely to do so in the earlier instar. However, once instar at maturation was determined, size at birth had no longer an effect on age at maturity. On the other hand, size at maturity is largely independent on size at birth, presumably because of the existence of maturation size threshold. The positive correlation between size at birth and size at maturity existed only within the groups of animals which matured in the same instar. Lack of interaction between size at birth and instar at maturation indicates that, within such groups, size at birth had no effect on how the individuals reacted to the fish kairomones. However, considering the combined data set revealed that pairs of siblings which were of intermediate size at birth reacted the strongest to the fish cue in size at maturity. This is most likely because individuals from those pairs most often responded to the fish kairomone by changing the instar at maturation, which in turn resulted in a large change in maturation phenotype. However, the association between size at birth and plasticity may depend on the growth conditions and range of sizes at birth. In different food conditions, for example, size at birth can be large enough so that all the individuals mature at the same instar, which may result in disappearance of the association between size at birth and plasticity.

The effect of size at birth on plasticity could also well have a bearing on the response to the fish kairomones over a few generations. I found that offspring size was reduced in the fish treatment. Those offspring, if themselves exposed to fish kairomones, might not be able to respond to it by changing instar at maturation, and, as a consequence, they would not show a decrease in age at maturity, although size at maturity could still be reduced. In this situation, additional maternal effects independent of body size are a possibility (e.g., see Agrawal et al. 1999). However, this hypothetical scheme corresponds to finding of De Meester and Weider (1999) who exposed *Daphnia* to fish kairomones for two generations. They found a significant effect of fish kairomones on size at maturity, but not age at maturity in the second generation of exposure.

Genetic variation for plasticity

The *D. galeata* population from Lake Constance did not reveal much genetic variation in plasticity of life history traits in response to fish kairomone, as indicated by lack of clone × treatment interactions as well as lack of clone effects on plasticity. Other studies reported more genetic variation for phenotypic plasticity (De Meester and Weider 1999). This could represent a real difference in levels of genetic variation in plasticity between study populations, or the fact that a hybrid harbors more genetic variation than the parental species. However, it is difficult to determine whether either is really the case, for the experimental methods of the two studies differed. In the study by De Meester and Weider (1999) the animals were grown in treatment conditions for two generations, so that the mothers of individuals that were eventually measured experienced the same conditions as did their offspring. Detecting genetic variation in plasticity would be facilitated if the difference between control and fish treatment tended to be magnified by this procedure in some clones, but not in others.

Although standard analyses detected no genetic variation in plasticity, several additional considerations indicated that it might be present, but obscured by complex interactions with phenotypic variation. First, in analysis of plasticity where nonlinear effects of size at birth were taken as covariates, clone effect became significant, indicating genetic variation for plasticity in size at maturity. That analysis as well as the analysis of instar at maturation revealed genetic variation for plasticity. This is surprising, because plasticity in the instar at maturation should have a large effect on plasticity of size and age at maturity. In this situation one could expect that variation in plasticity expressed in continuous traits would have also been detected. Determining how natural selection acts on variation so expressed is a question worth investigating. It requires analysis beyond the scope of this paper.

Conclusions

The main mechanism of the *D. galeata* plastic response to fish kairomones consists of shifting to earlier instar at maturation, probably by lowering the maturation threshold size. The changes in the other traits could be attributed to a large extent to this shift. Changing the instar at maturation may provide a simple mechanism affecting many life history traits at the same time in a presumably advantageous direction. Apart from reducing the number of instars at maturity, fish cue decreased instar increments, but only after the maturation had been initiated. That might be related to a shift towards larger allocation to reproduction. Siblings which were of intermediate size at birth showed the largest response to the fish cue in size at maturity. This was most likely because individuals from those pairs most often responded to the fish kairomone by changing the instar at maturation, which in turn resulted in a large change in maturation phenotype. Understanding causes and consequences of maturation in multiple instars is essential for predicting the response of *Daphnia* life history traits to the fish kairomones.

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