Size-selective predation accounts for intra- and inter-specific variation of inducible morphological defense of *Daphnia*

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Abstract. Inducible defense is adaptive when prey organisms cope with fluctuations of predation risk, and there exists ample variations of inducible defense both within a species and between species even in a single habitat. *Daphnia*, a freshwater zooplankton genus, shows remarkable inducible morphological defense when exposed to predators. This study explored the intra- and inter-specific variations of inducible defense of *Daphnia* and tested whether the variations can be explained by the size-selective nature of predation. In Lake Fukami-ike, Japan, *Daphnia ambigua* and *Daphnia pulex* coexist with planktivorous fish and *Chaoborus* larvae that have different preferences for prey size. We experimentally examined the ontogenetic change of inducible defense for ten clones of each of the two *Daphnia* species against the two different predators. The degree of defense expression evaluated based on the induction of multiple morphological traits showed remarkable differences between *Daphnia* species, instars and clones, and the intra- and inter-specific variations were attributed to size-selective predation from the different predators. The inducible defense of *Daphnia* showed the adaptive variations depending on the predation pressure that resulted from the size-selective predation by the coexisting predators.

Key words: clonal variation; *Daphnia*; inducible defense; phenotypic plasticity; phenotypic trajectory analysis; size-selective predation.

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INTRODUCTION

Phenotypic plasticity is the ability of organisms to alter their phenotype when exposed to a different environment such as the onset of predation (e.g., De Witt and Scheiner 2004). The predator-induced phenotypic plasticity (i.e., inducible defense) effectively reduces predation risk and leads to the improved survival of prey individuals (Adler and Harvel 1990, Tollrian and Harvell 1999), although there exists ample variations of inducible defense both within a species (Spitze 1992, Hammill et al. 2008) and between species even in a single habitat (Spaak et al. 2000). Given the adaptive role of inducible defense in coping with predation, organisms are expected to generate appropriate phenotypes (morphological, behavioral, and life-history traits) in response to a predatory cue because plasticity should have inherent significant costs (De Witt et al. 1998).

Researches on the inducible defense of genus *Daphnia*, freshwater zooplankton, have contributed to our understanding of the ecology and evolution of inducible defense (Tollrian and Harvell 1999, Lass and Spaak 2003, Riessen and Gilbert 2019). Predation pressure on *Daphnia* is characterized by its size-selective nature (Dodson 1974, Tollrian and Dodson 1999, Riessen and...
Trevett-Smith 2009, Weiss and Tollrian 2018, Riessen and Gilbert 2019). In general, Daphnia species are exposed to two different types of predation risk in lakes, by invertebrates that are usually gape size-limited predators (e.g., Chaoborus larvae) and by planktivorous fish that are visually hunting predators. These predators are size selective, and their size preferences of prey are different. For example, Chaoborus larvae prefer a narrow range of small-sized prey (Swift and Fedorenko 1975, Pastrok 1981), whereas fish prefer larger prey due to the visibility of prey (Brooks and Dodson 1965). Therefore, the expression of inducible defense of Daphnia should be related to their body size and size-selective predation. For instance, the induction of neckteeth is more profound at the second and third instars compared to other instars of Daphnia pulex (Tollrian 1993, 1995, Imai et al. 2009, Riessen and Trevett-Smith 2009) because these instars are more vulnerable to Chaoborus larvae than other sized instars. Daphnia ambigua forms an inducible helmet at the tip of the head from first to forth instar in response to Chaoborus larvae (Hebert and Grewe 1985, Hanazato 1991a, b, Hanazato and Ooi 1992). Daphnia ambigua is a small species and is susceptible to Chaoborus predation even in the adult stages (Hanazato 1990). Not only neckteeth and helmet but also the body size itself of Daphnia is functional for defense, and Daphnia with smaller body size are less detected by visually hunting fish predators (Brett 1992, Weber and Declerck 1997, Boersma et al. 1999, Fisk et al. 2007, Carter et al. 2013). Moreover, Daphnia can express inducible defense throughout the life when their size is always within the preferred size range of their predators (Laflorsch and Tollrian 2004, Rabus and Laforsch 2011). These previous studies suggest the possible role of size-selective predation in explaining the variations of inducible defense. Our study tests the importance of the size-selective predation in explaining the intra- and inter-specific variations of inducible defense that can be found in Daphnia species inhabiting a single lake.

A single individual of Daphnia can induce several defensive morphological traits simultaneously (Boersma et al. 1998, Boeing et al. 2006). The actual combination of induced morphological traits depends on the predator species (Laflorsch and Tollrian 2004, Boeing et al. 2006), Daphnia species (Spaak et al. 2000, Laflorsch and Tollrian 2004), and varies among clones (Spitze 1992, Boersma et al. 1998). Boersma et al. (1998) revealed that most of D. magna clones can express a significant response in only one to four of the twelve defensive traits including morphological, behavioral, and life history ones that they examined. In addition, different D. pulex clones can show the different combinations of multiple defensive traits (Boeing et al. 2006). However, the relationship between multiple defensive traits and the size-selective predation has not been examined so far.

Induction of several defensive morphological traits would also be in accordance with size-selective predation, although there would be a variation in the combination of different traits among clones and species. Thus, it is important to quantify the overall response of defensive morphological traits or “a degree of defense expression” as a whole when examining the induction of several traits at the same time. The degree of defense expression of Daphnia would be influenced by both the size of Daphnia individuals and the size preference of different predators. Here we hypothesize three simple patterns of the ontogenetic change of inducible defense by multiple traits that are predicted by Daphnia body size and size-selective predation (Fig. 1), in which we assume that multiple defense traits are equivalent in the effectiveness against predators as the relative effectiveness of these traits are not well known. When all instars of Daphnia are of vulnerable size against a specific predator, the degree of defense expression should be constant along the ontogenetic growth of Daphnia (Fig. 1a), because the defense is advantageous for all instars. When Daphnia body size partially overlaps with the preferred size of predation, the degree of defense expression should show an increasing (Fig. 1b) or a decreasing (Fig. 1c) pattern along the ontogenetic growth of Daphnia, because the inducible defense is advantageous only when they are exposed to predation. These hypothesized patterns are rather simple for the sake of ease in statistical analyses, and the actual patterns might be more complex such as curvilinear or idiosyncratic one.

To test the hypothesis, we examined the ontogenetic change of inducible defense for ten clones of each of the two Daphnia species (small-sized
D. ambiguа and large-sized D. pulex) against two different predators (Chaoborus larvae and planktivorous fish), inhabiting Lake Fukami-ike (Nagano Prefecture, Japan). This lake is a eutrophic lake, and the two Daphnia species coexist with Chaoborus flavicans, exotic bluegill sunfish (Lepomis macrochirus), exotic largemouth bass (Micropterus salmoides), and native crucian carp (Carassius sp.). We measured multiple morphological traits of Daphnia individuals and calculated the degree of defense expression by a multivariate analysis as the difference between morphologies when exposed to predator kairomone (from either Chaoborus larvae or fish) and those in the control without kairomone. The preferred prey size of C. flavicans is smaller than that of planktivorous fish. Chaoborus larvae feed on smaller planktonic prey, and their preferred size of prey depends on gape size. Sell (2000) reported that the gape size is 0.455–0.741 mm for the fourth (last) instar of C. flavicans, and Shei et al. (1988) found that about 98% of Daphnia eaten by fourth instar of C. flavicans were <1.25 mm in body length. Thus, the Daphnia prey size (body length) suitable for Chaoborus larvae is considered to be less than around 1.25 mm. In contrast, planktivorous fish selectively feeds on larger prey due to the better visibility (O’Brien et al. 1976, Drenner and McComas 1980, O’Brien 1987). Scott (1987) reported that small juvenile cyprinids fish of 25–30 mm size could not efficiently feed on planktonic prey of 0.3 mm, compared to those of 0.9 or 1.2 mm in body width (0.3 mm body width roughly corresponds to 0.6 mm body length). Similarly, bluegill sunfish (L. macrochirus) and largemouth bass (Micropterus salmoides) prefer the larger Daphnia individuals (Bremigan and Stein 1994, Nunn et al. 2012). The above argument leads to the testable hypothesis for our experiment on the ontogenetic pattern of inducible defense for the two Daphnia species (Fig. 2a). For small-sized D. ambiguа, almost all instars are vulnerable against Chaoborus larvae, but only older instars are vulnerable against fish. This would result in the ontogenetic pattern of inducible defense as constant against Chaoborus larvae and increasing as they grow against fish (Fig. 2b). In contrast, for large-sized D. pulex, only small young instars are vulnerable against Chaoborus larvae, whereas almost all instars are vulnerable against fish, and older large instars are more visible and thus more vulnerable. This would result in the ontogenetic pattern of inducible defense as decreasing as they grow against Chaoborus larvae and increasing as they grow against fish (Fig. 2b). We tested the hypothesis on how inducible defense of Daphnia is related to size-selective predation by different predators.

Methods

Daphnia clones

Two Daphnia species (D. ambiguа and D. pulex) inhabit the studied lake, Lake Fukami-ike (35°32’ N, 137°81’ E; maximum depth is 7.75 m and surface area 2.1 ha) with their predators, C. flavicans (Nagano et al. 2015) and planktivorous fish (crucian carp Carassius spp., bluegill sunfish L. macrochirus, and largemouth bass Micropterus salmoides (Kawanobe and Hosoe 2010)). Ten clones of each Daphnia species were isolated from live samples collected by vertically towing a plankton net (mesh size 100 µm, diameter 20 cm) or from individuals hatched in the laboratory from diapausing eggs collected by an Ekman grab sampler (15 × 15 cm) from the bottom sediments. Daphnia clones were maintained in the laboratory for several months before used
for the later experiments. They were cultured in aged tap water and fed by the algal food, *Scenedesmus* sp.

**Induction experiment**

Prior to the experiment, the animals were acclimated to each treatment condition (control without predator kairomone, kairomone of *Chaoborus* larvae, or fish kairomone). For each treatment condition, we prepared three 200-mL glass jars for each *Daphnia* clone. Each glass jar was filled with the designated treatment water, and three female individuals of *Daphnia* were introduced to each jar and reared for two generations (grandmother and mother generations) in order to let them stably induce the morphological changes. Three neonates born from the mother generation were used as experimental animals for each jar, and they were introduced to a new jar with the fresh treatment water to initiate the experiment. Thus, for each of ten clones of each *Daphnia* species, we had three replicates for each treatment (two species × ten clones × three treatments × three replicates). The preculture and experiment were conducted at 22°C on a 16:8 light:dark cycle. Daphnids were fed by algal food (*Scenedesmus* sp.), and they were transferred every other day to a new jar with the fresh treatment water and the food at a concentration of $2 \times 10^5$ cells/mL (ca. 0.55 mg C/L). One of three individuals in each jar was gently picked up and photographed under a microscope to measure the body length, head length, and tail length according to Laforsch et al. (2006) and to examine the presence or absence of head spine and neckteeth. We assumed that animals in the same jar had shown the same reactions to the treatment. The measurements were conducted every day until the daphnids grew to the sixth instar, which took seven to ten days. After the quick measurement, the living individuals were put back to the original jar gently. In total, only a few experimental animals actually died during the experiment, and in such a case, the experiment continued by measuring other animals in the same jar. Instars of experimental animals were determined based on the body length–instar relationship that we obtained prior to the experiment and the stepwise increment nature of body length.

*Chaoborus* kairomone water was prepared according to Hanazato (1991a). Larvae of *C. flavidans* were collected from the lake by a plankton net. Fourth-instar larvae were picked up from the living samples and were reared in the aged tap water at a density of 30 ind./L for two days at 22°C. Small living cladocerans were provided daily as food (three prey individuals per larva).

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Fig. 2. Ontogenetic body length change and predicted degree of defense expression as a function of body length of two *Daphnia* species against two different predators (planktivorous fish and *Chaoborus* larvae). (a) Ontogenetic body length change of *D. ambiguа* and *D. pulex* resulting in different predation pressure from *Chaoborus* larvae (range of their gape size is shown in black line) and fish (their preferred size in blue arrow). (b) Predicted degree of plasticity of two *Daphnia* species based on Fig. 1 (a): *D. ambiguа* (upper panel) and *D. pulex* (lower panel).
Then, the water was filtered through a Whatman GF/C filter and stored in a freezer at −30°C. For the induction experiment, the kairomone water was thawed at a room temperature and was immediately diluted to one-third with the aged tap water to be used for the experiment (equivalent to the final density of 0.5 ind./L). Fish kairomone water was prepared mostly according to Reede (1995). Two individuals of fish (Carassius auratus with the body length of <5 cm) were kept in the aged tap water (4 L) for 24 h at a room temperature. As reported by Von Elert and Loose (2000), fish kairomone can induce defense of Daphnia irrespective of the fish species used, so that we used this cyprinid species for the experiment as the representative of the planktivorous fish in the studied lake. The fish were fed by frozen cladocerans. Then, the water was filtered through a Whatman GF/C filter without prior freeze and immediately diluted to one-third with the aged tap water to be used for the experiment (equivalent to the final density of 0 ind./L). For the control water, the dechlorinated, aged tap water without any predator kairomone was used without prior freeze.

Data analysis

Principal component analysis (PCA) was performed for the morphological traits of the two Daphnia species including body length, head length, tail length, and presence or absence of head spine and neckteeth after the data were Z-transformed (mean = 0, standard deviation = 1). We used body length instead of instar as some individuals did not survive until sixth instar, compared to that in the control. The total degree of defense expression for each clone was calculated as the sum of the degree of defense expression from the first to sixth instar, except for one clone (no. 6) of D. ambigua that some individuals did not survive until sixth instar. As mentioned above, we assume that multiple defense traits are equivalent in the effectiveness against predators. It would be more desirable if we could weigh the effectiveness of each trait and then evaluate the total effectiveness of plastic morphological changes, but it was not possible as the relative effectiveness of every trait we measured was unclear.

Generalized linear mixed models (GLMMs) were used to assess the effect of Daphnia body length on the degree of defense expression for the two kairomone treatments for each Daphnia species (the identity-link and Gaussian distribution function). We used a mixed model that has a random effect term for clone, as we measured repeatedly at the clone level. Our hypothesis shows the simple linear relationships (Fig. 2b),

\[
|\text{kairomone} - \text{control}| = \sqrt{(PC_{1\text{kairomone}} - PC_{1\text{control}})^2 + (PC_{2\text{kairomone}} - PC_{2\text{control}})^2 + (PC_{3\text{kairomone}} - PC_{3\text{control}})^2}
\]

If the degree of defense expression is zero, the clone has no induced morphology for the respective instar, compared to that in the control. The total degree of defense expression for each clone was calculated as the sum of the degree of defense expression from the first to sixth instar, except for one clone (no. 6) of D. ambigua that some individuals did not survive until sixth instar. As mentioned above, we assume that multiple defense traits are equivalent in the effectiveness against predators. It would be more desirable if we could weigh the effectiveness of each trait and then evaluate the total effectiveness of plastic morphological changes, but it was not possible as the relative effectiveness of every trait we measured was unclear.

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but we performed GLMMs that included a quadratic term of body length in order to account for a potential nonlinear relationship. Thus, the full model included body length and its quadratic form as fixed effects, and clone as a random effect. The most parsimonious model was selected based on the Akaike’s information criterion (AIC). Wald Z-test was used to test the significance of the coefficients and the intercept. To examine the clonal variation of the degree of defense expression, the correlation between the degree of defense expression for *Chaoborus* larvae and that for fish was calculated. All analyses were conducted using R v. 3.3.1 (R Development Core Team 2011) with the lmer and cor functions for GLMs and correlation analysis, respectively.

**RESULTS**

Two *Daphnia* species showed intrinsic ontogenetic changes in body length, and their lengths overlapped when *D. pulex* was at first to third instar and *D. ambigua* was at fourth to sixth instar (Fig. 2a). The range of the body length was 427–866 μm for *D. ambigua* and 558–1823 μm for *D. pulex*. The variation of morphology explained by the first principal component (PC1) was 42.8%, and PC1 was positively associated with body length and tail length (Appendix S1: Table S1). The second principal component (PC2) explained 23.0% of the variance, and it was associated negatively with the head length and the presence of head spine, and positively with the presence of neckteeth (Appendix S1: Table S1). The third principal component explained 18.6% of the variance, and it was associated negatively with the presence of neckteeth and positively with the head length (Appendix S1: Table S1).

Two *Daphnia* species formed different clusters in the PCA plot of their morphology and showed different phenotypic trajectories as they grew (Fig. 3a). The phenotypic trajectory analysis revealed that *D. ambigua* altered the ontogenetic change of the morphology when exposed to either *Chaoborus* or fish kairomone (Fig. 3b, Appendix S1: Table S2). The difference between the control and the kairomone treatment was all significant in the angular, size, and shape of the trajectories, except for the size difference between the control and the fish kairomone (Appendix S1: Table S2). In contrast, *D. pulex*
showed the different ontogenetic trajectory when they were exposed to *Chaoborus* kairomone, whereas fish kairomone did not influence the trajectory of this species (Fig. 3c, Appendix S1: Table S2). The angular, size, and shape were all significantly different between the control and the *Chaoborus* kairomone, but none of them was different between the control and the fish kairomone (Appendix S1: Table S2).

The degree of defense expression, calculated for each instar using the principal component scores in the control and the treatment, was or was not related to the body length depending on the combination of *Daphnia* species and the type of predator kairomone (Fig. 4, Appendix S1: Table S3). The degree of defense expression of *D. ambigua* (Fig. 4a) when exposed to *Chaoborus* kairomone was not related to body length, as the most parsimonious model explaining the data was the model with an intercept and clone as a random effect, and other models including the body length effects were poorly supported (Appendix S1: Table S3). In contrast, the degree of defense expression of *D. ambigua* when exposed to fish kairomone increased with body length, as the most parsimonious model had a significant coefficient of body length (Appendix S1: Table S3). On the other hand, the degree of defense expression of *D. pulex* (Fig. 4b) showed the opposite pattern to that of *D. ambigua*. The degree of defense expression when exposed to *Chaoborus* kairomone was related to the body length, and it peaked at the second instar and then decreased as they grew. The most parsimonious model included a significant effect of body length, although the model with a quadratic term of body length was not selected as the top (Appendix S1: Table S3). In contrast, the
degree of defense expression was not related to body length in the fish kairomone treatment, as the most parsimonious model was the one without the body length effect (Appendix S1: Table S3).

The clonal variation of the degree of defense expression also differed between the two Daphnia species (Fig. 5). Among the clones of D. ambigua, the degree of plasticity in the fish and the Chaoborus treatments were not correlated ($r = 0.36$, $P = 0.31$, Fig. 5a), whereas those in D. pulex were positively correlated among the clones ($r = 0.79$, $P = 0.004$, Fig. 5b).

**DISCUSSION**

This study examined the variation in the degree of defense expression among Daphnia species, instars and clones, and demonstrated that the variation in the degree of defense expression was remarkably related to their size as a prey and the size-selective predation. Although previous studies have shown that some specific morphology of Daphnia can be induced when exposed to predator, our multivariate analysis showed that defensive morphology of Daphnia was frequently induced as predicted by the size-selective predation. For the predation by Chaoborus larvae, inducible defense is advantageous when daphnid size is susceptible to the predation by Chaoborus larvae (smaller than about 1.25 mm, Shei et al. 1988): All instars from first to sixth instar of D. ambigua were smaller than 1.25 mm, whereas only young instars of D. pulex were smaller than that. For the fish predation, inducible defense is advantageous when daphnid size is larger than the minimum prey size that fish can attack: Only older instars of D. ambigua were larger than the minimum prey size, whereas all instars of D. pulex were susceptible to fish predation. As predicted, the degree of defense expression of D. ambigua against Chaoborus larvae was relatively high and constant as they grew, and that against fish increased as the size of D. ambigua increased ontogenetically (Fig. 4a). Also, the degree of defense expression of D. pulex against Chaoborus larvae decreased as they grew. On the other hand, that against fish was constant over the range of the body length, although older instars of D. pulex are hypothesized to express more defense as they are more vulnerable to planktivorous fish (Fig. 4b). Size-selective predation is widely found to be relevant in other prey organisms including larva of dragonfly (Arnqvist and Johansson 1998), stickleback (Reist 1980), and tadpole (Semlitsch 1990), and the degree of defense expression can be different depending on the prey size as in dragonfly larvae (Arnqvist and Johansson 1998) and mayfly (Dahl and Peckarsky 2002). Adding to the previous studies, our study showed that the intra- and inter-specific variations in inducible defense of
Daphnia can be attributed to size-selective predation from different predators. In the studied lake, the two Daphnia species coexist with planktivorous fish and Chaoborus larvae, and the present results showed the adaptiveness of their inducible defense that varied depending on the predation pressure resulting from the size-selective predation. When understanding the nature of inducible defense, evaluating multiple traits of single individuals rather than a single remarkably changing trait was important as the previous studies suggested (Relyea 2001, Kishida and Nishimura 2005, Kishida et al. 2010).

Although the results were mostly in accordance with predictions, some of them were not explained by the size-selective predation. First, the degree of defense expression of D. pulex against fish was generally smaller and did not increase as they grew contrary to the prediction (Figs. 2b, 4b). Daphnia species can induce defense traits other than morphology, such as defensive behavior (e.g., vertical migration [De Meester et al. 1999]) and life-history trait (e.g., age and size at maturity [Hanazato et al. 2001, Weetman and Atkinson 2002]). Thus, D. pulex inhabiting the studied lake might induce other behavioral or life-history traits when exposed to fish predation; otherwise, they would not be able to coexist with fish predators in the lake. How these different defense strategies of morphology, behavior, and life history are combined to cope with predation is the interesting question (Boersma et al. 1998), but it remains unknown for the D. pulex population in the studied lake. Second, although the first instar of D. pulex is the smallest and more vulnerable against Chaoborus larvae than other older instars, the degree of defense expression against this predator was lower than second instar (Fig. 4b). This result is in line with the previous studies (Tollrian 1993, 1995, Riessen and Trevett-Smith 2009) that showed the induction of neckteeth was more profound at the second and third instars compared to the first one, although it is also the case in the multivariate phenotypic space examined in this study. The first instar might have lower encounter rate with predators owing to the slower swimming speed than other older instars (Dodson and Ramcharan 1991), so that the first instar with the smallest body size may not necessarily be most vulnerable against Chaoborus larvae compared to older instars.

Alternatively, for the very small-sized first instar, morphological defense can be completely ineffective in preventing predation by Chaoborus larvae or they can be too small to be eaten, leading to the absence of induced defense of the first instar (Riessen and Trevett-Smith 2009). In any case, the ontogenetic pattern of inducible defense can be more complex than we hypothesized in this study, although the size-selective nature of predation has crucial influences on the patterns.

Intraspecific variation of inducible defense of Daphnia has been found among clones (Weider 1984, Havel and Dodson 1984, 1987, Spitze 1992, Boersma et al. 1998, Declerck and Weber 2003, Duffy 2010, Dennis et al. 2011, Stoks et al. 2016), instars (Hanazato andb1990, 1991a, Hanazato and Ooi 1992, Tollrian 1995, Imai et al. 2009), or populations (Barry and Bayly 1985, Havel 1985, Parejko and Dodson 1991, Spitze 1992, Boersma et al. 1998, Declerck and Weber 2003, Boeing et al. 2006, Dennis et al. 2011, Miyakawa et al. 2015, Reger et al. 2017). The intraspecific variation among Daphnia populations has been considered as a result of local adaptation to local predation pressure that differs from lake to lake (Reger et al. 2017). Similar local adaptation to local predators is known in other organisms such as marine snails (Littorina obtusata) (Trussell 2000, Trussell and Smith 2000, Trussell and Nicklin 2002) and tadpoles (Kishida et al. 2007). The degree of defense expression exhibited by the studied two Daphnia species was also considered to be the local adaptation to different predators in the studied lake. In the previous studies of Daphnia, however, every defensive trait was not always found to be induced in an adaptive way. For example, the neckteeth of D. pulex seems to be almost always induced when they are exposed to Chaobours larvae (Tollrian 1995), whereas the neonate body size of the same species lacks the fixed pattern of induction when exposed to the same predator. The body length sometimes increases (Krueger and Dodson 1981, Havel and Dodson 1984, Riessen and Sprules 1990, Lüning 1992, Spitze 1992) which is thought to be adaptive against Chaoborus predation, or in other cases it decreases (Havel and Dodson 1987, Ketola and Vuorinen 1989, Boeing et al. 2006) which would be maladaptive. This contradictory pattern would be produced because of the development constraint or fitness cost (De Witt et al.
that can obscure the induction of some specific defensive trait. Thus, the local adaptation to the local predators should be better examined in the multivariate phenotypic space as performed in this study and previous studies (Krueger and Dodson 1981, Parejko and Dodson 1991, Boersma et al. 1998, Boeing et al. 2006, Hammill et al. 2008, Dennis et al. 2011); otherwise, we may not be able to observe the actual nature of the induced defense (Kishida et al. 2010). Although clonal variation in inducible defense has been commonly found (Weider 1984, Havel et al. 2008, Dennis et al. 2011), this study found different patterns of intraspecific variation of plasticity between the two species (Fig. 5). For D. pulex, the population consisted of well-defended and less-defended clones against two different predators, whereas there was no correlation in the degree of defense expression among clones of D. ambiguа. There might be some fitness tradeoff among D. pulex clones in the inducible morphological defense and other fitness components associated with resource competition and other defense strategy in behavior and life-history traits; otherwise, the coexistence of these clones would be difficult. In such a case, using these well- and less-defended clones, it may be possible to measure the cost of inducible defense, which is usually difficult to measure empirically (Van Kleunen and Fischer 2005). In any case, why there was the genetic correlation in the degrees of inducible defense expression in D. pulex but not in D. ambiguа is the interesting question that remains to be studied in future.

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