Interpopulation differences in developmental reaction norms of both predator and prey determine their trophic interaction

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Running head: Reaction norms in trophic interaction

Abstract

Phenotypic plasticity as a type of reaction norm creates variation in ecological interaction strengths across different environmental conditions. If populations of both interacting species (e.g., predator and prey) differ in the plasticity of their ecological traits, populations of both interacting species and environmental conditions can jointly determine their interaction strength. To examine this untested prediction, we experimentally investigated how geographic populations of both predator and prey species with differential reaction norms of offensive and defensive morphological traits, respectively, establish their trophic interactions, using salamander larvae (*Hynobius retardatus*) and their prey frog tadpoles (*Rana pirica*). Past studies showed that gigantism of salamander larvae as a result of cannibalism in their hatchling period triggers a salamander–tadpole trophic interaction because only gigantic salamanders with remarkably enlarged gape can swallow the large prey, frog tadpoles with an inducible morphological defense. By manipulating combinations of two geographic populations of the salamanders and tadpoles (*i.e.* Erimo and Chitose) and cannibalistic conditions of salamander hatchlings, we examined how developmental reaction norms of both predator and prey shape this trophic interaction. We found that geographic population identity of both salamander and tadpoles and the cannibalistic condition of salamanders interactively determined the trophic interaction strength between salamanders and tadpoles. Under cannibalistic conditions, giant salamander larvae emerged, and gigantism was more prominent in Erimo than Chitose salamanders. While the greater emergence of cannibalistic giant salamander larvae in Erimo salamanders resulted in significant predatory impacts on the prey tadpoles, the predatory impacts by cannibalistic Erimo salamander was larger for Chitose tadpoles than Erimo tadpoles because of Chitose tadpoles' lesser ability to develop morphological defenses. This study demonstrates that developmental

reaction norms of interacting partners interactively determine ecological interaction, and therefore suggests that genetic differences in reaction norms among geographic populations of both interacting partners jointly shape variation in the interaction strength among geographic regions.

Key words: Genotype×Genotype×Environment interplay; inducible offense; inducible defense; morphological plasticity; intraspecific variation; β-diversity; arms race; coevolution

Introduction

The factors determining the strength of ecological interactions have been a major interest in ecology because ecological interactions are principal mechanisms determining demography of the species involved in the interactions, and its effects propagate through the ecosystem (Nakano et al. 1999, Persson et al. 2007, Ushio et al. 2018). Although interaction strengths depend on the density of the interacting species, it is also strongly affected by the functional traits of the interacting species (Sinclair et al. 2003, Schmitz et al. 2015). Since expression of functional traits vary among species, species-specific values of functional traits (i.e. mean) have been a focus when disentangling community structure and dynamics (McGill et al. 2006, Schmitz et al. 2015). However, values in some key functional traits can vary considerably within species and this intraspecific trait variation can be involved in generation of complicated variation in the strength of ecological interactions (Miner et al. 2005, Miller and Rudolf 2011). Therefore, intraspecific trait variation is increasingly recognized as an important factor in determining community structure and dynamics (Bolnick et al. 2011, Des Roches et al. 2018, Raffard et al. 2018).

The effects of intraspecific trait variation on ecological interactions have been mainly investigated in terms of three interacting elements: size, phenotypic plasticity, and genotype. The first aspect is size variation among individuals. Because multicellular organisms generally shift their interacting partners as they grow larger, a species' size composition is a factor determining the types and strength of their ecological interactions (Persson et al. 2007, Miller and Rudolf 2011, Yamaguchi et al. 2016). Second is phenotypic plasticity as a form of reaction norm. Since individuals change their functional traits (e.g., behavior, life history and morphology) in response to different environmental conditions, interaction strengths can be altered by plastic responses of individuals to the environmental conditions they face (Miner et al. 2005, Winterhalter and Mousseau 2007, Kishida et al. 2014). For example, while many prey species enhance expression of defensive traits in the presence of a specific predator to effectively protect them against predators (*i.e.* inducible defense), some predator species enhance expression of offensive traits in the presence of particular prey items to effectively catch and consume them (*i.e.* inducible offense, Kishida et al. 2010). Both the expression of defensive traits of prey and offensive traits of predators can strongly determine the sign and strength of predator-prey interactions (Kishida et al. 2009b, 2014). The third context for intraspecific variation effects on ecological interactions is genetic trait variation. Expression of functional traits itself is under genetic control (Miner et al. 2005, Pigliucci 2005, Winterhalter and Mousseau 2007). If the value of a functional trait is different among genetic populations, these populations can differ in the strength of their ecological interactions (Yoshida et al. 2003, Hiltunen and Becks 2014, Bassar et al. 2017).

The importance of each of the entities to shape ecological interactions has been evidenced by numerous studies (Bolnick et al. 2011, Miller and Rudolf 2011, Des Roches et al. 2018). Although the effects of these entities have been considered independently, they also tightly link to each other. For morphological traits, in particular, we can acknowledge clear linkage among size variation, phenotypic plasticity and genetic variation. Changes in whole body size (i.e., ordinal isometric growth) or the size of organ parts (i.e., allometric growth) are sometimes facultative rather than constitutive (Kishida et al. 2006, Gerber et al. 2008), and such plastic change in morphological traits itself is under genetic control (i.e., reaction norm perspective, Miner et al. 2005, Pigliucci 2005). Hence, the ability to develop functional morphological traits can genetically differ among geographic populations (Kishida et al. 2007, Winterhalter and Mousseau 2007). Consequently, the strength of ecological interactions is determined by a combination of geographic populations and environmental conditions (i.e., G × E effect on interaction strengths) (Yamamichi et al. 2019). Although this integrative view is quite intuitive, there is little evidence of the expected effects of genetic variation in reaction norms of morphological traits on ecological interactions (Kasada et al. 2014). The first objective of this study is to test this prediction.

When investigating ecological interactions, focusing on the functional traits of both interacting partners rather than either one alone is essential because the sign and strength of the interaction should be determined by the performance of both interacting partners (Hiltunen and Becks 2014, Bassar et al. 2017). For example, in predator-prey relationships, the consequence of trophic interactions is determined by a balance of the predator’s foraging performance and prey’s defensive performance (Takatsu & Kishida 2013). If populations of both predator and prey differ in their functional trait reaction norms, populations of both interacting species and environmental condition can jointly determine their interaction strength. Furthermore, the reaction norms of both predator and prey may interactively operate on the interaction strength because functional trait values of an interacting partner affect expression of functional traits of the opponent

(Kishida et al. 2010). For example, some prey species intensify expression of their defensive traits when exposed to dangerous predators with more offensive traits (Kopp and Tollrian 2003, Kishida et al. 2006). This implies that populations of both interacting species and environmental conditions can interactively shape geographic variation in interaction strength (i.e., G × G × E interactive effects on ecological interaction). Although testing this prediction is important to advance our integrative understanding of the mechanisms shaping geographic variation in ecological interactions in natural systems, to our knowledge, this remains untested. We thus aimed to additionally test this prediction.

To test both predictions, we conducted an experiment using a trophic relationship between predatory larval salamanders (*Hynobius retardatus*) and prey frog tadpoles (*Rana pirica*) as a model system. Their trophic interaction is an excellent system to accomplish this objective, because both predator and prey species have remarkable phenotypic plasticity in their functional morphological traits (i.e., size of gape and body parts for salamanders and tadpoles, respectively) in the trophic interactions. While *H. retardatus* salamander larvae can exhibit gigantism characterized as enlarged gape in cannibalistic conditions (inducible offense), prey tadpoles can exhibit body enlargement by thickening epithelium tissue as an inducible defense. Importantly, expression ability of the antagonistic phenotypes vary among the geographic populations of both species (Michimae and Wakahara 2002, Kishida et al. 2007). To examine whether and how genetic differences in developmental reaction norms of predatory salamander and prey tadpoles shape their trophic interactions, we conducted an experiment in which combinations of two populations of the predatory salamanders and prey tadpoles and initial condition relevant to the emergence of gigantism of salamanders were manipulated.

Materials and Methods

Study System

Hynobius retardatus salamanders and *Rana pirica* frogs usually spawn in small ponds in early spring in Hokkaido, Japan. Although salamander larvae are carnivores, the trophic relationship with tadpoles is not always established even if the larvae of both species cohabit the same ponds. Since tadpoles typically hatch 3–4 weeks earlier than salamanders, tadpoles are too large to be consumed by salamander hatchlings (Nosaka et al. 2015). Therefore, a predator-prey interaction between two species occurs when salamander larvae grow rapidly. Typically, the rapid growth of salamander larvae is caused by cannibalism during their hatchling stage (Takatsu and Kishida 2015). Salamander hatchlings that successfully consume conspecifics tend to grow rapidly and become ‘giants’ with much larger body and gape. The emergence of cannibalistic giants occurs more frequently in the presence than absence of tadpoles, since disturbance effects of tadpoles enhance cannibalism among salamander hatchlings (Takatsu and Kishida 2020). Importantly, relative gape to body size of the cannibalistic giants is greater than that of the non-cannibalistic salamanders. That is, the cannibalistic salamanders have an extremely large gape (i.e., inducible offensive phenotype) and, hence, individuals with the offensive phenotypes can consume tadpoles as alternative large prey items (Takatsu and Kishida 2015).

Antagonistically, frog tadpoles exhibit inducible defense in this system. In the presence of the salamander larvae, frog tadpoles enlarge body and tail by thickening their epithelium tissue. This ‘bulgy’ phenotype makes it harder for the salamander larvae to swallow them (Kishida and Nishimura 2004). Importantly, expression of the defensive bulgy phenotype depends on predation risk. The tadpoles exhibit bulgier phenotype in the presence of offensive giant salamanders compared to non-offensive ones (Takatsu and Kishida 2015, Takatsu et al. 2017).

Thus, larvae of the two amphibian species exhibit antagonistic morphological plasticity, represented by condition-dependent allometric growth in functional traits (i.e. gape of salamanders and body of frog tadpoles), that greatly affects trophic interactions between them (Kishida and Nishimura 2004, Takatsu et al. 2017).

The potential to become an offensive giant salamander and to express the defensive bulgy phenotype in tadpoles differ among their geographic populations (Michimae and Wakahara 2002, Kishida et al. 2007). In particular, there is large variation in the emergence of offensive giants among the geographic populations of salamanders (e.g., Michimae 2006). Based on knowledge of the geographic variation in inducible offense of salamanders, we selected two localities of amphibians as experimental model populations: Erimo and Chitose. Compared to Chitose population of salamander (hereafter Chitose salamander), Erimo population (hereafter Erimo salamander) is characterized by higher frequency in the emergence of offensive giants (Michimae 2006; Atsumi and Kishida, unpublished data). Using these two populations of salamanders allows us to test our predictions, even though we had no prior knowledge about the geographic variation in the expression ability of defensive phenotype in tadpoles between these two geographic populations. We conducted the following experiment using the two geographic populations of salamanders and tadpoles by collecting their eggs from several ponds located in the Erimo (seven ponds around 42°6’ N; 143°16’E) and Chitose (three ponds around 42°48’ N; 141°35’ E) regions. Collection and keeping methods of experimental animals are described in Appendix S1.

Experimental Setting

The experiment was conducted in an experimental room in Tomakomai Experimental Forest, using semi-transparent polypropylene 22-L tanks (51.3 cm × 37.2 cm × 16.6 cm) of which the bottom was covered by sand to a depth of 2 cm as benthic substrate. Minimum natural water (ca. 10 ml per minute) was constantly supplied into each tank by using thin polypropylene hose and flowed out into an overflow pipe ($\varphi = 20$mm, 4cm height) set inside of the tank. The overflow pipe was covered with mesh net (mesh size 1mm) to prevent the experimental animals flowing out. Natural water was drawn using a pump from a well that is 5m away from a natural river. The overflow pipe kept water depth (from sandy bottom to water surface) at 4 cm. Two leaves of Japanese bigleaf magnolia (*Magnolia obovata*) (dry weight: 5 g) were provided as biotic structures.

To examine how genetic variation in developmental reaction norms of predatory salamander and prey tadpoles shape their trophic interactions, we conducted an three-way factorial experiment in which combinations of the two geographic populations of the predatory salamanders and prey tadpoles (i.e., Erimo and Chitose populations) and cannibalistic conditions that possibly affecting emergence of the offensive giant salamanders were manipulated (i.e., Cannibalism and No-cannibalism treatments). We haphazardly placed 30 three-week-old tadpoles that originated from either the Erimo (mean±SD snout-vent length, 7.32±0.60 mm, N = 20) or Chitose population (7.10±0.53 mm, N = 20) into each of all 80 tanks on May 17, 2018 (defined as day 1).

Cannibalistic conditions were controlled by manipulating size structure of salamander hatchlings; greater size heterogeneity among salamander hatchlings facilitates cannibalism (Kishida et al. 2015). Following a well-established method (Takatsu & Kishida 2015, Takatsu et al. 2017), we manipulated size structure of salamander hatchlings by using individuals that hatched at different times (i.e., early- or late-hatchlings) while keeping the total initial density constant across treatments. We set the following two treatments: (1) the “Cannibalism” treatment received 5 early- and 10 late- salamander hatchlings to create initial size heterogeneity and (2) the “No-cannibalism” treatment received either 15 early- or 15 late- salamander hatchlings. For this manipulation, we obtained the early- and late- salamander hatchlings by controlling the water temperature experienced by the embryos from a single egg cluster. The difference in hatch timing between early- (16th May) and late- (24rd May) salamander hatchlings was 8 days. The salamander hatchlings were assigned to the relevant treatments 1 day after they hatched (total length at day 8, N = 20 each: 25.36±2.40 mm and 20.33±1.16 mm in Erimo early and late hatchlings; 20.97±2.08 mm and 17.08±1.30 mm in Chitose early and late hatchlings). The method for obtaining the early and late- hatchlings is described elsewhere (Takatsu and Kishida 2015, Takatsu et al. 2017, Takatsu and Kishida 2020).

Although No-cannibalism treatment consisted of the two specific conditions (i.e., either early hatchlings only or late hatchlings only), we pooled the data of the two alternative hatch timing conditions, because our preliminary analyses showed non-significant effects of hatch timing of salamanders on survival of both species in the No-cannibalism treatment (Appendix S2) . Note that to avoid excessive use of the animals, we did not prepare a tadpole-only treatment for estimating background mortality of tadpoles. This is acceptable since previous studies repeatedly showed that mortality of tadpoles in the absence of predators was negligibly low in similar experimental settings (Nosaka et al. 2015; Takatsu & Kishida, 2015; Yamaguchi et al. 2016; Takatsu et al. 2017; Takatsu and Kishida 2020). Throughout the experiment, we added a piece of rabbit chow (dry weight: 0.2 g) and 100 frozen *Chironomid* larvae to all tanks on every Monday, Wednesday and Friday as an alternative food for the tadpoles and the salamanders, respectively. The experimental room was maintained under natural light-dark (ca. 14h/10h) conditions and water temperature ranged from 13 to 20 °C. At day 11, 18, 25, 32 and 39, we counted all surviving tadpoles and salamanders. We defined the period between successive days on which we counted surviving individuals as the experimental period: 1st (day 1-11), 2nd (day 11-18), 3rd (day 18-25), 4th (day 25-32) and 5th period (day 32-39). From the count data, we calculated survival rate of the salamanders and frogs in each experimental period by dividing the number of survivors at the end of each period by that at the beginning of the period. In addition, we photographed the dorsal side of surviving animals using a digital camera (Panasonic Lumix DC-TZ90) at the end of 2nd period (day 18) just before predation by salamanders on tadpoles began. The experiment was ended on day 39 because metamorphosis of tadpoles began in some tanks. Our conditions for hatch timing and density of the two amphibians species are relatively high but within a range in the natural habitats (Michimae 2006; Nosaka et al. 2015).

Statistical analyses – cannibalism and interaction strength

We investigated how the time-trajectory of the strength of salamander cannibalism and predator-prey interaction between salamander and tadpole was shaped by the three factors: Cannibalism condition, and populations of salamander and tadpole. Previous studies have shown that temporal change in the strength of cannibalism and tadpole consumption depends on the initial size structure of salamanders; when salamander hatchlings are uniform in their size, cannibalism and tadpole consumption rarely occurs and thus survival rate of two species are constantly high. In contrast, when size variation among salamander hatchlings is large, cannibalism occurs and subsequently tadpole consumption occurs as a result of emergence of offensive giant salamanders (Takatsu and Kishida 2015). Once offensive giant salamanders consume tadpoles, their predation become intense through time because consumption and growth of salamanders positively feedback (Nosaka et al. 2015). This process of the trophic interaction has been shown in experiments by using some geographic populations of salamanders and tadpoles which were different from those used in the present study (Takatsu and Kishida 2015, 2020, Takatsu et al. 2017). If cannibalism occurrence and emergence of offensive giants of salamanders and/or defensive performance of tadpoles are not uniform among the geographic populations, the temporal dynamics of salamanders' cannibalistic interaction and trophic interaction with tadpoles should vary depending on a combination of cannibalism condition and the populations of salamanders and tadpoles. We therefore predicted that salamander size structure and populations of two species jointly determine the time-trajectory of cannibalism and tadpole consumption strength. To investigate this prediction, we fit a linear mixed model with normal errors (LMM) to natural logarithms of salamander and tadpole survival rate in each experimental period (see below). The explanatory variables were the three factors above (i.e., cannibalism condition of salamanders, salamander population, and frog population), experimental period (centered [Schielzeth 2010]), and their interaction. Experimental tank identity was included as a random effect because we measured survival rate five times in each tank. We thus expect a significant effect of the interaction among cannibalism condition (i.e. $E_{\text{cannibalism}}$ representing Cannibalism and No-cannibalism treatment), the population of two species (i.e. $G_{\text{salamander}}$ and G_{tadpole} representing Erimo and Chitose populations) and experimental period. We firstly examined the significance of this interaction term, and then separatory analyzed Cannibalism and No-cannibalism treatment to interpret the biological meanings of complex interaction terms.

We assumed that the mortality of salamanders and tadpoles represent the strength of cannibalism and trophic interaction between two species, because previous experiments conducted in similar experimental settings showed that the mortality of two species is quite low in the absence of the trophic interactions (Kishida et al. 2011; Nosaka et al. 2015; Takatsu and Kishida 2015; Takatsu et al. 2017). Since our study investigated the effect of multiple interacting species on survivorship represented as proportion data, the multiplicative risk model was employed (Sih et al. 1998). In the multiplicative risk model, proportion data is log-transformed so that independent effects of different species can be assessed (Soluk and Collins 1988). Therefore, we used natural logarithms of survival rates of salamanders and tadpoles as response variables for the analyses of interaction strength. The statistical significance of each parameter in mixed models was computed via the Satterthwaite approximation (Satterthwaite 1946). All analyses in this study were performed in R 3.6.2 (R Development Core Team 2019). We used R package *lme4* (v. 1.1; Bates et al. 2015) to construct LMMs, and package *lmerTest* (3.1; Kuznetsova et al. 2017) to evaluate the significance of parameters within LMMs.

Phenotyping

To dissect mechanistic details of the trophic interactions, we assessed morphological traits of salamanders and tadpoles. From dorsal side photographs of the surviving amphibians at the end of 2nd period, we measured heart-vent length and gape width of salamanders and maximum body width and snout-vent length of tadpoles, using Image J software (Schneider et al. 2012). For tadpoles, as many individuals were measured as possible per tank (i.e., moving or inclining individuals on the photograph were not measured). For salamanders, the individuals with the four largest body length values were measured in each tank, because very few salamanders can become offensive giants as potential predators for tadpoles. Gape width of salamanders and maximum body width of tadpoles are considered as functional traits that are critically important for their trophic relationship, because size balance between gape of salamander and the largest body part of tadpoles strongly determines predation success (Kishida and Nishimura 2004, Takatsu and Kishida 2013). Heart-vent length of salamanders and snout-vent length of tadpoles are considered as body size (Takatsu and Kishida 2013, Kishida et al. 2015). In addition, we calculated relative gape width of salamanders (i.e., gape width / heart-vent length) and relative body width of tadpoles (i.e., maximum body width / snout-vent length) to represent the degree of salamander's offensive phenotype expression and that of tadpole's defensive phenotype expression (Kishida et al. 2009a, Takatsu and Kishida 2013). By focusing on morphological variables at the end of 2nd period (just before intense predation by salamanders on tadpoles started [see Results]), we dissected mechanistic causes underlying differentiated trophic interactions between salamanders and tadpoles among the treatments. We calculated mean values of the tadpole variables in each tank for use in the statistical analyses. We used variables of the salamanders whose body size was largest in each tank in the statistical analysis because the number of offensive giants was very few (0-2) in each tank if emerged. In addition, using the morphological data at the end of 2nd period, we examined the number of "potential predators" in each tank, which is defined as the salamander whose gape width exceeded the mean tadpole body width by 1.1 times (Takatsu and Kishida 2015). This count data is useful because it enables us to test the importance of size balance of gape of salamander and body of tadpoles for their trophic interaction.

Statistical analyses – morphology of both species

We conducted three-way analysis of variance (ANOVA) on mean body width, mean body size (snout-vent length) and mean relative body width of tadpoles and gape width, body size (heart-vent length) and relative gape width of the largest salamanders at the end of 2nd period, considering cannibalistic condition of salamanders, salamander population identity, frog population identity and their interaction as explanatory variables. Then, we conducted a Tukey post hoc test to examine how variables differ among treatments.

Results

The trajectory of salamander survival

Analyses of data including all of the treatments showed that the interaction between salamander cannibalism condition and salamander population identity altered the trajectory of salamander survival ($G_{\mathrm{salamander.Erimo}} \times E_{\mathrm{cannibalism}} \times$ Period, $\beta = 0.037 \pm 0.011$ SE, $P = 0.001$, Fig. 1a and 1b, Table S2a in Appendix S3). To biologically interpret the effect of this interaction, we separately analyzed the No-cannibalism and Cannibalism treatment. Analysis on the No-cannibalism treatment detected weak effects of period and an interactive effect of period and salamander population identity. This indicates that survival rate was slightly increased in the late experimental periods (Period, $\beta = 0.005 \pm 0.002$, $P = 0.012$), and that this tendency was slightly weaker in Erimo salamanders ($G_{\mathrm{salamander.Erimo}} \times$ Period, $\beta = 0.005 \pm 0.002$, $P = 0.045$, Table S2b in Appendix S3). The survival rate of salamanders remained constantly high under the No-cannibalism treatment in both salamander populations regardless of tadpole population (Fig. 1b, 39-day survival > 94 %, Table S4 in Appendix S4).

For the Cannibalism treatment, salamander population identity and period jointly shaped survival trajectory; survival of Erimo salamanders was lower in the early period but higher in the later experimental periods, compared to that of Chitose salamander ($G_{\mathrm{salamander.Erimo}} \times$ Period, $\beta =$

0.047±0.015, $P = 0.002$, Table S2c in Appendix S3, Fig. 1a). Tadpole population identity and their interactive terms had no effect on salamander survival ($P > 0.794$). Hence, irrespective of the tadpole population with which they coexist, the survival of Erimo salamanders was steeply reduced during the second period, whereas survival of Chitose salamanders was gradually reduced from the 1st to 4th period (Fig. 1a). The inter-population difference in survival trajectory resulted in a significant difference in net survival at the end of 2nd period, just before tadpole consumption started; in the Cannibalism treatment, the survival was 48.5±19.2 % in Erimo salamanders whereas 81.6±14.8 % in Chitose (pooled data for tadpole population, $P < 0.001$, Wilcoxon test). These results indicate that Erimo salamanders cannibalize conspecifics more frequently than Chitose salamanders particularly in the early experimental periods (Fig. 1a).

The trajectory of tadpole survival

Analyses of data including all treatments showed that the trajectory of tadpole survival was determined by the interaction among tadpole population identity, salamander cannibalism condition and salamander population identity (i.e. $G_{\text{tadpole.Erimo}} \times G_{\text{salamander.Erimo}} \times E_{\text{cannibalism}} \times \text{Period}$, $\beta = 0.005 \pm 0.002$, $P = 0.032$, Table S3a in Appendix S3, Fig. 1c and 1d). To dissect the higher-order interaction, we separately analyzed the No-cannibalism and Cannibalism treatment. The analysis of the No-cannibalism treatment detected a weak effect of the interaction between period and salamander population identity. Tadpole survival was slightly reduced in the presence of Erimo salamanders in the later experimental periods ($G_{\text{salamander.Erimo}} \times \text{Period}$, $\beta = -0.002 \pm 0.001$, $P = 0.035$, Table S3b, Fig. 1d). Still, tadpole survival was high in all treatments (> 96%, see Table S4). In the Cannibalism treatment, interaction between salamander population identity and tadpole population identity influenced the survival trajectory. Tadpole survival was lower when facing with Erimo salamanders ($G_{\text{salamander.Erimo}}$, β = -0.027±0.005, $P < 0.001$) and Erimo tadpole had higher survival when facing with Erimo salamanders ($G_{\text{tadpole.Erimo}} \times G_{\text{salamander.Erimo}}$, β = 0.014±0.007, $P = 0.044$); survival was greatly reduced through time when facing with Erimo salamanders ($G_{\text{salamander.Erimo}} \times$ Period, β = -0.015±0.002, $P < 0.001$) but this reduction was weaker in Erimo tadpoles ($G_{\text{tadpole.Erimo}} \times G_{\text{salamander.Erimo}} \times$ Period, β = 0.009±0.003, $P = 0.006$, Table S3c, Fig. 1c). In the Cannibalism treatment of Chitose salamander (where the potential predators did not appear at the end of 2nd period, see Results), tadpole survival was consistently high in both tadpole populations until the end of 4th period (i.e., day 32) (Fig. 1c). In contrast, in the Cannibalism treatment of Erimo salamander (where the potential predators appeared at the end of 2nd period, see Discussion), tadpole survival was continuously reduced after 2nd period (since day 18). Further, survival reduction was more severe in Chitose tadpoles than in Erimo (Fig. 1c). Additional analysis between the two treatments with Erimo salamanders under the Cannibalism treatment showed that tadpole survival was commonly reduced through time (Period, β = -0.018±0.002, $P < 0.001$), but this tendency was weaker in Erimo tadpoles than Chitose tadpoles ($G_{\text{tadpole.Erimo}} \times$ Period , β = 0.008±0.003, $P = 0.007$, Appendix S5). The complex interaction in survival trajectory resulted in the variation in net survival of tadpole at the end of experiment across the treatments. Under the Cannibalism treatment in Erimo salamanders, tadpole survival was markedly reduced, especially for Chitose tadpoles (39 days survival rate: 83.3±9.1 % and 71.9±13.8 % for Erimo and Chitose tadpole, respectively), whereas tadpole survival of both populations remained high in the other treatments (> 96 %, Table S4).

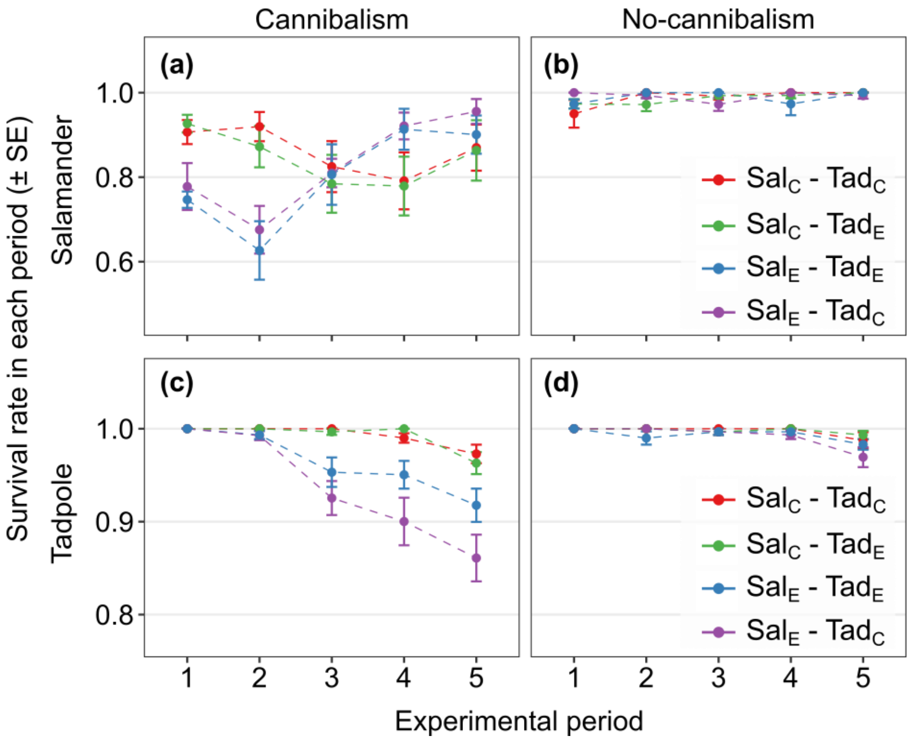

Fig. 1. Mean (±SE) of salamander (a and b) and tadpole (c and d) survival rates in each one-week experimental period. Salamander and tadpole mortality largely reflect cannibalism and tadpole consumption by salamanders, respectively. Trophic interactions occurred in Cannibalism treatment (a and c) while rarely occurred in No-cannibalism treatment (b and d). Colors of dots represent combination of salamander and tadpole population: Sal_C and Sal_E, Chitose and Erimo salamander; Tad_C and Tad_E, Chitose and Erimo tadpole.

Morphology of salamanders and tadpoles

We show results of three-way ANOVA on morphological variables of salamanders and tadpoles, while presenting the results of Tukey post hoc tests in Figures. Three-way ANOVA revealed that gape width of the largest salamander was larger in the Cannibalism treatment than

No-cannibalism treatment ($E_{\text{cannibalism}}$, $P < 0.001$, details are in Table S6a in Appendix S6) and was larger in the Erimo than the Chitose population ($G_{\text{salamander}}$, $P < 0.001$). Importantly, the interpopulation difference in gape width was enlarged in the Cannibalism treatment ($G_{\text{salamander}} \times E_{\text{cannibalism}}$, $P < 0.001$). Thus, Erimo salamander had the largest gape width in the Cannibalism treatment, regardless of the tadpole population with which they coexisted (Table S6a, Fig. 2a). Body length (heart-vent length) of the largest salamander was larger in the Cannibalism treatment than No-cannibalism treatment ($E_{\text{cannibalism}}$, $P = 0.006$, Table S6b) and also larger in Erimo population than Chitose population ($G_{\text{salamander.}}$, $P < 0.001$). Hence, regardless of the tadpole populations, body length was largest in the Erimo population under cannibalistic condition (Fig. 2b). Relative gape width of the largest salamanders was larger in the Cannibalism treatment than No-cannibalism treatment ($E_{\text{cannibalism}}$, $P < 0.001$, Table S6c) and in Erimo population than Chitose population ($G_{\text{salamander}}$, $P < 0.001$) and, importantly, the interpopulation difference in relative gape width was greater when the salamanders were in the Cannibalism treatment than No-cannibalism treatment ($G_{\text{salamander}} \times E_{\text{cannibalism}}$, $P < 0.001$). Thus, irrespective of the tadpole populations, morphology of the largest salamanders was the most offensive in the Erimo population under cannibalistic condition (Fig. 2c). These results clearly indicated that offensive giant salamanders emerged more easily in Erimo population than Chitose population if the salamander hatchlings grew under cannibalistic condition.

Erimo tadpoles had a greater body width than Chitose tadpoles (G_{tadpole}, $P < 0.001$, Table S7a in Appendix S6). The interpopulation difference in body width of tadpoles was more apparent under cannibalistic salamander conditions than non-cannibalistic conditions ($G_{\text{tadpole.}} \times E_{\text{cannibalism}}$, $P = 0.005$) and was also more apparent in the presence of Erimo salamanders than Chitose salamanders ($G_{\text{salamander}} \times G_{\text{tadpole}}$, $P = 0.004$). As a result, Erimo tadpoles exposed to

Eriomo salamanders that grew under cannibalistic conditions had the widest body among treatments (Fig. 2d). Erimo tadpoles had larger body length (Snout-vent length) than Chitose tadpoles ($G_{tadpole}$, $P < 0.001$, Fig. 2e, Table S7b). The interpopulation difference in body length of tadpoles was significantly larger in the presence of Erimo salamanders than Chitose salamanders ($G_{salamander.} \times G_{tadpole.}$, $P = 0.021$) and tended to be larger when facing salamanders under cannibalistic condition than non-cannibalistic condition ($G_{tadpole} \times E_{cannibalism}$, $P = 0.072$). In the presence of Erimo salamanders, tadpoles exhibited bulgier phenotype (i.e., greater ratio of body width to body length) than in the presence of Chitose salamanders ($G_{salamander}$, $P = 0.003$, Table S7c). Erimo tadpoles tended to express the bulgy phenotype more strongly when Erimo salamanders were under cannibalistic condition than under non-cannibalistic condition ($G_{salamander} \times G_{tadpole} \times E_{cannibalism}$, $P = 0.086$). Thus, Erimo tadpoles subjected to the presence of cannibalistic Erimo salamander exhibited the greatest defensive bulgy morph (Fig. 2f). These results indicate that Erimo tadpoles attained the most defensive state (i.e., largest body width) when exposed to Erimo salamanders that grew under cannibalistic situation. This was achieved by strongly accelerating both isometric growth (i.e., body width increase proportional to body length) and allometric growth (i.e., expression of bulgy phenotype) of body width.

At the end of the 2nd period, salamanders with the potential to consume tadpoles (salamanders with gape width exceeding mean tadpole body width at 1.1 times [Nosaka et al. 2015]) appeared only in the Erimo population under cannibalistic condition with Chitose tadpoles (appeared in 4 of 8 tanks, one individual on average). This result explains why the strongest tadpole consumption by salamanders occurred in that treatment.

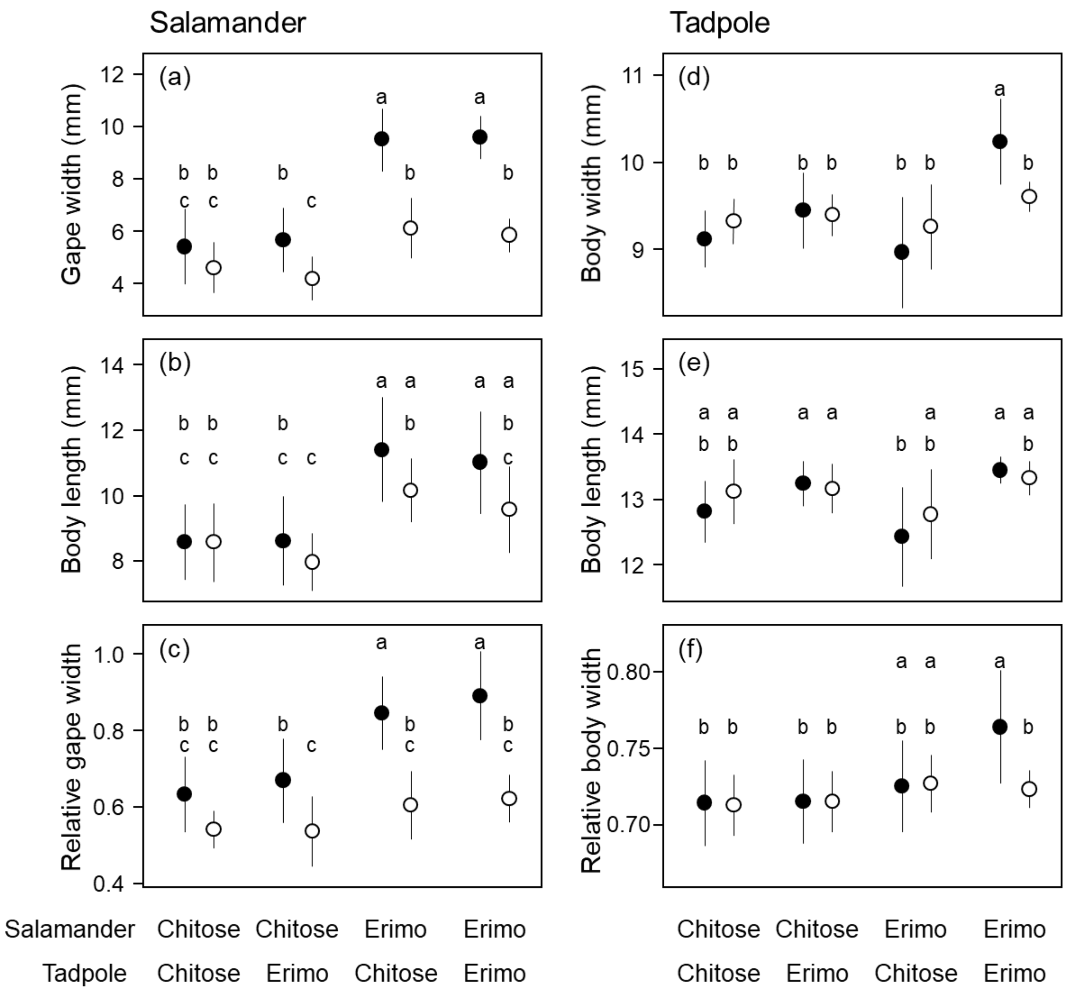

Fig. 2. Functional trait size (a. gape width and d. body width), body length (b and e) and functional trait size relative to body size (c and f) of predator salamander and prey tadpole in each combinations of geographic populations (Chitose and Erimo) and cannibalism treatments (black and white dots represents Cannibalism and No-cannibalism treatment, respectively). The largest salamander in each tank, and all measurable tadpoles were measured at the end of 2nd period (day18), just before the tadpole consumption started.

Discussion

Since interaction strength depends on the functional traits of both interacting partners, identifying factors affecting trait values is imperative to better understand the mechanisms shaping geographic patterns of ecological interactions (Miner et al. 2005, Hendry 2016, Bassar et al. 2017). Since individuals change their trait values according to their external and internal conditions (Miner et al. 2005) and such reaction norms are under the genetic controls (Pigliucci 2005, Winterhalter and Mousseau 2007), trait values expressed by individuals is determined by the specific combination of genotypes of the individuals and environmental conditions to which the individuals are subjected. Importantly, trait values of an interacting species sometimes affects the traits of their interacting partner, operating as an external factor selecting the latter (Kopp and Tollrian 2003, Kishida et al. 2006, see also Kishida et al. 2010). This implies that interspecific interaction strengths can be determined not only by the reaction norms of both interacting partners but also through their interaction. Consequently, genotypes of both interacting species and environmental conditions jointly and interactively shape geographic variation in interaction strength (i.e., G × G × E interactive effects on ecological interaction). Our experiment using predatory salamander larvae (*H. retardatus*) and prey frog tadpoles (*R. pirica*) provided experimental evidence supporting this prediction; temporal changes in survival of prey tadpoles were determined by the combinations of geographic population of both tadpole and salamander and growth condition of the predatory salamanders (Fig. 1).

Though in our experiment survival rate of salamanders remained quite high across all No-cannibalism treatments for the whole experimental period (>94% at the end of experiment), it was significantly reduced in the Cannibalism treatments. This result indicates that our manipulation of size structure of salamander hatchlings successfully controlled a cannibalistic interaction and therefore allows us to conclude that mortality of salamanders in this experiment was largely caused by cannibalism. Importantly, intensity of salamander cannibalism differed between the two salamander populations. In the Cannibalism treatment, although survival of Chitose salamanders remained high, survival of Erimo salamanders strongly decreased in the early periods. As a result, in the Cannibalism treatment, more than half of Erimo salamanders died but only 20% of Chitose salamanders died due to cannibalism by the end of 2nd period. The interpopulation difference in salamander cannibalism in the early period translated into interpopulation variation in size of a functional trait of the cannibalistic salamander. At the end of 2nd period, the largest individuals of Erimo salamanders in Cannibalism treatment had the widest gape, regardless of the tadpole populations with which they coexisted.

Because Erimo salamanders had larger body length than Chitose salamanders across treatments, the widest gape of the cannibalistic Erimo salamanders is partly explained by their larger body size. In addition, the widest gape of the cannibalistic Erimo salamander is achieved through greater allocation to enlargement of their gape than Chitose salamanders. The widest gape of Erimo salamander in the Cannibalism treatment likely determined their significant predatory effects on tadpoles in the subsequent period, because only the tadpoles in these treatments suffered significant mortality. By the end of experiment, both Erimo and Chitose tadpoles in the Cannibalism treatment of Erimo salamanders suffered significant mortality; while tadpole mortality of both populations was negligibly low in the other treatments (less than 4%, Table S4).

We found that predatory impacts of salamanders on tadpoles differed between tadpole populations. Comparison of tadpole mortality between the two Cannibalism treatments of Erimo salamander (i.e., Erimo tadpole-Erimo salamander-Cannibalism treatment and Chitose tadpole-Erimo Salamander-Cannibalism treatment) revealed that Chitose tadpoles suffered predation mortality 1.7 times higher than Erimo tadpoles (i.e., mortality rate of Chitose and Erimo tadpoles were 28.1% and 16.6 %, respectively. Fig. 1). The largest Erimo salamanders had similar morphology between the two treatments and Erimo tadpoles had 1.11 times wider body than Chitose tadpoles in the Cannibalism treatment of Erimo salamanders just before salamander predation on tadpoles started (i.e., end of 2nd period). Hence, differences in the mortality of tadpoles between treatments were likely caused by variation in defensive performance between tadpole populations. In support of this, the potential predatory salamanders whose gape was large enough to swallow the coexisting tadpoles appeared only in the Cannibalism treatments of Erimo salamanders faced with Chitose tadpoles. The difference in body width were the results of greater isometric and allometric growth of Erimo tadpoles than Chitose tadpoles under the risky situation with cannibalistic Erimo salamanders. Although body length of tadpoles as indicator of body size was similar between the two populations at the beginning of the experiment, Erimo tadpoles had longer body than Chitose tadpoles at the end of 2nd period and this trend was intensified in the presence of Erimo salamander. This means that Erimo tadpoles accelerated their ordinal growth in the riskier situation than Chitose tadpoles, implying that the Erimo tadpoles more enlarged their body width isometrically with increase in their body size. Furthermore, the fact that Erimo tadpoles in the Cannibalism treatment of Erimo salamanders had the largest relative body width among the treatments (Fig. 2d) represents that Erimo tadpoles expressed defensive bulgy phenotype more strongly than Chitose tadpoles under the riskiest situation (facing with cannibalistic Erimo salamanders), implying that Erimo tadpoles more enlarged their body width allometrically than Chitose tadpoles.

As discussed above, we argue that population specific reaction norms in morphological traits of both predator and prey are the mechanisms underlying G × G × E interplay in their trophic interactions. Although population specific behavioural plasticity (e.g., development of aggressiveness) can be an alternative mechanism (Bell and Stamps 2004), its importance is negligible in our study since densities of experimental animals are relatively high. In the high-density situation, the salamanders and tadpoles should have encountered frequently and thus opportunity of salamanders to attack tadpoles would be frequent. In this case, occurrence of successful predation events should have exclusively depended on the size balance between salamander gape and tadpole body, since salamander is a representative swallowing-type predator and the tadpoles are their large prey items (Takatsu and Kishida 2013). Importance of predator-prey size balance in the trophic interaction was also evidenced by the additional analysis (Appendix S7). Although effects of the treatment on tadpole mortality was significant in our original analysis, the treatment effects became no longer significant by including the number of potential predators as an additional predictor (Appendix S7). This result strongly suggests that size balance between salamander gape and tadpole body is the exclusive mediator of the treatment effects on the trophic interaction of salamanders on tadpoles.

Our experimental result predicts multiscale variation in interaction strength across the geographic regions. In the comparison of interaction strength among the treatments with natural population pairs of salamander and tadpole, Erimo pair (i.e., Erimo salamanders and Erimo tadpoles) more differed in the interspecies interaction strength (tadpole survival) between Cannibalism and No-cannibalism treatment than Chitose pair (i.e., Chitose salamanders and Chitose tadpoles) (Appendix S8). That is, while Erimo and Chitose pairs showed similarly weak interaction in No-cannibalism treatment, Erimo pair much more intensively interacted than

Chitose pair in Cannibalism treatment (Fig. 1c and d, see also Fig. S1). As a conditional factor involving salamander cannibalism, our study featured size structure (i.e., hatch timing variation) of salamander hatchlings, which should vary among ponds within geographic regions. Likewise, other prospective conditional factors affecting cannibalism (e.g., conspecific density, alternative prey abundance, structural complexity and predator presence: Fox 1975, Polis 2003, Kishida et al. 2011) are generally spatially heterogenous within geographic regions. If within-region spatial heterogeneity in those factors is equal across the geographic regions, interaction strength should more vary in Erimo (no to strong interaction) than Chitose region (no to weak interaction). Interaction strength variability across ponds may further create variability in pond communities because amphibian larvae can strongly influence pond communities due to the far the largest biomass among pond animals. Trophic interactions between the two larval amphibians can impacts other species through alterations in their density and individual traits. Hence, population-specific reaction norms of the two amphibian species can create regional variation in the heterogeneity of their trophic interaction, and potentially further shape regional variation in β-diversity of pond communities in nature.

More broadly, our study illuminates the importance of the interplay between environmental conditions and genotypes of both interaction partners as the factors causing heterogeneity in the strength of ecological interactions. If environmental heterogeneity is similar across geographic regions, individuals with higher trait plasticity can exert more variable impact on interaction strength than those with lower trait plasticity. In fact, compared to Chitose salamanders (i.e., less plastic inducible offense — non- to poorly-offensive), Erimo salamanders (i.e., highly plastic inducible offense — non- to highly-offensive) exerted more variable effects to intensify the trophic interaction with tadpoles. Similarly, compared to Chitose tadpoles (i.e., less plastic inducible defense — non- to poorly-defensive), Erimo tadpoles (i.e, greater plasticity — non- to highly-defensive) exerted more variable effects to weaken the trophic interaction with salamanders. As a result, while Erimo salamanders experienced larger variation in the interspecies trophic interaction strength across the treatments than Chitose salamanders, Erimo tadpoles experienced smaller variation in the interspecies trophic interaction strength across the treatments. In trophic interactions, we can generally expect that higher plasticity in predator inducible offense will increase the variation of trophic interaction strength, but higher plasticity in prey inducible defense will decrease the variation of trophic interaction strength. This suggests that the combination of reaction norms of both interaction partners can shape multiscale spatial variation of interaction strength (i.e., heterogeneity in interaction strength within a geographic region vary among different geographic regions). For example, if predators with highly plastic inducible offense (i.e., from non- to highly-offensive) and prey with less plastic inducible defense (i.e., from non- to poorly-defensive) cohabit in a geographic region, heterogeneity in interaction strength within the region is expected to be considerably large (i.e., from no to quite strong interaction). Conversely, if predators with less plastic inducible offense (i.e., from non- to poorly-offensive) and prey with highly plastic inducible defense (i.e., from non- to highly-defensive) cohabit in the other region, heterogeneity in interaction strength within this region is expected to be quite small (i.e., from no to quite weak interaction).

Developmental reaction norms can be the target of natural selection (Urban 2008, 2010). Geographic variation of differential developmental reaction norms of both salamander and tadpole may be a result of differential coevolution history of the predator and prey amphibians. While larger body size is necessary for tadpoles to avoid predation by giant salamanders, much larger gape is required for salamanders to consume the defensive tadpoles with larger body

(Takatsu and Kishida 2015, Takatsu et al. 2017). Although only two geographic populations were examined, the intrapopulation pattern of developmental reaction norms of predator and prey follows an arms race scenario; Erimo salamanders had a higher ability to develop the offensive phenotype (i.e., the salamander became giant with an enlarged gape) than Chitose salamanders, and Erimo tadpoles grew more rapidly and expressed more defensive phenotypes than Chitose tadpoles. This phenotypic pattern implies a geographic mosaic in coevolution with Erimo as a coevolutionary hotspot (a region where coevolution is escalated) and Chitose as a coldspot. In this coevolutionary scenario where evolutionary enhancement of antagonistic phenotype expression imposes stronger selective pressure on the opponent, ecological interactions may be stronger in coevolutionary hotspots than in coldspots. Therefore, describing geographic patterns of developmental reaction norms of the two amphibians and testing the coevolutionary hypothesis should advance our understanding of the mechanisms promoting variation in the strength of ecological interactions.

Our study suggests that genetic variations in reaction norms of both species shape regional variation in heterogeneity of the interaction strength within regions. Under different selection regimes, populations can have evolved different reaction norms for their functional traits (e.g., plastic or fixed phenotypes along environmental gradient) (Kishida et al. 2007, Winterhalter and Mousseau 2007). Investigating how reaction norms of functional traits for interacting partners vary geographically and how the combination of reaction norms of the interacting partners control their interactions is fruitful to disentangle complex geographic mosaics of ecological interactions around the globe.

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