Interpopulation differences in developmental plasticity of carnivores determine the emergence of a trophic interaction

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Abstract

1. Through its influence on trait expression, phenotypic plasticity can shape variation in strengths of interspecific interactions across environmental gradients. If species exhibit interpopulation differences in phenotypic plasticity, their genotypes and environmental conditions may jointly determine the strength of interspecific interactions.
2. To examine this prediction, we experimentally investigated the trophic interactions between different populations of predators and prey, using amphibians that vary in the plasticity of offensive and defensive morphological traits, respectively. Cannibalism-induced gigantism of *Hynobius retardatus* salamander larvae can produce salamanders with wide enough gapes to consume *Rana pirica* frog tadpoles, an otherwise inaccessible large prey species that, in turn, possesses an inducible morphological defense.
3. By manipulating combinations of two populations of salamanders and tadpoles and the size distribution—hence, prevalence of cannibalism—of salamander hatchlings, we found an interactive effect of salamander population identity and size distribution on the trophic interaction between salamanders and tadpoles across the entire cohabitation period.
4. Early life salamander size heterogeneity caused cannibalism in only one salamander population, resulting in interpopulation differences in salamander gigantism. Salamanders from the population with weaker cannibalism-induced gigantism were largely unable to consume tadpoles across the entire larval period of frog tadpoles. However, salamanders from the population exhibiting striking offensive gigantism consumed tadpoles from both populations, though tadpole inducible defenses were stronger in the population with higher prevalence of salamander gigantism.

5. Our results suggest that the likelihood of emergence of a trophic interaction between salamanders and tadpoles differs among salamander populations due to genetic variation in inducible offense. Ultimately, this implies that geographic variation in trait plasticity can determine geographic variation in interspecific interaction strengths.

Key words: Genotype × Environment interaction; phenotypic plasticity; reaction norm; intraspecific variation; arms race; coevolution

Introduction

The factors determining the strength of interspecific interactions are of broad relevance as such interactions influence population demographics and their effects can propagate through ecological communities (Nakano, Kuhara, & Miyasaka, 1999; Persson et al., 2007; Ushio et al., 2018). Although interaction strengths depend on the densities of interacting species, they are also influenced by those species' functional traits (Schmitz, Buchkowski, Burghardt, & Donihue, 2015; Sinclair, Mduma, & Brashares, 2003). Functional traits are phenotypic characteristics that contribute to individual fitness and species niches including their interspecific interactions (McGill, Enquist, Weiher, & Westoby, 2006; Violle et al., 2007) and that drive ecological processes or characterize species responses to environmental conditions (Mori, Furukawa, & Sasaki, 2013; Ross et al., 2017; Suding et al., 2008). Since trait expression varies among species, species-specific (i.e., mean) functional trait values are primarily used when investigating community structure and dynamics (McGill et al., 2006; Ross et al., 2017; Schmitz et al., 2015). Yet, functional traits often vary considerably within species, and such intraspecific trait variation

63 can alter the strength of interspecific interactions (Miller & Rudolf, 2011; Miner, Sultan,
64 Morgan, Padilla, & Relyea, 2005). Intraspecific trait variation is thus increasingly recognized as
65 an important factor in determining community structure and dynamics (Bolnick et al., 2011; Des
66 Roches et al., 2018; Raffard, Santoul, Cucherousset, & Blanchet, 2018; Ross et al., 2017).
67 The effects of intraspecific trait variation on predator-prey interactions have been mainly
68 investigated in terms of two interacting elements: genotype and phenotypic plasticity. The
69 expression of functional traits is under genetic control (Miner et al., 2005; Pigliucci, 2005;
70 Winterhalter & Mousseau, 2007). Distinct functional trait values between genetically distinct
71 populations will result in interpopulation differences in interspecific interaction strengths
72 (Bassar, Simon, Roberts, Travis, & Reznick, 2017; Hiltunen & Becks, 2014; Yoshida, Jones,
73 Ellner, Fussmann, & Hairston, 2003). Some individuals can change their functional traits in
74 response to the presence or absence of predator and prey (i.e., phenotypic plasticity: changes to
75 foraging and defensive behavior, physiology or morphology for instance), with consequences for
76 predator-prey interaction strengths (Kishida, Costa, Tezuka, & Michimae, 2014; Miner et al.,
77 2005; Winterhalter & Mousseau, 2007). For example, while some prey species enhance
78 expression of defensive traits in the presence of a specific predator (i.e., inducible defense), some
79 predator species can also enhance expression of offensive traits in the presence of particular prey
80 items (i.e., inducible offense, Kishida et al. 2010). Since biotic and abiotic conditions influence
81 expression of functional traits (e.g., Kishida et al., 2011), predator-prey interaction strengths can
82 vary due to differential trait expression through time or space.
83 Numerous studies have demonstrated the importance of the above attributes (genotype and
84 phenotypic plasticity) as causal mechanisms behind variation in predator-prey interaction
85 strengths (e.g., Miner et al., 2005, Des Roches et al., 2018). These concepts have been primarily

studied independently, yet are also tightly linked. Phenotypic plasticity *per se* is under genetic control and, thus, the degree of phenotypic plasticity can vary among genotypes (Pigliucci, 2005). This is true when considering the phenotypic plasticity of predator and prey. In many prey species with inducible defense strategies, genetic variation in the induction ability of defensive traits has been documented, even though evidence of genetic variation in predator phenotypic plasticity (i.e., inducible offense) is relatively limited (e.g. Kishida, Trussell, & Nishimura, 2007; Michimae, 2006; Relyea, 2005). Therefore, predator-prey interaction strengths can be determined through an interaction between population (i.e. genotype) of predator and/or prey and environmental conditions (Yamamichi, Klauschies, Miner, & van Velzen, 2019). Although this integrative view is intuitive, there is little evidence of how predator-prey interaction strengths are affected in nature by genetic variation in the condition-dependent development of predator and/or prey traits (Kasada et al. 2014).

Here, we investigate the potential for genetic variation in phenotypic plasticity of both predator and prey to mechanistically shape variation in predator-prey interaction strengths. We focused on genetic variation of phenotypic plasticity of both predator and prey species simultaneously, since predators and prey can jointly shape the sign and strength of their interaction (Bassar et al., 2017; Hiltunen & Becks, 2014). To address this objective, we conducted an experiment using a model trophic relationship between predatory larval salamanders (*Hynobius retardatus*) and their frog tadpole prey (*Rana pirica*). Both predator and prey species exhibit morphological plasticity that directly determines the success of the predator's consumption on prey (Takatsu & Kishida, 2013). Salamander larvae can develop gigantism with an enlarged gape as an inducible offense (Michimae and Wakahara 2002, Takatsu and Kishida 2015) and tadpoles develop "bulgy" bodies (i.e., a thickened epithelium) as an

inducible defense (Kishida and Nishimura 2004). The magnitude of the induced response varies among populations of both species (Kishida et al., 2007; Michimae, 2006), but whether population-level differences in the morphological plasticity influence the strength of trophic interactions remains unknown. We examined whether and how population genetic differences in the morphological plasticity of predatory salamander and prey tadpoles affects their trophic interaction by conducting a controlled multifactorial experiment manipulating the identity of both predator and prey population, as well as initial biotic conditions relevant to the emergence of offensive salamander phenotypes.

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. This is because tadpoles typically hatch 2–4 weeks earlier than salamanders and grow to a large size before the salamander larvae hatch (Nosaka et al. 2015). In such a typical phenological scenario, tadpoles are too large to be consumed by salamander hatchlings (Nosaka, Katayama, & Kishida, 2015). A predator-prey interaction between the two species thus occurs only when salamander larvae grow rapidly. Rapid growth of salamander larvae typically results from cannibalism during their hatchling stage; individuals that successfully consume conspecifics tend to grow rapidly and become ‘giants’ with much larger body and gape width than non-cannibals (Takatsu & Kishida, 2015). The ratio of salamander gape to tadpole body size

is greater for cannibalistic giants than for non-cannibalistic salamanders. The disproportionately large gape (i.e. inducible offensive phenotype) of giant salamanders can facilitate consumption of tadpoles as alternative large prey items (Takatsu & Kishida, 2015). We also note than tadpole consumption by salamanders can occur and, hence, the offensive phenotype of salamanders can be induced without the cannibalistic interaction of salamanders when salamanders hatch as early as tadpoles (Nosaka et al., 2015), though we focus on the cannibalism-induced trophic interaction herein.

Just as *H. retardatus* salamanders have an inducible offense, *R. pirica* tadpoles have an inducible defense. *R. pirica* tadpoles induce defense in the presence of salamander larvae, enlarging their body and tail by thickening their epithelium tissue. Tadpoles with this 'bulgy' phenotype are difficult for predatory salamander larvae to swallow (Kishida & Nishimura, 2004). Notably, expression of the defensive bulgy phenotype depends on predation risk; tadpoles exhibit bulgier phenotypes in the presence of giant (offensive phenotype) salamanders compared to non-offensive ones (Takatsu & Kishida, 2015; Takatsu, Rudolf, & Kishida, 2017). Thus, larvae of the two amphibian species exhibit antagonistic morphological plasticity that characterizes both the presence and strength of their trophic interactions (Kishida & Nishimura, 2004; Takatsu et al., 2017).

We examined whether genetic differences in antagonistic morphological plasticity of salamanders and tadpoles affects their trophic interaction. Previous studies reported that the potential to become a giant salamander greatly differs among geographic populations, at least partly due to genetic differences between these populations (Michimae 2006, Atsumi K. and Kishida O., unpublished data). Moreover, previous work in this system demonstrated that tadpoles' potential to express the defensive phenotype genetically differs between populations

(Kishida et al., 2007). Based on our knowledge of geographic variation in salamander inducible offense, we selected two localities of amphibians as experimental model populations: Erimo and Chitose. Giant salamanders are more common in the Erimo population (hereafter Erimo salamanders) than in the Chitose population (hereafter Chitose salamanders; Michimae 2006; Atsumi K. and Kishida O., unpublished data). We thus expected that frog tadpole inducible defense is stronger in Erimo than in Chitose.

Assumed scenario and predictions

In our experiment, we assumed the typical phenological scenario for the emergence of predator-prey interaction between the two amphibian species; owing to cannibalism of salamander hatchlings, the offensive giant salamander phenotype can emerge, in turn allowing tadpole predation by giant salamanders (Takatsu & Kishida, 2015). We considered the conditions associated with cannibalism of salamander hatchlings to be an environmental factor. While various factors affect cannibalism of carnivores, including their size-distribution, conspecific density, alternative prey abundance, habitat complexity, and predation pressure (Fox 1975, Polis 2003, Kishida et al. 2011), we used size distributions (i.e., heterogeneous or homogeneous distributions of body size) as a manipulative conditional factor because it should vary among ponds within geographic regions and is unlikely to cause confounding effects (Takatsu & Kishida, 2015). In our experiment, we expected that the strength of the predator-prey interaction between salamanders and tadpoles would be higher (more predation) when the Erimo salamanders are under cannibalistic conditions (i.e., heterogeneous size distributions allowing consumption of smaller salamanders by larger conspecifics) during their hatchling period, facilitating the emergence of offensive giants of salamanders.

Experimental Setting

We collected eggs of both species 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 in mid- to late-April 2018. From each region, we collected 50 salamander egg clusters and 10 tadpole egg masses. We mimicked natural hatching timing so that frog tadpoles hatched 2–3 weeks earlier than salamanders (Nosaka et al., 2015). We kept frog egg masses in a separate 22 L semi-transparent polypropylene tank (51.3 × 37.2 × 16.6 cm high) filled with 5 L of aged tap water, and the tanks were placed in an indoor facility which was maintained at 15 °C on a natural light-dark (14h/10h) regime. Tadpole eggs hatched in late April (Chitose: April 23–27, Erimo: April 25–27). For each tadpole population, we mixed all tadpoles after they hatched, and reassigned 100 individuals to each 22 L tanks. We cultured tadpoles under the conditions described above for two weeks prior to the start of the experiment by putting eight pieces of rabbit chow (dry weight: 1.6 g) into each tank as food and changing the water every 2 days. Each salamander egg cluster was placed separately in a draining net which we put into 4 L semi-transparent polypropylene tanks (33.4 × 20 × 10 cm high; 5 nets per tank) filled with 3 L of aged tap water. We then placed the salamander tanks in a refrigerator with glass door and maintained at 3 °C under natural light-dark conditions.

The experiment was conducted in an experimental room in Tomakomai Experimental Forest, using semi-transparent polypropylene 22-L tanks as above. We covered the bottom of each tank with 2 cm of sand as benthic substrate, and provided two leaves of Japanese Bigleaf Magnolia (*Magnolia obovate*, dry weight: 5 g) as biotic structures. Minimum natural water (ca. 10 ml per minute), drawn from a well ~5m from a natural river, was constantly supplied into

each tank using thin polypropylene hoses and water flowed out of an overflow pipe (φ = 20mm, 4cm height) inside each tank. To prevent the experimental animals leaving the system, we covered the overflow pipe with mesh net (mesh size 1mm). Overflow pipes kept water depth at 4 cm (from sandy bottom to water surface). The experimental room was maintained under natural light-dark (ca. 14h/10h) conditions and water temperature ranged from 13 to 20℃.

Experimental design

To examine how genetic variation in expression of antagonistic phenotypes of predatory salamander and prey tadpoles shape their trophic interactions, we conducted a three-way factorial experiment with 8 treatment combinations. We manipulated combinations of the two geographic populations of the predators (Erimo versus Chitose salamanders) and prey (Erimo versus Chitose tadpoles) and salamander size distribution (size homogenous versus heterogeneous). Each treatment was replicated ten times.

We put 30 tadpoles and 15 salamanders in each tank. We haphazardly placed three-week-old tadpoles into each of our 80 tanks on 18 May 2018 (day 1, see Fig. 1). Tadpoles originated either all from Erimo (mean±SD snout-vent length, 7.32±0.60 mm, N = 20) or all from Chitose (7.10±0.53 mm, N = 20). Following well-established methods (Takatsu & Kishida, 2015; Takatsu et al., 2017), we manipulated size structure of salamander hatchlings by using individuals that hatched at different times (early- and late-hatchlings, placed into tanks at day 1 and 8, respectively) while keeping the total initial salamander density constant across treatments (n = 15). Briefly, we obtained early- and late-hatchlings by controlling the water temperature experienced by embryos; late-hatchlings resulted from longer exposure to low water temperature during the embryonic stages compared to early-hatchlings (see Takatsu and Kishida 2015). We

produced the following size distribution treatment levels: a size heterogeneity treatment which included 5 early- and 10 late- salamander hatchlings and a homogeneity treatment with either 15 early- or 15 late- salamander hatchlings (Fig. 1). Our conditions for hatch timing and density of the two amphibian species were within the range found in natural habitats (Michimae 2006; Nosaka et al. 2015). Although the size homogeneous treatment level included two conditions (either early or late hatchlings)—each condition was replicated 5 times for each combination of the two geographic populations of salamanders and tadpoles—we pooled all data within this treatment level because preliminary analyses revealed no difference between the two conditions in morphology or mortality of either tadpoles or salamanders (Appendix S1). In all cases, salamanders within each tank were siblings to standardize genetic variance among treatments.

Previous studies repeatedly report negligible tadpole mortality in the absence of predatory salamanders in similar experimental settings (Takatsu & Kishida, 2015, 2020). Thus, to avoid excessive use of animals, we did not include a tadpole-only treatment to estimate background mortality. Throughout the experiment, we added a piece of rabbit chow (dry weight: 0.2 g) and 100 frozen Chironomid larvae to each tank three times per week as alternative food for tadpoles and salamanders, respectively. We omitted 1 replicate for Erimo salamander–Chitose tadpole–size heterogenous and 2 for Chitose salamander–Chitose tadpole–size homogenous treatments in the all analyses because of a counting error.

At day 11, 18, 25 and 32, we counted all surviving tadpoles and salamanders. From the count data, we calculated cumulative salamander mortality by day 18 (just before predation by salamanders on tadpoles began) and cumulative tadpole mortality at the end of experiment (day 32)**.** On day 18, we also photographed the dorsal side of surviving animals using a digital camera (Panasonic Lumix DC-TZ90). The experiment ceased on day 32 as metamorphosis of tadpoles

247 began in several tanks, allowing us to evaluate interaction strength across almost the entire
248 period of cohabitation.
249
250 *Phenotyping*
251 We assessed morphological traits of salamanders and tadpoles. From dorsal side
252 photographs of the surviving amphibians at day 18, we measured salamander trunk (heart-vent)
253 length—which controls for the disproportionate scaling of offensive salamander heads—and
254 gape width, as well as maximum body width and snout-vent length of tadpoles (Kishida et al.,
255 2014; Kishida & Nishimura, 2004), using Image J software (Schneider, Rasband, & Eliceiri,
256 2012). For tadpoles, we measured as many individuals as possible, but for 11 tanks, we could not
257 assess tadpole morphology because they were moving or inclining (Table S1). We also focused
258 on the morphology of the salamander with the largest body length in each tank, by visually
259 selecting and measuring the four largest salamanders per tank and then excluding data on all but
260 the largest individual. This was sufficient to capture the extent of salamander gigantism because
261 typically only one salamander per tank expresses the offensive giant phenotype if cannibalism
262 occurs (Kishida et al., 2011; Takatsu & Kishida, 2015). We measured trunk length of
263 salamanders and snout-vent length of tadpoles as proxies for body size (Kishida, Tezuka, Ikeda,
264 Takatsu, & Michimae, 2015). Finally, we calculated relative gape width of salamanders (gape
265 width / trunk length) and relative body width of tadpoles (maximum body width / snout-vent
266 length per tadpole) as measures of the salamander offensive phenotype and the tadpole defensive
267 phenotype, respectively (Takatsu & Kishida, 2013).
268 We measured these morphological variables at day 18—prior to the start of tadpole
269 predation by salamanders with the offensive phenotype—as this allows us to infer the

mechanisms underlying salamander-tadpole trophic interactions based on their size balance. We calculated mean values of the tadpole variables in each tank, which were used in the statistical analyses (6-10 tanks/treatment, see Table S1), whereas to analyze treatment effects on salamander phenotype, we used only the data from the largest salamander per tank. Moreover, we used the above morphological data to calculate the number of *potential predators* per tank, defined as the number of salamanders whose gape width exceeded mean tadpole body width. This count data allows us to test whether the size balance between salamander gape and tadpole body affects the propensity for salamanders to consume tadpoles (Takatsu & Kishida, 2015).

Statistical analysis

To check for treatment effects on tadpole and salamander morphological traits, we first conducted three-way ANOVA after confirming that morphology data met the assumptions of normality and homogeneity of variances. In each case, we used tadpole population (Tad_{pop}), salamander population (Sal_{pop}), salamander size distribution ($Size_{sal}$), and their three-way interaction as predictor variables. The following response variables were modelled separately: tadpole mean body width, mean body size (snout-vent length), and mean relative body width, and salamander gape width, body size (trunk length), and relative gape width of the largest (by trunk length) salamander per tank. Where ANOVA results revealed significant treatment effects, we conducted a Tukey HSD post hoc test to examine how variables differ among treatment levels.

We next asked whether tadpole population, salamander population, and salamander size distribution affected the strength of the predator-prey relationship within and between species. Specifically, we tested for the effect of a three-way interaction between size distribution and both

salamander and tadpole populations. We first tested whether salamander mortality (by cannibalism) differed among treatments. A score test for zero inflation (van den Broek, 1995) revealed that our salamander mortality data was significantly zero-inflated (Score = 202.5, $p < 0.001$). To test for treatment effects on salamander mortality prior to tadpole predation, we then used a Scheirer–Ray–Hare (SRH) test on salamander mortality at day 18. The SRH test is a nonparametric equivalent of multifactorial analysis of variance (ANOVA) that extends the rank-based Kruskal-Wallis test to allow consideration of more than one factor (and potential interactions) as predictor variables (Scheirer, Ray, & Hare, 1976). The SRH test is a conservative estimate of among group differences—the test strength is considerably lower than the equivalent parametric ANOVA (Dytham, 2003)—so observed differences are likely to be true effects (i.e. low Type I error rate at the expense of increased Type II error). Where we identified significant terms, we made pairwise post hoc comparisons of treatment levels using a Tukey HSD test on the ranked data from our SRH test (Tukey, 1949).

We also conducted the above analyses (multifactorial comparisons and post hoc Tukey HSD on significant terms) to determine whether treatment groups differed in their tadpole mortality values. Tadpole mortality was zero-inflated (Score = 64.8, $P < 0.001$), so we again used the SRH test which is robust to the underlying data distribution. We modelled cumulative tadpole mortality at the end of experiment against tadpole population, salamander population, salamander size distribution, and their pairwise and three-way interactions. All analyses were conducted in R (ver 4.0.2) using packages *MASS* (ver 7.3-51.6; Venables and Ripley 2002) and *Stats* (R Core Team, 2020).

Results

Salamander and tadpole morphology

Our ANOVA test of treatment effects on the morphology of the largest salamander per tank revealed that the offensive giant salamander phenotype was most strongly expressed in the Erimo salamander population when hatchlings grew under a heterogeneous size distribution (Fig. 2A–C). While trunk length was larger in Erimo salamanders irrespective of initial size distribution (ANOVA: Sal_{pop} $F_{1,69} = 53.77$, $P < 0.001$; Fig. 2B), the offensive phenotype expression—large gape relative to trunk length—and gape width was determined by salamander population, initial size distribution and their interaction (Gape width: Sal_{pop} $F_{1,69} = 135.68$, $P < 0.001$; $Size_{sal}$ $F_{1,69} = 89.6$, $P < 0.001$; $Sal_{pop} \times Size_{sal}$ $F_{1,69} = 24.43$, $P < 0.001$; Fig. 2A. Relative gape width: Sal_{pop} $F_{1,69} = 47.19$, $P < 0.001$; $Size_{sal}$ $F_{1,69} = 71.17$, $P < 0.001$; $Sal_{pop} \times Size_{sal}$ $F_{1,69} = 10.68$, $P < 0.001$; Fig. 2C). A post hoc Tukey HSD test on the significant $Sal_{pop} \times Size_{sal}$ interaction revealed that Erimo salamanders under the size heterogeneous treatment had larger relative gape widths than Erimo salamanders reared under a homogenous size distribution (Tukey HSD: $P_{adj} < 0.001$) and than Chitose salamanders under the size heterogeneous treatment ($P_{adj} < 0.001$; Fig. 2C). This was also true for salamander gape width (Tukey HSD $P_{adj} < 0.001$ in both cases). Tadpole population did not affect salamander morphology (ANOVA: $P > 0.05$).

Three-way ANOVA on tank-averaged tadpole phenotypes revealed that Erimo tadpoles had a larger body than Chitose tadpoles (Tad_{pop}: body width $F_{1,58} = 28.24$, $P < 0.001$; body length, $F_{1,58} = 20.76$, $P < 0.001$; Fig. 2D,E). These interpopulation difference in body length and width were greater in the presence of Erimo salamanders than Chitose salamanders ($Sal_{pop} \times Tad_{pop}$: body width $F_{1,58} = 10.08$, $P = 0.002$; body length $F_{1,58} = 5.76$, $P = 0.021$) and tended to be greater under heterogeneous salamander size distributions ($Tad_{pop.} \times Size_{sal}$: body width $F_{1,58} =$

8.43, $P = 0.005$; body length $F_{1,58} = 3.26$, $P = 0.076$). Indeed, Erimo tadpoles exposed to Erimo salamanders grown under cannibalistic conditions had the widest bodies among all treatments (pairwise Tukey HSD comparisons: $P_{adj} < 0.036$), while the remaining treatments did not differ in body width ($P_{adj} > 0.05$) (Fig. 2D). However, a post hoc Tukey HSD test revealed body length was similar across treatments (Fig. 3E). The discordance between body width and size arose because tadpoles expressed the more defensive 'bulgy' phenotype (greater body width:length ratio) when faced with Erimo salamanders than with Chitose salamanders (ANOVA Sal_{pop}: $F_{1,58} = 11.78$, $P = 0.001$; Fig. 3F). There was also a tendency for Erimo tadpoles to express the defensive phenotype more strongly in the presence of Erimo salamanders reared under a heterogeneous size distribution than a homogeneous one (i.e. a three-way $Tad_{pop.} \times Sal_{pop} \times Size_{sal}$ interaction; Fig. 3F), though this interaction only approached statistical significance ($F_{1,58} = 3.04$, $P = 0.086$).

At day 18, just before tadpole predation began, *potential predators* (salamanders with gape width larger than mean tadpole body width; Nosaka et al. 2015) appeared only in the Erimo salamander treatment under heterogeneous size distribution (for Chitose and Erimo tadpoles, present in 6/8 and 4/10 tanks [1.38 and 0.5 potential predator/tank on average], respectively).

Salamander mortality

A Sheirer-Ray-Hare test of among-group differences in salamander mortality at day 18 found a significant effect of initial salamander size distribution and its interaction with salamander population (Fig. 3A), though salamander population alone was marginally nonsignificant (Sal_{pop}: $SRH_{1,69} = 3.28$, $P = 0.07$). Salamander mortality was significantly higher in the heterogeneous salamander size distribution treatment than the homogeneous treatment

362 ($Size_{sal}$: $SRH_{1,69}$ = 35.1, P < 0.001). A post hoc Tukey HSD test on the significant Sal_{pop} × $Size_{sal}$
363 interaction ($SRH_{1,69}$ = 8.3, P = 0.004) revealed that salamander mortality tended to be higher
364 under the heterogenous than the homogeneous size distribution conditions for both the Erimo
365 salamander treatment (Tukey HSD: P_{adj} < 0.001) and the Chitose treatment (P_{adj} = 0.007; Fig.
366 3A). Moreover, Erimo salamanders experienced higher mortality than Chitose salamanders under
367 heterogenous size distribution treatments (P_{adj} < 0.001), while there was no difference in
368 mortality between salamander populations under the homogeneous size distribution (P_{adj} = 0.62;
369 Fig. 3A).
370
371 *Tadpole mortality*
372 Our Sheirer-Ray-Hare test of among-group differences revealed significant effects of
373 salamander population, salamander size distribution, and their interaction, on tadpole mortality
374 (Fig. 3B). Tadpole mortality was significantly higher in the heterogeneous salamander size
375 distribution treatment than the homogeneous treatment ($Size_{sal}$: $SRH_{1,69}$ = 14.1, P < 0.001), and
376 was higher in the Erimo salamander treatment than the Chitose treatment (Sal_{pop}: $SRH_{1,69}$ = 24.8,
377 P < 0.001). A post hoc Tukey HSD test on the significant Sal_{pop} × $Size_{sal}$ interaction ($SRH_{1,69}$ =
378 8.42, P = 0.004) identified significantly higher tadpole mortality under the heterogeneous than
379 the homogeneous salamander size distribution treatment for the Erimo salamander population
380 treatment (Tukey HSD: P_{adj} < 0.001), but there was no difference between size distribution
381 treatment levels for the Chitose salamander population (P_{adj} = 0.69; Fig. 3B). We also found that
382 for the heterogeneous salamander size distribution treatment, tadpole mortality was significantly
383 higher under Erimo than Chitose salamanders (P_{adj} < 0.001), but there was no difference between
384 Erimo and Chitose salamander treatments under homogeneous salamander size distribution (P_{adj}

385 = 0.12; Fig. 3B). We did not find statistical significance for the main and interactive effects of
386 tadpole populations on the tadpole mortality.
387
388

389 **Discussion**

390 Although there is increasing recognition of genetic variation in the conditional
391 development of functional traits (Pigliucci, 2005; Winterhalter & Mousseau, 2007), little is
392 known about how genetic differences in development of predator and prey functional traits
393 influence their trophic interaction strengths. Through an experimental study using predatory
394 *Hynobius retardatus* salamander and prey *Rana pirica* frog tadpoles from two geographic
395 regions (Eriomo and Chitose), we found that population genetic differences in the inducible
396 offense of predatory salamanders shape predator-prey interaction strengths during their
397 cohabitation period. Our experiment revealed that tadpole survival was significantly reduced
398 during their larval stage only when they were exposed to one population (Erimo), and hence
399 genotype, of predatory salamanders under a particular environmental context (heterogeneity in
400 initial predator size distribution). That is, predator-prey interaction strengths between the two
401 amphibian species were jointly determined by salamander genotype and environmental
402 conditions (Fig. 3B). Further, we showed that this interactive effect on salamander predation was
403 explained by the phenotypic differentiation of the salamanders among treatments. Owing to
404 cannibalism during the early life stages of salamanders (Fig. 3A), the largest Erimo salamanders
405 in the size heterogeneous treatment attained a far wider gape and larger body than those in the
406 other treatments before the onset of the salamander-tadpole trophic interaction (Fig. 2A).
407 Consequently, the induced offensive phenotype of Erimo salamanders in the size heterogeneous

408 treatment successfully consumed frog tadpoles that would otherwise be inaccessible prey items
409 due to size constraints. The causal relationship between salamander phenotype and interaction
410 strength (predation) is also evidenced by the exclusive appearance of potential predators in the
411 Erimo salamander population only under initial size heterogeneity. We thus revealed the
412 potential of genetic variation in predator inducible offense to mechanistically shape predator-
413 prey interaction strengths.

414 The antagonistic nature of predator-prey interactions produces the intuitive expectation that
415 genotypes of both predators and prey influence interaction strengths (Bassar et al., 2017;
416 Hiltunen & Becks, 2014). However, we detected significant population effects on the interaction
417 strength only for predatory salamanders (Fig. 3A). Though differential expression of defensive
418 bulgy phenotypes of Erimo and Chitose tadpoles resulted in a significant difference between
419 tadpole populations in their body width under high predation risk (i.e. Erimo salamanders and
420 initial salamander size heterogeneity; Fig. 2D), this did not translate into variation in tadpole
421 mortality. Asymmetry between predator and prey population effects on interaction strengths may
422 result from differences in the timing of inducible offense and defence. While salamanders
423 express offensive phenotype before consuming tadpoles, tadpoles exhibit inducible defence in
424 response to the emergence of giant salamanders (Takatsu & Kishida, 2015; Takatsu et al., 2017).
425 Due to this time lag between the trait expression of potential prey and their would-be predators,
426 population-level differences in offensive trait expression by predatory salamanders should more
427 strongly influence interaction strengths than population differences in tadpoles' inducible
428 defence.

429 We argue that population-specific morphological plasticity of predators underlies the
430 observed salamander population-by-size distribution (G×E) interaction in our model predator-

prey system. Although population-specific behavioural plasticity (e.g. development of aggressiveness) can offer an alternative explanation (Bell & Stamps, 2004), its importance is negligible here since densities of experimental animals are relatively high. In such a context, our salamanders and tadpoles frequently encounter each other, providing ample opportunity for salamander predation. As such, predation success should exclusively depend on the balance between salamander gape and tadpole body size, since *H. retardatus* is a representative swallowing-type predator and *R. pirica* tadpoles are their largest prey items (Takatsu & Kishida, 2013).

Our findings suggest that interaction strengths vary across species' geographic ranges. When comparing predation between naturally co-occurring salamanders and tadpoles, the Erimo pair (Erimo salamanders and Erimo tadpoles) differed in predator-prey interaction strengths between the size heterogeneous and homogeneous salamander treatments, while the Chitose pair did not (Fig. 3B). That is, while the Erimo and Chitose pairs showed similarly weak trophic interactions in the absence of salamander cannibalism (i.e. under homogeneous size distribution), the Erimo pair exhibited stronger predation by salamanders than the Chitose pair where early-life cannibalism resulted in salamander gigantism (Fig. 3B). As one of the factors involving salamander cannibalism, our study featured salamander size distributions resulting from hatch timing variation of salamander hatchlings, which should vary among ponds within geographic regions (Nosaka et al., 2015). Likewise, other prospective factors affecting cannibalism (e.g. conspecific density, alternative prey abundance, habitat complexity, predation pressure; Fox, 1975, Polis, 2003, Kishida et al., 2011) are generally spatially heterogeneous within geographic regions. If within-region spatial heterogeneity in such factors is equivalent across regions, interaction strengths should be more variable in Erimo (where there is potential for strong

predator-prey interactions) than in the Chitose region (potential only for weaker interactions) for these species. Interaction strength variability across ponds may further impact pond communities due to the large biomass of the two amphibian species (Kishida & Nishimura, 2006; Michimae, 2011); predation by salamanders on tadpoles can shift the densities and trait distributions of both species, with knock-on consequences for pond communities (Petranka & Kennedy, 1999). Hence, population-specific condition-dependent expressions in functional traits (i.e. reaction norms) of salamanders can create regional variation in the heterogeneity of their trophic interaction, with the potential to further shape regional variation in β-diversity of natural pond communities (Carstensen, Sabatino, Trøjelsgaard, & Morellato, 2014).

Condition-dependent development of morphological traits as a reaction norm can be the target of natural selection (Urban, 2008, 2010). Our finding of geographic variation in the inducible offense of predators and inducible defence of prey may be due to differences in the coevolutionary history of our model species. While larger body size is necessary for tadpoles to avoid predation by giant salamanders, much larger gape is required for salamanders to consume tadpoles expressing the 'bulgy' defensive phenotype (Takatsu & Kishida, 2015; Takatsu et al., 2017). Although we only examined two geographic populations, the inter-population variation in predator inducible offense and prey inducible defence suggests an arms race scenario; Erimo salamanders more frequently expressed the offensive phenotype than Chitose salamanders, and Erimo tadpoles grew more rapidly and more commonly expressed the defensive phenotype than Chitose tadpoles. This phenotypic pattern implies a geographic mosaic of coevolution (Thompson, 1999; Thompson & Cunningham, 2002), with Erimo as a coevolutionary hotspot (a region where coevolution is escalated). In this scenario where evolutionary enhancement of antagonistic phenotype expression imposes stronger selective pressure on the opponent,

interspecific interactions should be strongest in coevolutionary hotspots. Describing geographic patterns of antagonistic phenotypic plasticity as we have here and testing coevolutionary hypotheses should therefore advance our understanding of the mechanisms underpinning interspecific interaction strengths.

Our study suggests that genetic variation in expression of offensive phenotypes of predators shapes variation in predation pressure within and across regions. Under different selection regimes, divergent reaction norms for functional traits (e.g. plastic versus fixed phenotypes) emerge along environmental gradients (Kishida et al., 2007; Winterhalter & Mousseau, 2007). Investigating geographic variation in reaction norms of functional traits for interacting species, and the consequences of pairwise combinations of such developmental reaction norms, shows promise in disentangling complex geographic mosaics of interspecific interactions around the globe.

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Authors' contributions

KA and OK conceived the ideas and designed methodology; KA and OK collected the data; KA and SRP-JR analysed the data; All authors wrote the manuscript, and gave final approval for publication.

Data availability

All analytic code and data can be downloaded from https://osf.io/26yrc/.

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FIGURE LEGENDS

Fig. 1. Timeline of our experiment. We illustrate a typical scenario for salamander cannibalism, followed by salamander gigantism with offensive phenotype, tadpole predation and tadpole

induced defense. Note that such a scenario of the trophic interaction occurred when Erimo salamander population was used.

Fig. 2. Treatment effects on salamander and tadpole morphology (functional traits). Salamander gape width (A), trunk length (B), and relative gape width (C), and tadpole body width (D), body length (E), and relative body width (F) for each combination of geographic populations (Chitose and Erimo) and initial salamander size distribution treatments (heterogeneous size distribution [to facilitate salamander cannibalism] = black, homogeneous [to suppress cannibalism] = grey). Boxplots represent the median and interquartile range of treatment groups, with points showing individual tanks. The largest salamander in each tank and all measurable tadpoles were measured at day18, prior to the start of predation (see Materials and Methods).

Fig. 3. Mortality (count) of predatory salamanders by day 18 (A), prior to the onset of predation, and prey tadpoles by the end of experiment (B), for each combination of geographic populations (Chitose and Erimo) and initial salamander size distribution treatments (heterogeneous size distribution [to facilitate salamander cannibalism] = black, homogeneous [to suppress cannibalism] = grey). Boxplots represent the median and interquartile range of treatment groups, with points showing individual tanks. Salamander and tadpole mortality largely reflect cannibalism and tadpole consumption by salamanders, respectively.

677 Fig. 1

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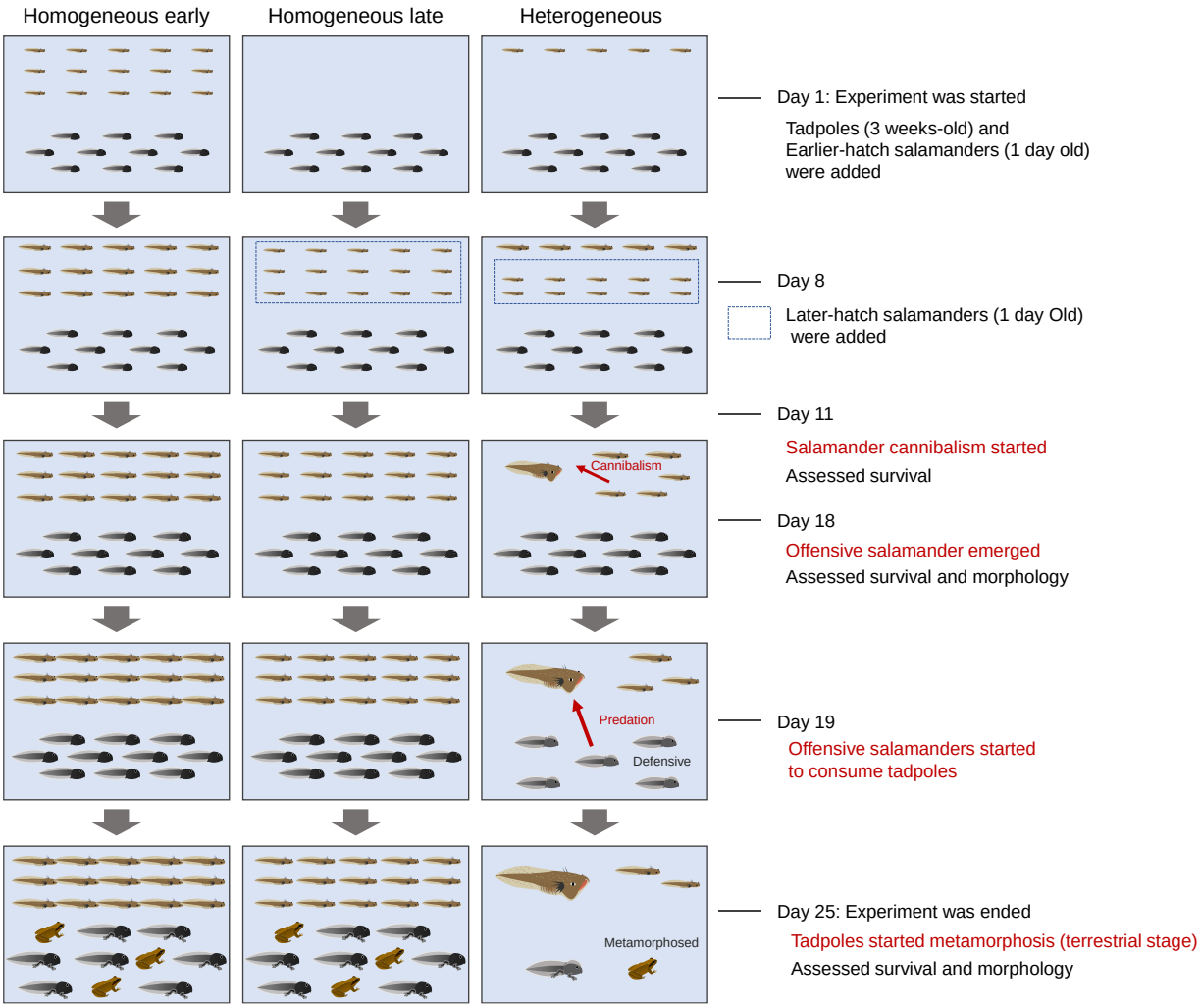

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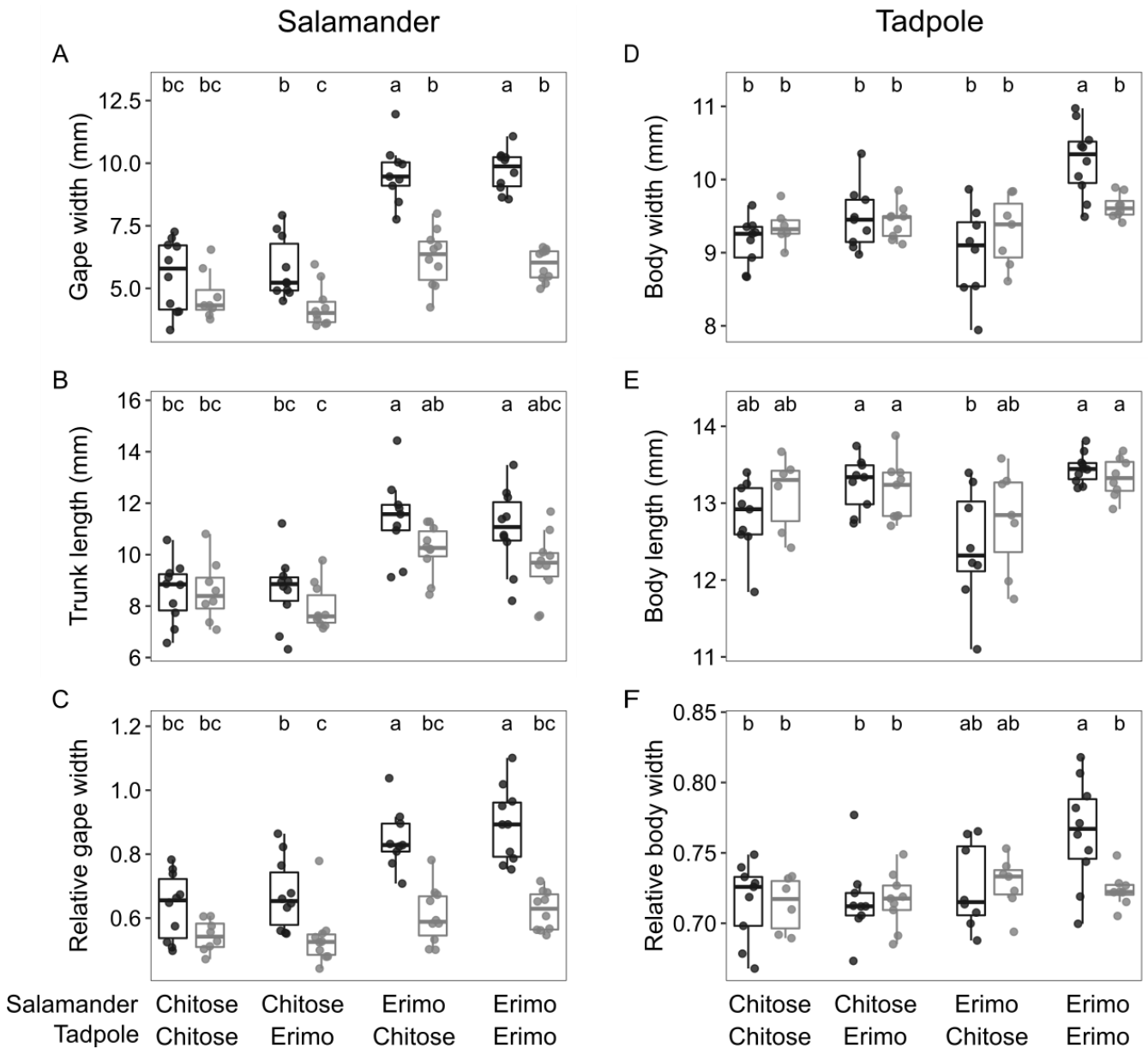
681 Fig. 2



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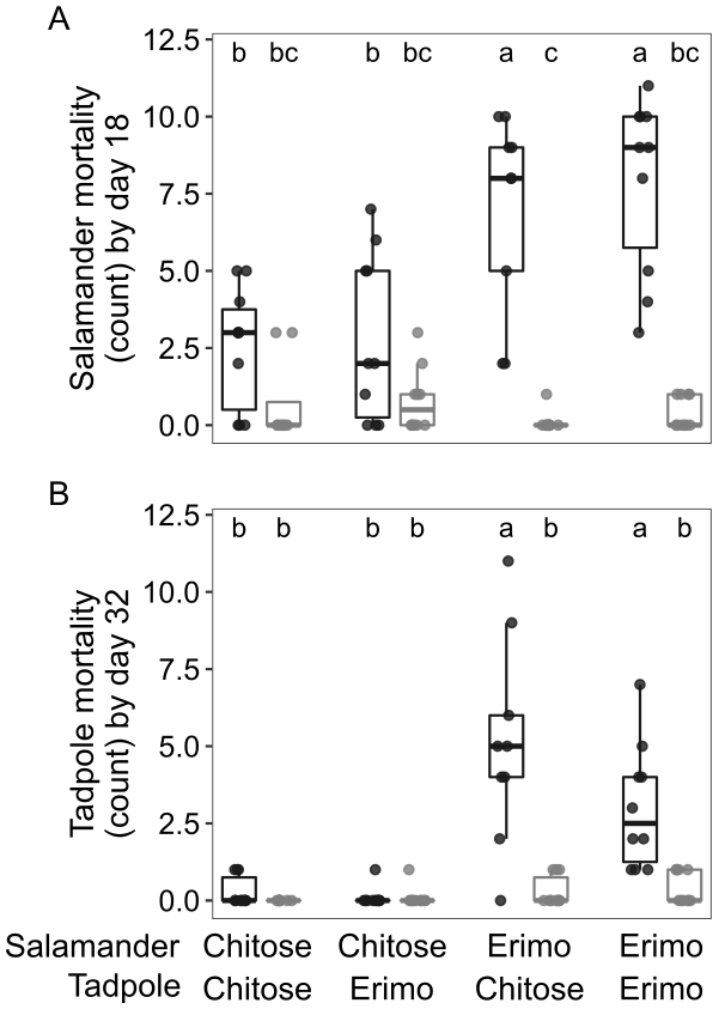
685 Fig. 3



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