Transgenerational plasticity of inducible defenses: combined effects of grand-parental, parental and current environments

Juliette TARIEL[1,*], Sandrine PLÉNET[1], Émilien LUQUET[1]

Abstract

While an increasing number of studies highlights that parental environment shapes offspring phenotype (transgenerational plasticity TGP), TGP beyond the parental generation has received less attention. Studies suggest that TGP impacts population dynamics and evolution of phenotype, but these impacts will depend on how long an environmental effect can persist across generations and whether multi-generational effects are cumulative. Here we tested the impact of both grand-parental and parental environments on offspring reaction norm in a prey-predator system. We exposed three generations of *Physa acuta* snails without and with predator-cues according to a full factorial design and measured offspring inducible defenses. We found that both grand-parental and parental exposure to predator cues impacted offspring anti-predator defenses, but their effects were not cumulative and depended on the defenses considered. We also highlighted that grand-parental environment could alter reaction norm of offspring shell thickness, demonstrating a grand-parental TGP and WGP interaction. We called for more studies covering the combine effects of multigenerational environments.

Keywords

transgenerational plasticity — grand-parental effect — inducible defenses — predator-prey interactions — *Physa acuta*

[1] *Université de Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5023, ENTPE, Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés, Villeurbanne, France*

*__Corresponding author__: juliettetariel@gmail.com

Introduction

Organisms may respond to fluctuating environments by adapting through genetic evolution over generations or through phenotypic plasticity. This last is traditionally defined as the capacity of a given genotype to render alternative phenotypes under different environmental conditions (within-generational plasticity, WGP) (Pigliucci, 2005; West-Eberhard, 2003). Plasticity may also occur across generations (transgenerational plasticity, TGP), when the phenotype of offspring is influenced by carry-over effects of past environments experienced by the previous generation(s) (Agrawal et al., 1999; Galloway and Etterson, 2007; Salinas et al., 2013). Ancestors can alter the phenotype of their offspring without involving genetic changes through a range of non-genetic processes as parental effects, e.g. transmission of nutrients, hormones, proteins (Crean and Bonduriansky, 2014; Mousseau, 1998), or by any form of epigenetic inheritance, e.g. DNA methylation marks, histone protein modifications, non-coding small RNAs (Holeski et al., 2012; Schlichting and Wund, 2014). TGP has been shown for diverse organisms and traits in response to various environments (Bonduriansky and Day, 2009; Donelson et al., 2018; Salinas et al., 2013). When past environmental cues provide reliable proxies about future offspring conditions, TGP may enable organisms to cope with fast-changing environments because it refines offspring phenotypes in anticipation of the environmental conditions they are likely to experience (Bonduriansky and Day, 2009; Donelson et al., 2018; Herman and Sultan, 2011).

Most studies on TGP focused on the effect of parental environment on offspring phenotype (e.g. Donelson et al., 2018; Herman and Sultan, 2011; Mousseau, 1998; Wolf and Wade, 2009) and on offspring reaction norm (interaction TGP x WGP; Salinas et al., 2013). But transgenerational effects are not confined to the parental generation. Few examples showed that TGP can persist for multiple successive generations (Plaistow et al., 2006; Remy, 2010; Sarker and Peleg-Raibstein, 2019), even though the majority is restrained to three generations (i.e. grand-parental effect) (e.g. Hafer et al., 2011; Herman and Sultan, 2011; Kou et al., 2011; Lock, 2012; Shama et al., 2014; Walsh et al., 2014). However, it is unclear how multigenerational effects can interact (Prizak et al., 2014) and experimental works controlling for combination of multigenerational effects always showed complex patterns of phenotypic offspring responses. For example, Hafer et al. (2011) and Walsh et al. (2014) in collembolan (*Folsomia candida*) and cladoceran (*Daphnia ambigua*) respectively, demonstrated that some life-history traits were affected by an interactive effect between grandparental and parental environment, leading to phenotypic landscapes that do not fit simple adaptive scenarios. Moreover, such complex patterns of phenotypic responses can depend on the offspring context. Plaistow et al. (2006), for instance, showed in the soil mite *Sancassania berlesei* that the persistence of past environments (across four generations) differed between high- and low-food offspring contexts. Consequently, understanding how multigenerational combined environments interact to shape the offspring phenotype is still limited and challenging.

Predator-induced plasticity is a well-known model in WGP study (e.g. Harvell, 1990; Hoverman et al., 2005; Relyea, 2001) and allows an individual to fine-tune its phenotype when faced to a predation risk (Benard, 2004; Lima, 1998; Tollrian and Harvell, 1999). Predator-induced defenses are also widely used to investigate TGP over two generations (parental and offspring generations) (e.g. Agrawal et al., 1999; Bell and Stein, 2017; Colicchio, 2017). However, to our knowledge, only two studies have been interested in TGP of predator-induced traits beyond two consecutives generations (Sentis et al., 2018; Walsh et al., 2015).

We focused on a hermaphroditic gastropod *Physa acuta*. Physidae are well-known to develop adaptive phenotypes in response to predation risk (Auld and Houser, 2015; Auld and Relyea, 2008, 2011; Beaty et al., 2016; DeWitt et al., 1999; Gustafson et al., 2014). Predator (crayfish) cues induce WGP of *Physa sp.* life-history traits (larger age and size at first reproduction; Auld and Relyea, 2008), shell thickness (thicker shell; Auld and Relyea, 2011), shell shape (narrower shape; DeWitt, 1998), and behavior (crawling-out the water; Alexander and Covich, 1991; DeWitt et al., 1999). We exposed, to environments without and with predator-cues, three successive generations of snails from hatching to sexual maturity according to a full factorial design. The results concerning the first two generations have demonstrated a predator-induced TGP in *P. acuta* (Luquet and Tariel, 2016) that has been confirmed in a concomitant study (Beaty et al., 2016). Here, we focused on the F3 generation to investigate how the effects of grand-parental, parental and offspring environments combine to influence morphological and behavioral traits.

Material & Methods

Animal collection and experimental design

Adult *P. acuta* snails were collected (n total = 150) on March 2015 in a lentic backwater of the Rhône river (45˚48’6"N, 4˚55’33"E) in Lyon, France. Crayfish predators are present in this natural environment. These wild-caught adult snails constituted our F_0 generation. We pooled them overnight in a 10L-aquarium to ensure that offspring result from outcrossing (*P. acuta* is a preferential outcrosser; Jarne et al., 2010). Then, we individually isolated all F_0 snails in 70 mL plastic vials filled with reconstituted water (2.4 g NaHCO3, 3 g CaSO4, 1.5 g MgSO4, 0.1 g KCl to 25 L deionized water) in a 25˚C experimental room with 12h light-dark photoperiod. After 24 hours, we removed the F_0 adults from the vials and we randomly choose 15 vials with one egg capsule each. These 15 egg capsules (hereafter called "families") developed until hatching ($\sim$ 7 days) and constituted the F_1 generation. Two days after hatching, we randomly sampled 12 siblings per family and split them into two environments: 6 snails remained in a no-predator environment (control environment) while 6 others were moved in a predator-cue environment. These F_1 snails were reared in 70 mL plastic vials with their siblings until 28 days old where they were isolated in the same type of plastic vials (n total = 156) until the end of the experiment (35 days after hatching). We then generated the second F_2 generation. The F_1 snails were divided into copulation groups of 15 individuals (one snail from each of the 15 families). We made 6 copulation groups per treatment and each reproduced in

a 5L-aquarium for 24h in no-predator water to insure embryos are not exposed to predator environment. Then, individuals were isolated, and we randomly selected 18 F_1 snails that had laid eggs from each treatment. We then followed the same protocol as previously to rear F_2 snails in control and predator-cue environments according to a full factorial design (n total = 395) until 49 days after hatching. The F_3 generation was then generated and reared using the procedure described above (n total = 375). As growth rate was slowing down every generation under our laboratory conditions and we wanted snails in a reasonably sufficient size, we reared the F_3 generation at a later age (74 days after hatching). This F_3 generation was represented by eight combinations of grand-parental (E1), parental (E2) and offspring (E3) environments: CCC, CPC, PCC, PPC, CCP, CPP, PCP and PPP with each time "C" for control environment and "P" for predator-cue environment. The number of families per combination of environments spreads from 11 to 17, except for PCC and PCP where the number of families were 3 and 4, respectively (details in Table S1).

Water and food (ad libitum, chopped and boiled lettuce) were renewed for all experimental snails twice a week. Predator-conditioned water with predator cues was obtained by individually rearing crayfishes (*Procambarus clarkii*) in 4L reconstituted water and feeding with one *P. acuta* adult one day before water change (Auld and Relyea, 2011). This crayfish-conditioned water was used for the predator-cue treatment while only reconstituted water was used for the control treatment.

Anti-predator behavior assessment and morphological measurements

We assessed anti-predator behavior three times in 70-day-old F_3 snails through three consecutive days starting one day after the water change. Crawling-out of the water (position above water surface) is considered as allowing to escape from benthic predators like crayfishes (DeWitt et al., 1999). We then measured anti-predator behavior by recording the position above/on or below the water surface in the rearing boxes with predator cues present or absent according to the treatment.

At 74 days old, we weighted the snail weight with an electronic scale at the nearest 0.001mg. Shell and aperture size (shell and aperture length, shell and aperture width) was assessed using a photography analyzed with the software ImageJ (Schneider et al., 2012). We estimated the change of the shell and aperture shape by calculating the shell and aperture length/width ratio. Shell thickness was measured with an electronic caliper at the nearest 0.01mm. Shorter and narrower shell and aperture dimensions (after adjusting for mass) and thicker shell are adaptive anti-predator responses (Auld and Relyea, 2011).

Statistical analysis

The multigenerational effect of predator cues on anti-predator behavior (i.e. snail position above/on or below the water surface) was analyzed using generalized linear mixed models (GLMM) assuming a binomial distribution (logit link function). The multigenerational effect of predator cues on weight, shell thickness, shell size and shape,

and aperture size and shape was analyzed using linear mixed models (LMM) with restricted maximum likelihood estimation and Kenward and Roger's approximation for degrees of freedom. Grand-parental (E1), parental (E2), offspring (E3) environments, and all interactions were considered as fixed effects. Interactions with P-value > 0.10 were removed from models. Because shell thickness and all shell and aperture size traits were significantly correlated with weight (see in Table S2), weight (ln transformation) was added as a continuous fixed effect (analyses of covariance) in these models. Family was considered as a random intercept effect in all models. Individual identity was added as a random intercept model in the GLMM model (anti-predator behavior analysis) to account for repeated measures on the same individual. We used type 2 F-tests for significance of fixed effects in the LMM and likelihood ratio tests in the GLMM (lmerTest package; Kuznetsova et al., 2017). We tested significance of random effects with likelihood ratio tests comparing models with or without the tested random effect in the full fixed effect structure. All statistical analyses were performed with R 3.4.1 (R Core Team, 2017) and the glmer() and lmer() functions from the lme4 package (Bates et al., 2015).

Results

Anti-predator behavior

The offspring exposure to predator cues (E3) significantly increased by 105% the proportion of snails crawling-out the water (Table 1a; Fig. 1a). The parental environment (E2) did not affect the proportion of crawling-out behavior (Table 1a; Fig. 1a). However, grand-parental exposure to predator cues (E1) significantly increased by 28% the proportion of crawling-out behavior (Table 1a; Fig. 1a). Family and individual random effects were significant (Table 1a).

Snail weight

The offspring exposure to predator cues significantly decreased by 18% the snail weight (Table 1b; Fig. 1b). Neither the parental environment nor the grand-parental environment influenced snail weight (Table 1b; Fig. 1b). Family random effect was significant (Table 1b).

Shell thickness

After accounting for snail weight, grand-parental and parental environments interacted both with the offspring environment (Table 1c; Fig. 1c), demonstrating that both could affect the offspring response to predator cues. In the offspring control environment, the grand-parental exposure to predator cues increased by 7% the offspring shell thickness whereas the parental exposure to predator cues decreased by 11% the offspring shell thickness. And in the offspring predator-cue environment, the grand-parental exposure to predator cues decreased by 9% the offspring shell thickness whereas the parental exposure to predator cues increased by 11% the offspring shell thickness. Regarding

the direct effect of offspring environment, offspring from predator-cue environment had a thicker shell than those from current control environment (Table 1c; Fig. 1c). Family random effect was not significant (Table 1c).

Shell shape

The shell shape (ratio shell length/width) was significantly affected by a parental-by-offspring environments interaction (Table 1d; Fig. 1d). The parental exposure to predator cues induced a narrower shell shape (increase by 1.6% of the ratio shell length/width) for offspring from control environment whereas it induced a rounder shell shape (decrease by 0.9% of the ratio) for offspring from predator-cue environment. The grand parental exposure to predator cues had no effect on shell shape (Table 1d; Fig. 1d). Regarding the direct effect of offspring environment, the offspring exposure to predator cues induced a narrower shell (Table 1d; Fig. 1d) but did not affect significantly the shell length and width (for details see Table S3 and Figure S1). Family random effect was significant (Table 1d).

Aperture shape

The aperture shape (ratio aperture length/width) was significantly affected by a parental-by-offspring environments interaction (Table 1e; Fig. 1e; for details about aperture length and width see Table S3 and Figure S1). The offspring from parental control environment reacted to predator cues by increasing of 1.1% their aperture shape (narrower aperture), whereas offspring from parental predator-cue environment reacted to predator cues by decreasing by 2.5% their aperture shape (rounder aperture). From a parental environment point of view, the parental exposure to predator cues induced a narrower aperture of offspring from offspring control environment (increase of 3.5%) whereas it did not significantly influence the aperture shape of offspring from offspring predator-cue environment. The grand-parental environment had no effect on aperture shape (Table 1e; Fig. 1e). Family random effect was significant (Table 1e).

Discussion

We first confirm that the exposure to predator cues induces well-known defenses against crayfish predation in *P. acuta*. Our key finding is that predator cues alter also offspring defenses two generations later but depending on the offspring environment (WGP x TGP) and the defensive traits considered. Our experimental work highlights that TGP effects can be complex beyond the parental generation and that the offspring phenotype results from a combination of multigenerational effects.

Corroborating several studies on anti-predator defenses of *Physa sp.* (Auld and Relyea, 2011; Dalesman et al., 2009; DeWitt et al., 1999, 2000; Turner et al., 1999), the offspring exposure to predator cues induced higher crawling-out behavior, shell-crushing resistance (thicker shell) and entry-resistant shell (narrower shell and aperture shape). Moreover, offspring exposed to predator cues were lighter, suggesting a trade-off, i.e. a lower energetic investment in growth due to a potential cost to produce these defenses (as showed in other gastropod species: Brönmark et al.,

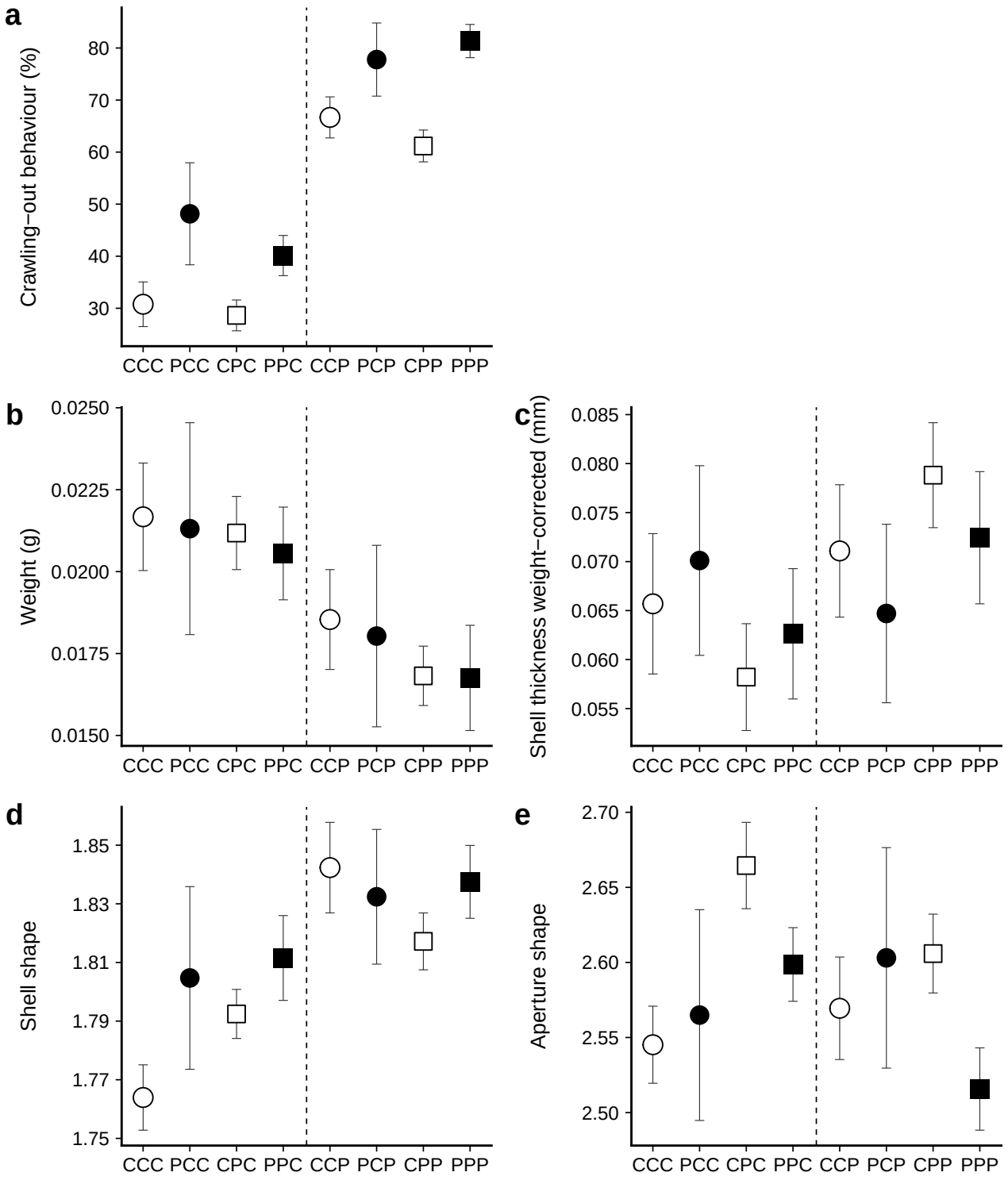

Figure 1. The effect of multi-generational exposure to predator-cues on offspring **a** crawling-out behaviour (proportion of snails out the water in %), **b** total weight, **c** least-mean squares of shell thickness in the model accounting for weight described in Table 1, **d** shell shape (ratio shell length/width), and **e** aperture shape (ratio aperture length/width). CCC, PCC, CPC, PCC, CCP, PCP, CPP and PPP represent the eight combinations of grand-parental (E1), parental (E2) and offspring (E3) environments with "C" for control environment and "P" for predator-cue environment for every generation. White shapes are for grand-parental control environment and black shapes for grand-parental predator-cue environment. Circles are for parental control environment and squares for parental predator-cue environment. Data are means ± SE.

a.	Crawling-out	*Fixed effects*	*Estimate (SE)*	*Df*	*Chisq*	*P*
		Grand-parental env. (E_1)	0.778	1	8.29	0.004*
		Parental env. (E_2)	−0.165 (0.261)	1	0.40	0.527
		Offspring env. (E_3)	1.81 (0.181)	1	114.00	< 0.001*
		Random effects	*Variance*	*Df*	*Chisq*	*P*
		Individual	4.89×10^{-1}	1	8.26	0.004*
		Family	3.91×10^{-1}	1	14.98	< 0.001*
b.	Weight	*Fixed effects*	*Estimate (SE)*	*Numdf, Dendf*	*F*	*P*
		Grand-parental env. (E_1)	−0.0006 (0.00141)	1,44.55	0.16	0.688
		Parental env. (E_2)	−0.0011 (0.00141)	1,50.40	0.60	0.442
		Offspring env. (E_3)	−0.0039 (0.00100)	1,357.66	14.96	< 0.001*
		Random effect	*Variance*	*Df*	*Chisq*	*P*
		Family	8.59×10^{-6}	1	6.60	0.010*
c.	Shell thickness	*Fixed effects*	*Estimate (SE)*	*Numdf, Dendf*	*F*	*P*
		Weight (ln transformation)	0.03(0.002)	1,366.81	238.25	< 0.001*
		Grand-parental env. (E_1)	0.004(0.004)	1,41.60	0.12	0.733
		Parental env. (E_2)	−0.01(0.004)	1,51.13	0.02	0.878
		Offspring env. (E_3)	0.01(0.005)	1,359.15	24.56	< 0.001*
		E_1 x E_3	−0.01(0.005)	1,352.60	3.91	0.049*
		E_2 x E_3	0.02(0.006)	1,365.59	7.25	0.007*
		Random effect	*Variance*	*Df*	*Chisq*	*P*
		Family	1.51×10^{-5}	1	0.32	0.573
d.	Shell shape	*Fixed effects*	*Estimate (SE)*	*Numdf, Dendf*	*F*	*P*
		Grand-parental env. (E_1)	0.0194(0.01347)	1,45.76	2.07	0.157
		Parental env. (E_2)	0.0271(0.01700)	1,49.75	0.04	0.837
		Offspring env. (E_3)	0.0707(0.01642)	1,355.01	18.54	< 0.001*
		E_2 x E_3	−0.0467(0.01937)	1,363.86	5.77	0.017*
		Random effect	*Variance*	*Df*	*Chisq*	*P*
		Family	9.85×10^{-4}	1	15.07	< 0.001*
e.	Aperture shape	*Fixed effects*	*Estimate (SE)*	*Numdf, Dendf*	*F*	*P*
		Grand-parental env. (E_1)	−0.0559(0.03675)	1,47.03	2.30	0.136
		Parental env. (E_2)	0.1017(0.04476)	1,49.42	1.71	0.197
		Offspring env. (E_3)	0.0298(0.04066)	1,352.49	4.04	0.045*
		E_2 x E_3	−0.103(0.04792)	1,361.09	4.63	0.032*
		Random effect	*Variance*	*Df*	*Chisq*	*P*
		Family	8.22×10^{-3}	1	23.34	< 0.001*

Table 1. Results of generalized mixed model (a) and linear mixed model (b, c, d and e) analyses on offspring predator-induced traits.

2012). This result stresses the fitness advantage of WGP which allow the production of costly defenses only in case of predation (Harvell, 1990).

Grand-parental and parental effects on anti-predator defenses

TGP is expected to evolve when the ancestral environment is a good proxy of offspring environment (Bonduriansky and Day, 2009; Dey et al., 2016; English et al., 2015; Harvell, 1990; Leimar and McNamara, 2015; Uller, 2008), allowing a pre-adaptation of offspring to predation risk (Agrawal et al., 1999). In our predator-prey system, crayfish has a long lifespan (ca. 3 years) compared to the generation time of *P. acuta* (ca. 50 days) and a relatively sedentary lifestyle (Vioque-Fernández et al., 2009). This suggests that generational cues of crayfish presence can be a good proxy of predation risk across several snail generations and thus that TGP could have long-lasting effects on the anti-predator defenses. Consistently, in *P. acuta*, parental exposure to predator cues induces a more crush-resistant shell and an higher escape behavior in offspring (Beaty et al., 2016; Luquet and Tariel, 2016). In this study, as expected, TGP went further than the parental generation: the grand-parental environment also influenced the anti-predator behavior and the shell thickness of offspring.

How long can persist transgenerational effects on anti-predator responses is a remaining open question. To our knowledge, the study of Sentis et al. (2018) on the pea aphid (*Acyrthosiphon pisum*) is the only one to investigate predator-induced TGP over a large number of generations (> 25). They found that the defensive phenotype - a high frequency of winged aphids in the population - persists for one generation after removing predators whatever the induction time is, i.e. the previous number of successive generations experiencing the novel environment (predator presence). However, three generations are needed after removing predators for the frequency of winged phenotypes to come back to the control level, and this number of generations increases with the induction time. Together, these results suggest that multigenerational environmental effects on inducible defenses are broader than just a parental effect and could persist for many generations.

Combination of multigenerational effects on anti-predator defenses

As previous theoretical and experimental works (Hafer et al., 2011; Kou et al., 2011; Lock, 2012; Prizak et al., 2014; Shama and Wegner, 2014; Walsh et al., 2014), we showed that the offspring phenotype results from a combination of multigenerational effects (grand-parents, parents and offspring). However, the grand-parental and parental effects did not interact together in our study. They acted rather independently, either only one affected the offspring phenotype or the two acting in opposition, and sometimes in interaction with the offspring environment (WGP x TGP). This results in complex offspring phenotypic patterns that do not fit with simple anti-predator scenarios. Morphological traits were only influenced by a parental and offspring environment interaction (WGP x TGP; non-significant grand-parental effect). Parental exposure to predator cues induced a more elongated shell and aperture shape in offspring control environment whereas it induced a rounder shell shape and didn't affect

aperture shape in offspring predator environment. By contrast, the offspring crawling-out behavior increased with offspring and grand-parental exposure to predator cues while the parental environment did not alter this behavior. Shell thickness was influenced by both grand-parental and parental environments, but in opposing directions and depending on the offspring environment (grand-parental and parental WGP x TGP interactions). In offspring control environment, grand-parental exposure to predator cues increased the offspring shell thickness whereas parental exposure reduced the shell thickness. The effects were opposite in the offspring predator-cue environment.

Firstly, these results confirm that offspring reaction norms can be altered by parental environment (morphological traits and shell thickness; Donelson et al., 2018; Luquet and Tariel, 2016; Salinas et al., 2013) but expand for the first time the WGP x TGP interaction to grand-parental environmental cues (shell thickness). Secondly, opposing directions of grand-parental and parental effects found on shell thickness are not rare in empirical studies (Magiafoglou and Hoffmann, 2003; Shama and Wegner, 2014) and illustrate the complexity to determine the adaptive significance of multigenerational effects. Such opposing effects may reflect different mechanisms underlying the transfer of environmental information (Shea et al., 2011). This complex opposing relationship between grand-parental and parental environmental effects could be also theoretically beneficial by reducing the phenotypic variance which allow the population to stay closer to the target phenotype (Prizak et al., 2014). Moreover, focusing on few generations in short-term experiments artificially focuses the interpretations of such effects while they could only be transient over larger timescales in a population dynamic framework. For example, Sentis et al. (2018), after removing predators, observed that the frequency of winged aphids remained high for one generation before dropping abruptly below the control levels (grand-parental effect), and then converging with the winged aphid frequencies of the control lines (great-grand-parental effect). Consequently, in focusing on only three consecutive generations as in our study, these results could be interpreted as a negative grand-parental effect (decrease of winged aphid frequency) opposing to a positive parental effect (increase of winged individual frequency) on the offspring phenotype. These findings highlight the need to develop empirical studies on larger timescales and controlling for the combination of multigenerational effects.

Trait-dependence of transgenerational plasticity

Our results show that the pattern of TGP depends on the traits (anti-predator behavior, shell thickness and shell/aperture shapes). Behavioral traits, which are often labile and exhibiting reversible WGP within developmental or adult stages, are predicted to be influenced by current environment rather than by past environmental experience (Dingemanse and Wolf, 2013; Piersma and Drent, 2003). Behavioral WGP in response to current environmental cues should rapidly by-pass the behavioral TGP (Beaman et al., 2016). By contrast, the traits that are more constrained during the development and exhibiting irreversible variations, as morphological traits, are predicted to be relatively more influenced by past environments (Kuijper and Hoyle, 2015). TGP on morphological traits could irreversibly

engage the offspring on developmental trajectories that this effect could not be compensated by WGP. In *P. acuta*, crawling-out behavior is indeed very flexible and reversible at a time scale of hours while a thicker shell and a rounder or narrower shell shape are irreversible changes in the developmental trajectory (DeWitt et al., 1999; Relyea, 2003). Surprisingly in our study, the escape behavior of offspring is influenced by the grand-parental environment while morphological (shell and aperture shapes) traits are only influenced by the parental environment. This highlights that transgenerational effects on morphological traits may have a short persistence over generations while behavioral TGP may be much more prevalent than currently realized. Parental TGP on behavioral traits has been sometimes observed (e.g. Bestion et al., 2014; Donelan and Trussell, 2015; Giesing et al., 2011; Storm and Lima, 2010) and few times with long-lasting effects over generations (Dias and Ressler, 2014; Remy, 2010).

Conclusion

In our study, we demonstrated that the effects of multigenerational (grand-parental, parental and offspring) exposure to predator cues on a variety of offspring defensive traits (escape behavior, shell thickness and shape) do not fit simple anti-predator scenarios. The multigenerational effects combined, sometimes in opposing directions and depending on the traits, to shape the offspring anti-predator defenses. We call for more theoretical and empirical studies integrating the combined effects of multigenerational environments on larger number of generations, investigating the underlying mechanisms (epigenetic inheritance) and evaluating their evolutionary importance.

Acknowledgments

Thanks to P. Gibert for advice on the manuscript. We thank L. Bensousoussan for help on data analysis. We also thank Mathias Legrand for the LateX template.

Author contribution

S.P. and E.L. conceived of and designed the study. All authors contributed to the data collection. J.T. and E.L. carried out the data analysis and interpretation. All authors drafted, revised and approved the article for publication.

Conflict of interest

We have no conflicts of interests.

Data and materials

Data and the R script used for data analysis will be deposited in Zenodo upon publication.

References

Agrawal, A. A., Laforsch, C., and Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. Nature 401, 60-63. doi:10.1038/43425.

Alexander, J. E., and Covich, A. P. (1991). Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish *Procambarus simulans*. Oecologia 87, 435-442.

Auld, J. R., and Houser, R. (2015). Age-dependent effects of predation risk on reproductive success in a freshwater snail. Evolution 69, 2793-2798. doi:10.1111/evo.12769.

Auld, J. R., and Relyea, R. A. (2008). Are there interactive effects of mate availability and predation risk on life history and defence in a simultaneous hermaphrodite? J. Evolution. Biol. 21, 1371-1378. doi:10.1111/j.1420-9101.2008.01562.x.

Auld, J. R., and Relyea, R. A. (2011). Adaptive plasticity in predator-induced defenses in a common freshwater snail: altered selection and mode of predation due to prey phenotype. Evol. Ecol. 25, 189-202. doi:10.1007/s10682-010-9394-1.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1-48. doi:10.18637/jss.v067.i01.

Beaman, J. E., White, C. R., and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. Trends Ecol. Evol. 31, 237-249. doi:10.1016/j.tree.2016.01.004.

Beaty, L. E., Wormington, J. D., Kensinger, B. J., Bayley, K. N., Goeppner, S. R., Gustafson, K. D., et al. (2016). Shaped by the past, acting in the present: transgenerational plasticity of anti-predatory traits. Oikos 125, 1570-1576. doi:10.1111/oik.03114.

Bell, A. M., and Stein, L. R. (2017). Transgenerational and developmental plasticity at the molecular level: lessons from *Daphnia*. Mol. Ecol. 26, 4859-4861. doi:10.1111/mec.14327.

Benard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. Annu. Rev. Ecol. Evol. S. 35, 651-673. doi:10.1146/annurev.ecolsys.35.021004.112426.

Bestion, E., Teyssier, A., Aubret, F., Clobert, J., and Cote, J. (2014). Maternal exposure to predator scents: offspring phenotypic adjustment and dispersal. Proc. R. Soc. B-Biol. Sci. 281, 20140701. doi:10.1098/rspb.2014.0701.

Bonduriansky, R., and Day, T. (2009). Nongenetic inheritance and its evolutionary implications. Annu. Rev. Ecol. Evol. S. 40, 103-125. doi:10.1146/annurev.ecolsys.39.110707.173441.

Brönmark, C., Lakowitz, T., Nilsson, P. A., Ahlgren, J., Lennartsdotter, C., and Hollander, J. (2012). Costs of inducible defence along a resource gradient. PLoS One 7, e30467. doi:10.1371/journal.pone.0030467.

Colicchio, J. (2017). Transgenerational effects alter plant defence and resistance in nature. J. Evolution. Biol. 30, 664-680. doi:10.1111/jeb.13042.

Crean, A. J., and Bonduriansky, R. (2014). What is a paternal effect? Trends Ecol. Evol. 29, 554-559. doi:10.1016/j.tree.2014.07.009.

Dalesman, S., Rundle, S. D., and Cotton, P. A. (2009). Crawl-out behaviour in response to predation cues in an aquatic gastropod: insights from artificial selection. Evol. Ecol. 23, 907-918. doi:10.1007/s10682-008-9280-2.

DeWitt, T. J. (1998). Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. J. Evolution. Biol. 11, 465-480. doi:10.1046/j.1420-9101.1998.11040465.x.

DeWitt, T. J., Robinson, B. W., and Wilson, D. S. (2000). Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evol. Ecol. Res. 2, 129-148.

DeWitt, T. J., Sih, A., and Hucko, J. A. (1999). Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. Anim. Behav. 58, 397-407. doi:10.1006/anbe.1999.1158.

Dey, S., Proulx, S. R., and Teotónio, H. (2016). Adaptation to temporally fluctuating environments by the evolution of maternal effects. PLOS Biol. 14, e1002388. doi:10.1371/journal.pbio.1002388.

Dingemanse, N. J., and Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: causes and consequences. Anim. Behav. 85, 1031-1039. doi:10.1016/j.anbehav.2012.12.032.

Donelan, S. C., and Trussell, G. C. (2015). Parental effects enhance risk tolerance and performance in offspring. Ecology 96, 2049-2055. doi:10.1890/14-1773.1.

Donelson, J. M., Salinas, S., Munday, P. L., and Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: where do we go from here? Glob. Change Biol. 24, 13-34. doi:10.1111/gcb.13903.

English, S., Pen, I., Shea, N., and Uller, T. (2015). The information value of non-genetic inheritance in plants and animals. Plos One 10, e0116996. doi:10.1371/journal.pone.0116996.

Galloway, L. F., and Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild. Science 318, 1134-1136. doi:10.1126/science.1148766.

Giesing, E. R., Suski, C. D., Warner, R. E., and Bell, A. M. (2011). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. P. Roy. Soc. B-Biol. Sci. 278, 1753-1759. doi:10.1098/rspb.2010.1819.

Gustafson, K., Kensinger, B., Bolek, M., and Luttbeg, B. (2014). Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions. Evol. Ecol. Res. 16, 77-89.

Hafer, N., Ebil, S., Uller, T., and Pike, N. (2011). Transgenerational effects of food availability on age at maturity and reproductive output in an asexual collembolan species. Biol. Lett. 7, 755-758. doi:10.1098/rsbl.2011.0139.

Harvell, C. D. (1990). The ecology and evolution of inducible defenses. Q. Rev. Biol. 65, 323-340.

Herman, J. J., and Sultan, S. E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. Front. Plant Sci. 2, 102. doi:10.3389/fpls.2011.00102.

Holeski, L. M., Jander, G., and Agrawal, A. A. (2012). Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol. Evol. 27, 618-626. doi:10.1016/j.tree.2012.07.011.

Hoverman, J. T., Auld, J. R., and Relyea, R. A. (2005). Putting prey back together again: integrating predator-induced behavior, morphology, and life history. Oecologia 144, 481-491. doi:10.1007/s00442-005-0082-8.

Jarne, P., Pointier, J. P., David, P., and Koene, J. M. (2010). Basommatophoran gastropods. The evolution of primary sexual characters in animals, 173-196.

Kou, H. P., Li, Y., Song, X. X., Ou, X. F., Xing, S. C., Ma, J., et al. (2011). Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa L.*). J. Plant Physiol. 168, 1685-1693. doi:10.1016/j.jplph.2011.03.017.

Kuijper, B., and Hoyle, R. B. (2015). When to rely on maternal effects and when on phenotypic plasticity? Evolution 69, 950-968. doi:10.1111/evo.12635.

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82, 1-26. doi:10.18637/jss.v082.i13.

Leimar, O., and McNamara, J. M. (2015). The evolution of transgenerational integration of information in heterogeneous environments. Am. Nat. 185, E55-E69. doi:10.1086/679575.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. what are the ecological effects of anti-predator decision-making? BioScience 48, 25-34. doi:10.2307/1313225.

Lock, J. E. (2012). Transgenerational effects of parent and grandparent gender on offspring development in a biparental beetle species. Biol. Lett. 8, 408-411. doi:10.1098/rsbl.2011.0920.

Luquet, E., and Tariel, J. (2016). Offspring reaction norms shaped by parental environment: interaction between within- and trans-generational plasticity of inducible defenses. BMC Evol. Biol. 16, 209. doi:10.1186/s12862-016-0795-9.

Mousseau, T. (1998). The adaptive significance of maternal effects. Trends Ecol. Evol. 13, 403-407. doi:10.1016/S0169-5347(98)01472-4.

Piersma, T., and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. Trends Ecol. Evol. 18, 228-233. doi:10.1016/S0169-5347(03)00036-3.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20, 481-486. doi:10.1016/j.tree.2005.06.001.

Plaistow, S. J., Lapsley, C. T., and Benton, T. G. (2006). Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. Am. Nat. 167, 206-215. doi:10.1086/499380.

Prizak, R., Ezard, T. H. G., and Hoyle, R. B. (2014). Fitness consequences of maternal and grandmaternal effects. Ecol. Evol. 4, 3139-3145. doi:10.1002/ece3.1150.

R Core Team (2017). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing Available at: https://www.R-project.org/.

Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82, 523-540. doi:10.1890/0012-9658(2001)082[0523:MABPOL]2.0.CO;2.

Relyea, R. A. (2003). Predators come and predators go: the reversibility of predator-induced traits. Ecology 84, 1840-1848. doi:10.1890/0012-9658(2003)084[1840:PCAPGT]2.0.CO;2.

Remy, J.-J. (2010). Stable inheritance of an acquired behavior in *Caenorhabditis elegans*. Curr. Biol. 20, R877-R878. doi:10.1016/j.cub.2010.08.013.

Salinas, S., Brown, S. C., Mangel, M., and Munch, S. B. (2013). Non-genetic inheritance and changing environments. Non-Genetic Inheritance 1, 38-50. doi:10.2478/ngi-2013-0005.

Sarker, G., and Peleg-Raibstein, D. (2019). Maternal overnutrition induces long-term cognitive deficits across several generations. Nutrients 11, 7. doi:10.3390/nu11010007.

Schlichting, C. D., and Wund, M. A. (2014). Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. Evolution 68, 656-672. doi:10.1111/evo.12348.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of Image Analysis. Nat Methods 9, 671-675.

Sentis, A., Bertram, R., Dardenne, N., Ramon-Portugal, F., Espinasse, G., Louit, I., et al. (2018). Evolution without standing genetic variation: change in transgenerational plastic response under persistent predation pressure. Heredity 121, 266. doi:10.1038/s41437-018-0108-8.

Shama, L. N. S., Strobel, A., Mark, F. C., and Wegner, K. M. (2014). Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. Funct. Ecol. 28, 1482-1493. doi:10.1111/1365-2435.12280.

Shama, L. N. S., and Wegner, K. M. (2014). Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations. J. Evolution. Biol. 27, 2297-2307. doi:10.1111/jeb.12490.

Shea, N., Pen, I., and Uller, T. (2011). Three epigenetic information channels and their different roles in evolution: epigenetic mechanisms and evolution. J. Evolution. Biol. 24, 1178-1187. doi:10.1111/j.1420-9101.2011.02235.x.

Storm, J. J., and Lima, S. L. (2010). Mothers forewarn offspring about predators: a transgenerational maternal effect on behavior. Am. Nat. 175, 382-390. doi:10.1086/650443.

Tollrian, R., and Harvell, C. D. (1999). The Ecology and Evolution of Inducible Defenses. Princeton University Press.

Turner, A. M., Fetterolf, S. A., and Bernot, R. J. (1999). Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia 118, 242-247. doi:10.1007/s004420050724.

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. Trends Ecol. Evol. 23, 432-438. doi:10.1016/j.tree.2008.04.005.

Vioque-Fernández, A., Alves de Almeida, E., and López-Barea, J. (2009). Assessment of Doñana National Park contamination in *Procambarus clarkii*: integration of conventional biomarkers and proteomic approaches. Sci. Total Env. 407, 1784-1797. doi:10.1016/j.scitotenv.2008.11.051.

Walsh, M. R., Cooley, F., Biles, K., and Munch, S. B. (2015). Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory? P. Roy. Soc. B-Biol. Sci. 282, 20142205. doi:10.1098/rspb.2014.2205.

Walsh, M. R., Whittington, D., and Funkhouser, C. (2014). Thermal transgenerational plasticity in natural populations of *Daphnia*. Integr. Comp. Biol. 54, 822-829. doi:10.1093/icb/icu078.

West-Eberhard, M. J. (2003). Developmental plasticity and evolution. Oxford University Press.

Wolf, J. B., and Wade, M. J. (2009). What are maternal effects (and what are they not)? Phil. Trans. R. Soc. Lond. B 364, 1107-1115. doi:10.1098/rstb.2008.0238.