PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Walsh MR, Cooley IV F, Biles K, Munch SB. 2015 Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory? *Proc. R. Soc. B* **282**: 20142205.
http://dx.doi.org/10.1098/rspb.2014.2205

Received: 4 September 2014
Accepted: 15 October 2014

Subject Areas:
ecology, evolution

Keywords:
ecological epigenetics, life-history evolution, maternal effects, *Daphnia*

Author for correspondence:
Matthew R. Walsh
e-mail: matthew.walsh@uta.edu

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2014.2205 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory?

Matthew R. Walsh[1], Frank Cooley IV[1], Kelsey Biles[1] and Stephan B. Munch[2]

[1]Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA
[2]National Marine Fisheries Service, 110 Shaffer Road, Santa Cruz, CA 95060, USA

Much work has shown that the environment can induce non-genetic changes in phenotype that span multiple generations. Theory predicts that predictable environmental variation selects for both increased within- and across-generation responses. Yet, to the best of our knowledge, there are no empirical tests of this prediction. We explored the relationship between within- versus across-generation plasticity by evaluating the influence of predator cues on the life-history traits of *Daphnia ambigua*. We measured the duration of predator-induced transgenerational effects, determined when transgenerational responses are induced, and quantified the cues that activate transgenerational plasticity. We show that predator exposure during embryonic development causes earlier maturation and increased reproductive output. Such effects are detectable two generations removed from predator exposure and are similar in magnitude in response to exposure to cues emitted by injured conspecifics. Moreover, all experimental contexts and traits yielded a negative correlation between within- versus across-generation responses. That is, responses to predator cues within- and across-generations were opposite in sign and magnitude. Although many models address transgenerational plasticity, none of them explain this apparent negative relationship between within- and across-generation plasticities. Our results highlight the need to refine the theory of transgenerational plasticity.

1. Introduction

It is becoming increasingly clear that environmental changes, due to such factors as invasive species, rising temperatures and habitat loss, pose significant threats to biodiversity. Much research has evaluated the mechanisms that allow organisms to adapt to environmental change. This work has focused on the ability of organisms to evolve or alter the expression of traits (phenotypic plasticity) in response to environmental changes [1–3]. Yet, there is now much evidence demonstrating that the environment can induce non-genetic phenotypic changes that span multiple generations. Such 'transgenerational plasticity' occurs when the environment experienced by parents alters the phenotypes of subsequent generations [4,5]. Transgenerational responses have been documented in many organisms [6] for a variety of environmental perturbations [3] and are postulated to have far-reaching consequences for population dynamics [7], community interactions [8] and the rate and direction of evolutionary change [9–11]. Yet, despite widespread appreciation for the existence of transgenerational plasticity ([11,12], but see [13,14]), our understanding of the evolution of transgenerational responses is limited.

Theory predicts that similar conditions favour the evolution of phenotypic responses that occur within- and across-generations. Within-generation responses are favoured when environmental conditions are variable but predictable [15,16] and such predictions have received empirical support (e.g. [17–19]). Transgenerational responses are expected to be favoured when there is environmental heterogeneity across generations and when offspring environmental conditions are predictable from parental environmental conditions [2,3,20–22]. Current

theory that explicitly incorporates phenotypic plasticity and 'maternal effects' [23,24] indicates that predictable environmental variation will simultaneously select for increased plasticity within- *and* across-generations. However, the covariation between within- and across-generation plasticities has not yet received any empirical evaluation.

The interplay between freshwater species of zooplankton and their predators has long served as a model for studying phenotypic plasticity. Notably, many species of water fleas (*Daphnia*) respond to the presence of predators by producing morphological defences (head and tail spines) and altering life-history traits [25,26]. Specifically, *Daphnia* sp. respond within a generation to fish chemical cues (kairomones) by developing at a faster rate and investing more heavily into reproduction [25,26]. There is also evidence that parental exposure to predator cues significantly modifies the phenotypes of offspring [27–29], though the duration of such effects is unclear. A key virtue of using asexually reproducing *Daphnia* to explore transgenerational responses is that the results within clones are unambiguously due to plasticity; they cannot result from selection, recombination or genetic drift. Thus, *Daphnia* offer great promise to explore the connection between plasticity within- and across-generations.

Here we quantified patterns of within- and across-generation predator-induced plasticities across an array of experimental contexts in natural populations of *Daphnia*. We reared more than 25 clones of *Daphnia ambigua* from lakes in Connecticut in a common garden setting for two generations. We then performed a series of experiments that were designed to: (i) measure the duration of predator-induced transgenerational effects, (ii) evaluate the mechanism of transgenerational induction (i.e. maternal versus embryonic exposure to predators), and (iii) determine whether similar responses are generated by exposure to cues emitted by injured conspecifics versus cues emitted by predators. All experiments produced direct evaluations of the magnitude of within- versus across-generation plasticity.

2. Material and methods

(a) Overview

This study used clones of *D. ambigua* from six lakes in Connecticut: Bride, Dodge, Gardner, Long, Quonnipaug and Wyassup [30,31]. Note that not all lakes are represented in each experiment. *Daphnia* in these lakes naturally experience a variety of fish predators including strong predation intensity by the alewife (*Alosa pseudoharengus*; in Bride, Dodge, Long and Quonnipaug) as well as a variety of generalist planktivorous fish predators such as bluegill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*), redbreast sunfish (*Lepomis auritus*) and white perch (*Morone Americana*) in all lakes [30,31]. We have previously explored life-history variation in *D. ambigua* as a function of changes in the severity and duration of predation intensity [32–34]. The goal of this study was to use a genetically diverse array of clones across many lakes to characterize transgenerational responses in this species. All of the experiments described below used clones of *Daphnia* that were originally hatched from ephippia (diapausing resting eggs), reared for several generations in a common garden setting, and then experienced multiple generations of experimental manipulation.

(b) Experiment 1: predator cue removal

The aim of this first experiment was to characterize the transgenerational effects of predator cues and to quantify the duration of such effects. To establish our laboratory populations, we hatched 26 clones of *D. ambigua* from sediment samples that were collected using an Ekman grab from each lake. These sediment samples were all collected approximately in August 2009. Even though we do not have information on the age of these surface sediment samples, all of our populations have experienced consistent exposure to fish predators over the past century [35]. We hatched seven clones each from Bride and Dodge Lake and six clones each from Long and Quonnipaug Lake. For each clone, the first laboratory generation consisted of a single post-ephippial female that was reared individually in a 90 ml jar containing COMBO media [36] and fed ample quantities of *Scenedesmus obliquus* (concentration: more than 0.8 mg C l^{-1} d^{-1}). These individuals were reared under common temperature (18°C) and photoperiod regimes (photoperiod: 14 L : 10 D). All clones were transferred to fresh media and algae every day. The second laboratory generation was established by collecting replicate sets of two newly born neonates from the second clutch of each clone (i.e. multiple females were available to produce the experimental treatments). The density of individuals was reduced to one individual per container on day 3. These individuals experienced the same conditions (temperature, photoperiod, food quantities) and frequency of food/media replenishment (every day) as the previous generation.

We evaluated the transgenerational effects of predator cues on the life-history traits of *D. ambigua* beginning with third generation laboratory-reared individuals. This experiment ran for a total of four generations (F_0, F_1, F_2 and F_3). To begin the experiment, we collected nine newly born individuals (less than 12 h old) per clone and individually placed them into 90 ml jars containing COMBO media [36]. Each individual was randomly assigned to one of three treatments: (i) predator (P), (ii) predator removal (PR), and (iii) no predator (N). The P treatment received conditioned media containing fish kairomones daily throughout the entire experiment. The PR treatment received fish predator cues during the F_0 generation of the experiment. The N treatment never received fish chemical cues. To ensure that there was no contamination across treatments and generations (for all experiments), we created separate sets of glassware/pipettes/dishes for P and N treatments. Each treatment was replicated 3× per clone per generation (26 clones × 9 individuals per clone = 234 jars per generation; 936 total jars over 4 generations). The experimental conditions were the same as described above (temperature = 18°C, photoperiod = 14 L : 10 D). All individuals were fed specified quantities of *S. obliquus* (0.8 mg C l^{-1} d^{-1}) and were transferred to fresh media and algae every day.

Beginning on day 3, all *Daphnia* were evaluated 2× daily for maturation (defined as the release of the first clutch into the brood chamber). When an individual matured, the timing of maturation as well the size of the first clutch was recorded. All individuals were subsequently monitored daily for the production of clutches 2 and 3. To initiate the F_1 generation (and subsequent generations), we collected newly born (<12 hours) individuals from the second clutch of each jar and placed them into a new jar containing fresh media and algae (and kairomones when appropriate). For the PR treatment, the transition between the F_0 and F_1 generations represents the point at which each lineage was transferred to media that did not contain predator cues. In this treatment, F_1 individuals were exposed to predator cues during embryonic development and very early life-history stages (up until 12 h old). The timing of maturation and production of offspring was monitored using the same procedures as described above.

(c) Kairomone collection

COMBO medium conditioned by the presence of planktivorous fish was collected daily from a tank containing two to four

redbreast sunfish (*L. auritus*; approx. 3 cm in total length) in 130 l of water. Each morning, more than 200 *D. ambigua* were added as prey to the aquaria. Injured *Daphnia* emit chemical cues that contribute to the magnitude of the phenotypic response to predation [37]. Our conditioned media probably contained both fish kairomones and *Daphnia* 'alarm cues'. Following the consumption of these *Daphnia*, media was removed from the aquaria and filtered using membrane filters (47 mm diameter, 0.45 µm mesh). The fish-conditioned media was filtered down to 0.45 µm because it has been shown that this mesh removes bacteria that are large enough for *Daphnia* to ingest [38]. This filtering is thus intended to preclude the fish-conditioned media from providing supplemental nutrients. The concentration of kairomones that was used in this experiment equalled 0.0025 fish l^{-1}.

(d) Experiment 2: cue switching

We performed an additional experiment where we manipulated exposure to predator cues at maturation to better understand the timing of induction of transgenerational responses (i.e. maternal versus environmental induction) [27]. This experiment used 27 clones of *Daphnia* from six lakes. This included five clones each from Bride and Dodge, four each from Long and Wyassup, six from Gardner and three from Quonnipaug. There was a 42% overlap in clone identity between the predator-removal and cue switching experiments. All clonal lines were reared in a common environment for a period of two generations prior to the initiation of the experiment (temperature = 18°C, photoperiod = 14 L : 10 D. A key difference is that the 'cue switching' experiment started the multiple generations of laboratory rearing by using females from existing stock cultures. These cultures were established in the laboratory by hatching resting eggs from sediment samples (same as the predator-removal experiment) and were maintained in 250 ml glass jars at 18°C for two months prior to start of the cue switching experiment. The cultures were changed to fresh media and algae weekly and were maintained at moderate densities (less than 60 adults l^{-1}). We removed approximately two adult *Daphnia* from each stock culture. One neonate was removed from the first clutch produced by each adult and was subsequently reared as described above for the 'predator-removal' experiment for a period of two generations.

The 'cue switching' experiment began by collecting 12 newly born individuals (less than 12 h old) per clone and individually placing them into 90 ml jars containing COMBO media [36] and algae (*S. obliquus*, 0.8 mg C l^{-1} d^{-1}). Each individual was randomly assigned to one of four treatments: (i) predator (P), (ii) predator to no predator (P to N), (iii) no predator to predator (N to P), and (iv) no predator (N). The P treatment received fish chemical cues daily throughout the experiment. The P to N and N to P treatments were transferred to media containing the presence/absence of predator cues when the individual attained maturation. The N treatment never received fish chemical cues. Using the same procedures as described above, we quantified the timing of maturation for all treatments. We evaluated the effects of the four predator treatments on the timing of maturation across two generations (F_0 and F_1). To initiate the F_1 generation, we collected a newly born individual (less than 12 h) from the second clutch of each jar and placed them into a new jar containing media and algae. This timing is important because individuals in the second clutch will develop in the presence of predator cues in the N to P treatment but in the absence of such cues in the P to N treatment. Each treatment was replicated 3× per clone per generation (27 clones × 12 individuals per clone = 324 jars per generation; 648 total jars over two generations). The experimental conditions (temperature, photoperiod, feeding schedule) were identical to the predator-removal experiment. The concentration of kairomones used in this experiment was 0.0043 fish l^{-1}).

(e) Experiment 3: alarm cue versus fish kairomone

Daphnia are known to respond phenotypically to chemicals emitted by predators (i.e. kairomones) as well as the chemical cues emitted by injured conspecifics (*Daphnia* 'alarm cues' hereafter) [37]. To quantify the influence of each cue on transgenerational plasticity, we measured life-history responses when *Daphnia* were exposed to alarm cues or kairomones for a period of two generations. We hatched 23 clones from ephippia (Bride = 10 clones, Dodge = 5 clones, Long = 5 clones, Quonnipaug = 4 clones) and reared them in a common garden at 15°C setting for two generations (same procedures as described above).

We evaluated the transgenerational effects of predator cues on the life-history traits of *Daphnia* beginning with third generation laboratory-reared individuals. We collected nine newly born individuals (less than 12 h old) per clone and randomly assigned each individual to one of three treatments: (i) *Daphnia* alarm cues only, (ii) fish kairomones only, and (iii) no predator. The '*Daphnia*-only' treatment received water containing the cues emitted by macerated *Daphnia* (100 *Daphnia* l^{-1}) every other day throughout the experiment. The 'fish-only' treatment received media conditioned by fish every other day. The conditioned water was created by isolating a redbreast sunfish (*L. auritus*) in an aquaria for 12 h. Water was then removed from this aquaria and filtered as described above. The concentration of kairomones used in this treatment was 0.05 fish l^{-1}. The 'no predator' treatment never received fish chemical cues. Each treatment was replicated 3× per clone per generation (23 clones × 9 individuals per clone = 207 jars per generation; 414 total jars). All individuals were fed quantities of *S. obliquus* (0.8 mg C l^{-1} d^{-1}) and were transferred to fresh media and algae every other day.

(f) Statistical analyses

Variation in age at maturation and clutch size was analysed using linear mixed models (SPSS v. 21) implemented using restricted maximum-likelihood estimation. Predator treatment, generation and the predator × generation interaction were entered as fixed effects. Clone identification was entered as a random effect. We used Satterthwaite approximations for the denominator degrees of freedom. When we observed a significant generation × predator treatment interaction, we used tests of simple main effects to examine differences of one factor at each level of the other in the interaction [39]. In these tests, we used false discovery rates to adjust our *p*-values for multiple tests. A likelihood ratio test was used for tests of significance of the random effects. Data for age at maturation were log-transformed while all clutch size data were square-root transformed to improve fits with normality and homogeneity of variances. r^2 values for all analyses were calculated using the method developed by Xu [40].

(g) Correlations between within- and across-generation predator effects

To explore the relationship between within- and across-generation predator responses, we evaluated Pearson correlations between the F_0 and F_1 generations. For all experiments, we quantified the 'within-generation response' to predators for each genotype as: F_0 predator trait value—F_0 non-predator trait value. We then examined the average clonal response across generations by calculating the difference in trait values for the predator treatments between the F_1 and F_0 generation (i.e. F_1 predator trait value—F_0 predator trait value). One potential shortcoming of these correlations is that the F_0 predator trait value appears in the *x*- and *y*-axes. As a consequence, a negative correlation may arise solely because of noise in the F_0 predator data.

To independently assess whether *Daphnia* genotypes respond within- or across-generations, we removed the effect of measurement error by estimating the within- and across-generation

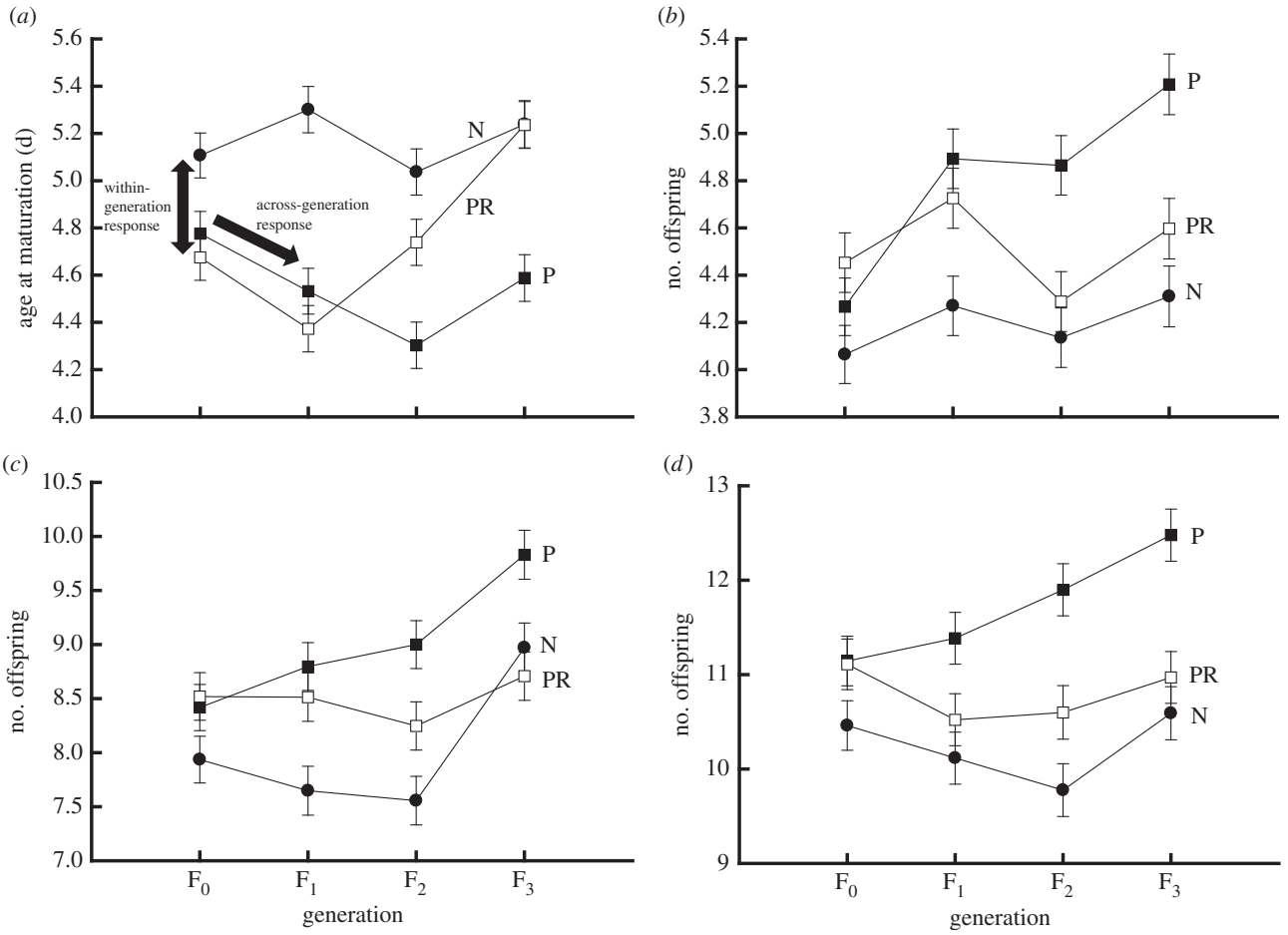

Figure 1. Life-history variation in the predator-removal experiment. (*a*) Age at maturation, (*b*) clutch 1, (*c*) clutch 2, and (*d*) clutch 3. Closed squares = predator (P), open squares = predator removal (PR) (*Daphnia* exposed to predator cues for a single generation), closed circles = non-predator (N). Significant ($p < 0.05$) interactions between predator treatment and generation were observed for age at maturation, clutch 1 and clutch 3. *Daphnia* in the predator and predator-removal treatments matured earlier and produced larger clutches of offspring than the no-predator treatment in the F_0 generation. Such differences in the timing of maturation and clutch size (clutch 1 only) increased in the following generation. Black arrows in panel (*a*) designate the within- and across-generation responses to predator cues. Error bars = ± 1 s.e.

effects for each clone in the context of a general linear model. Specifically, we fitted the model

$$X_{c,t,g,i} = \mu_c + \varphi_c 1_{(t=p)} + \gamma_c 1_{(g=2)} + \theta_c 1_{(t=p \text{ and } g=2)} + \epsilon_{c,t,g,i},$$

where $X_{c,t,g,i}$ is the response (age at maturation or clutch size) for clone c in generation g (1 or 2) and treatment t (N or P) and $\epsilon_{c,t,g,i} \sim N(0, \sigma_c^2)$ accounts for variation among replicates for a given clone. The indicator variables $\{1_{(t=p)}$, etc.$\}$ take on the value 1 when the subscripted condition is true and 0 otherwise and the parameters μ_c, φ_c, γ_c and θ_c account for the clone-specific baseline and effects of treatment, generation and their interaction. Note that this analysis is precisely analogous to treating these terms as random effects without the added assumption that they are normally distributed. To make the analogy between this approach and the direct correlation explicit, we note that the within-generation response is given by φ_c. Similarly, the difference between the predator treatment in generation 2 and generation 1 is given by $\gamma_c + \theta_c$. Thus the correlation across clones between φ_c and $\gamma_c + \theta_c$ yields the correlation in within- and across-generation responses with the added benefit of explicitly accounting for noise that might have biased the direct calculation.

3. Results

Our results show that *Daphnia* reared in the presence of fish chemical cues produced offspring that matured faster in the subsequent generation (figures 1 and 2), although this influence of predator on prey depended upon the number of generations of predator exposure (figure 1) as well as the timing of initial exposure to predator cues (figure 2). Most significantly, all experiments revealed a negative correlation between the magnitude of within- versus across-generation plasticity (figure 3).

(a) Predator-removal experiment

We observed significant ($p < 0.05$) 'predator × generation' interactions for age at maturation and offspring production (clutch number 1 and 3 only; electronic supplementary material, table S1; figure 1). The predator × generation interaction was marginally non-significant ($p > 0.05$) for the second clutch of offspring. Tests of simple main effects revealed significant effects of predator cues within each experimental generation save the lack of divergence in clutch size in the F_0 generation (electronic supplementary material, table S1). In the F_0 generation, *Daphnia* reared in the presence of predator cues matured faster and produced more offspring than *Daphnia* from the N treatment. This initial exposure to a predator cue (in the F_0 generation) is then correlated with earlier maturation and the production of larger clutches of offspring in the following generation (figure 1). For instance, the differences in age at

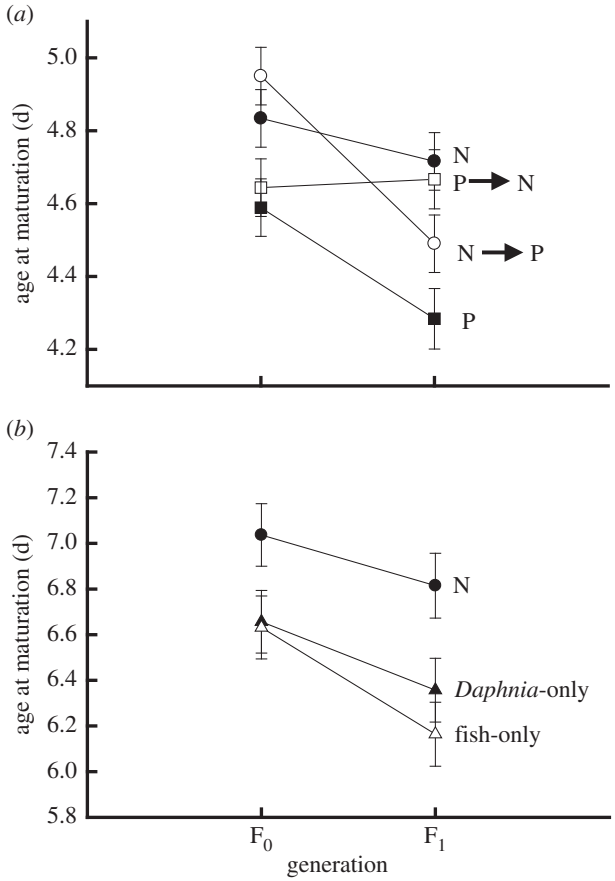

Figure 2. Differences in age at maturation across generations in the 'cue switching' and 'alarm versus fish kairomone' experiment. (*a*) Cue switching: closed squares, predator (P); open squares, predator to non-predator (P to N); open circles, non-predator to predator (N to P); closed circles, non-predator (N). Exposure to predator cues was manipulated at maturation. A significant ($p < 0.05$) predator × generation interaction was observed. *Daphnia* in the predator treatment matured earlier than *Daphnia* not exposed to predator cues and such differences increased in the F_1 generation. *Daphnia* that were exposed to predator cues starting at maturation (N to P) matured faster in the following generation while *Daphnia* transferred to non-predator media at maturation (P to N) did not alter the timing of maturation between generations. (*b*) Alarm versus fish kairomone: closed circles, non-predator (N); closed triangles, *Daphnia*-only; open triangles, fish-only. A significant treatment effect was observed as *Daphnia* from the *Daphnia*-only and fish-only treatments matured significantly ($p < 0.05$) earlier than *Daphnia* from non-predator treatments. Error bars = ± 1 s.e.

maturation between the P and N treatments increased from approximately 8 to 19% between the F_0 and F_1 generations. Similar trends were observed between the PR and N treatments for age at maturation and the size of clutch 1 and 2 between the F_0 and F_1 generations. The size of clutch 3 did not increase in the PR treatments between generation F_0 and F_1. Post-hoc tests revealed significant ($p < 0.05$) differences in age at maturation between the P (i.e. P and PR) and N treatments in the F_0 and F_1 generation (see the electronic supplementary material, table S1). For clutch size, post-hoc tests revealed non-significant differences in the F_0 generation, but both P and PR treatments differed significantly from the N treatment in the F_1 generation (clutch 1 and 2 only; electronic supplementary material, table S1).

Differences in age at maturation and clutch size between the P and N treatments were maintained in generations in F_2 and F_3 (figure 1). Post-hoc tests showed that *Daphnia* from the P treatment continued to mature significantly ($p < 0.05$) earlier and produce more offspring than the N treatment (electronic supplementary material, table S1). Differences in age at maturation between the PR and N treatments were small but significant in the F_2 generation, and disappeared entirely by generation F_3. By contrast, there were no significant differences in the production of offspring between the PR and N treatments in the F_2 and F_3 generations (electronic supplementary material, table S1).

(b) Cue switching experiment

We observed a significant interaction between predator treatment and generation for age at maturation (figure 2; $F_{3,590.9} = 4.12$, $p = 0.007$; model $r^2 = 0.071$). Tests of simple main effects revealed significant effects of predator treatments in the F_0 ($F_{3,288.2} = 5.17$, $p = 0.002$) and F_1 generation ($F_{3,278.1} = 6.86$, $p < 0.001$). *Daphnia* reared in the presence of fish predator cues matured approximately 4% faster than *Daphnia* reared in the absence of fish predator cues in the F_0 generation. Such differences subsequently increased in the F_1 generation; *Daphnia* from the P treatment matured 10% faster than *Daphnia* in the N treatment in the F_1 generation (figure 2). Post-hoc tests revealed significant differences between P and N treatments in the F_0 and F_1 generations. The two treatments that experienced a change in conditions at maturation (i.e. N to P, P to N) exhibited divergent responses across generations (figure 2). The N to P treatment accelerated development across generations (a 10% change), while the P to N treatment did not. As a result, the P to N treatment matured faster than the N to P treatment in the F_0 generation, but such trends reversed in the F_1 generation (figure 2). Comparisons between these two treatments revealed significant differences in the F_0 generation but non-significant differences in the F_1 generation.

(c) Alarm cue versus kairomones experiment

There was a significant influence of cue treatment on age at maturation in *Daphnia* (cue effect: $F_{2,382.2} = 9.67$, $p < 0.001$; model $r^2 = 0.044$; figure 2). Post-hoc tests revealed that *Daphnia* from 'alarm cue' and 'fish-only' treatments matured significantly (approx. 7%) earlier than *Daphnia* from the non-predator treatment. Differences between the alarm cue and fish only treatments were minor ($p > 0.05$). Significant differences in age at maturation were observed across generations ($F_{1,381.9} = 16.53$, $p < 0.001$) as *Daphnia* matured 5% faster in the F_1 generation compared with F_0 generation. The cue treatment × generation was non-significant ($F_{2,381.9} = 1.52$, $p = 0.22$).

(d) Correlation between within- and across-generation responses

We evaluated the correlation between within- and across-generation responses (for F_0 and F_1 generations) in all experiments. We measured these correlations directly from the trait responses recorded for each clone (see the electronic supplementary material, figure S1) and also via a general linear model (GLM) approach that accounts for measurement error (see Material and methods). Both approaches yielded very similar trends (compare figure 3 versus electronic supplementary material, figure S1; see the electronic supplementary material, figure S2, for error bars on correlational plots).

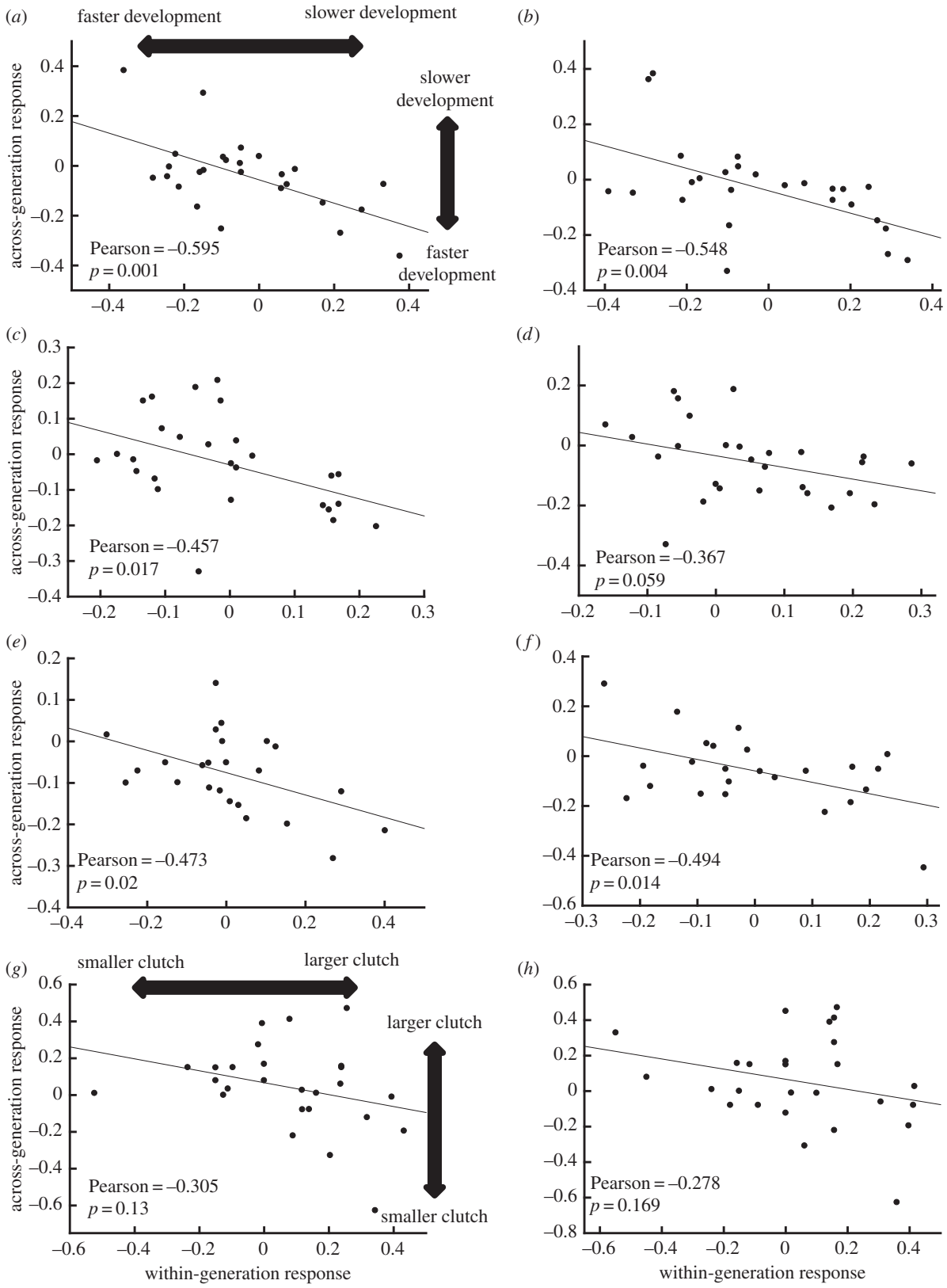

Figure 3. Correlations between within- and across-generation responses for age at maturation (*a*–*f*) and clutch size (*g*,*h*). (*a*) Predator-removal experiment 'predator' treatment, (*b*) predator-removal experiment 'predator-removal' treatment, (*c*) cue switching experiment 'predator' treatment, (*d*) cue switching experiment 'non-predator to predator' treatment, (*e*) *Daphnia* alarm versus fish kairomones experiment 'fish only' treatment, (*f*) *Daphnia* alarm versus fish kairomones '*Daphnia*-only' treatment, (*g*) predator-removal experiment 'predator' clutch size (clutch 1 only), and (*h*) predator removal 'predator-removal' clutch size (clutch 1 only). Predator-removal' clutch size (clutch 1 only). 'Within-generation' and 'across-generation' responses were calculated by using general linear models (predator treatment and generation entered as fixed effects) for each combination of clone and experiment to explicitly account for clonal variation. 'within-generation' = predator treatment parameter, 'across-generation' = generation + predator by generation interaction parameters (see Material and methods).

Because the GLM approach represents an unbiased estimate of the relationship between within- versus across-generation responses, we will emphasize those results. This analysis revealed a negative relationship between within- and among-generation responses to predator cues for all combinations of traits and experiments (figure 3). For 5 out of 6 assessments

of age at maturation, this correlation was significant and negative ($p < 0.05$). Such correlations indicate that clones which mature earlier upon initial exposure to predator cues, will often exhibit the opposite response in the following generation (delayed maturation; figure 3). Similarly, there was a negative relationship between within- and among-generation responses to predator cues for the size of the first clutch, although these correlations were not significant (figure 3*g*,*h*). Such correlations qualitatively indicate that clones which responded strongly to predators by increasing offspring production in the F_0 generation, produced comparatively smaller clutches in the F_1 generation.

4. Discussion

Our results demonstrate that prey adaptively respond to the threat of predation by modifying the expression of life-history traits across generations (figures 1 and 2). *Daphnia* reared in the presence of predator cues, on average, matured earlier and produced larger clutches of offspring than *Daphnia* reared in the absence of predator cues (figures 1 and 2). These initial, within-generation effects of predator on prey were generally modest (approx. 6–10% response). Parental exposure to predator cues then resulted in a significantly earlier age at maturation in the following generation thus doubling the difference between predator and non-predator treatments (figures 1 and 2). The magnitude of these responses is noteworthy as our previous studies of life-history evolution have shown that similar changes in trait values provide a direct link to ecological processes via altered consumer–resource dynamics and primary production [34]. Furthermore, these cross-generational responses were detectable two generations following cue removal (figure 1), were probably induced during embryonic development (compare 'P to N' versus 'N to P' in figure 2*a*) and were similar in magnitude, whether *Daphnia* were exposed to cues emitted by injured *Daphnia* (alarm cues) or predators (kairomones; figure 2*b*). All of these results provide evidence for adaptive transgenerational plasticity when the phenotypic responses of prey to predator cues are averaged across many clones of *Daphnia*.

Most surprisingly, our correlational plots that explored clone-specific patterns of plasticity revealed negative relationships (figure 3). Clones that initially matured earlier (or produced larger clutches) when exposed to predator cues (i.e. within-generation response) exhibited no response or the opposite response across generations. Conversely, strong transgenerational responses, as measured by declines in the timing of maturation across generations, were displayed by clones that delayed the timing of maturation when initially exposed to predators cues (figure 3). That is, clones responded to predator cues by modifying their timing of maturation within- or across-generations, but not both. Contrasting phenotypic responses within- and across-generations (in sign and magnitude) were evident for multiple traits and experimental contexts and were independently replicated using a distinct collection of genotypes (figure 3). Such trends thus appear to be robust and require further investigation.

Theory predicts that similar conditions will favour the evolution of within- and across-generation plasticities (i.e. [2,3,15,16,20–22]). However, only one extant framework considers both within- and across-generation plasticities and makes specific predictions regarding the connections between

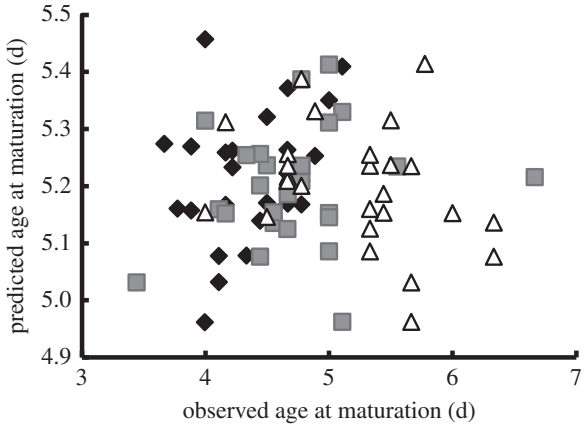

Figure 4. Fit of predicted versus observed maturation data based upon the Hoyle & Ezard model [23]. We fitted the model [23] to the *Daphnia* maturation data from the predator and no-predator treatments of the predator-removal experiment. The black (generation 2), grey (generation 3) and white (generation 4) markers indicate the results for generations 2–4. The fitted model does not predict any of the variability in maturation for the predator-removal treatment (prediction $r^2 < 0$ for all generations).

the two [23,24]. The negative correlation we observed between within- and across-generation responses is consistent with the model of Hoyle & Ezard [23] but only when the maternal effect coefficient is negative and the genetic covariance among reaction norm coefficients is positive. We evaluated these conditions by fitting the Hoyle & Ezard model [23] to the *Daphnia* maturation data from the predator and no-predator treatments of experiment 1 (predator removal). Doing so, results in a maximum-likelihood estimate of the maternal effects coefficient that is not significantly different from zero (see the electronic supplementary material, appendix S1). Moreover, the fitted model is incapable of predicting age at maturation for *Daphnia* that were initially exposed to predators for a single generation (i.e. predator removal; figure 4; see the electronic supplementary material, appendix S1). Thus, existing theory cannot explain the observed negative covariation between within- and across-generation phenotypic responses (figure 3). While these results do not diminish the conceptual contribution made by Hoyle & Ezard [23,24], they clearly highlight the need for more mechanistic theory on the transduction of environmental information within- and across-generations.

Transgenerational plasticity is generally considered adaptive when current environmental conditions accurately reflect future conditions and parents modify the traits of offspring (or future generations) to best match those conditions. This may include a positive or negative covariation (i.e. negative maternal effect) in trait values across generations depending upon the extent to which cues experienced by parents foreshadows similar conditions or a change in conditions for offspring. There are clear examples that provide strong evidence for adaptive variation in transgenerational plasticity (i.e. [5,41,42]). Yet, the results of a recent meta-analysis revealed that, overall, the evidence for adaptive transgenerational plasticity is surprisingly weak ([13], see also [14]). Uller *et al*. [13] identify several reasons as to why the evidence for adaptive transgenerational plasticity may be limited. This includes the extent to which researchers impose environmental treatments that reflect ecologically relevant variation and the statistical robustness of designs that are typically used to detect transgenerational

responses. Our results provide an additional explanation as to why experiments can fail to yield evidence for adaptive variation in transgenerational plasticity. In all of our experiments, we observed significant genetic variation in the magnitude and direction of transgenerational responses to predator cues (figure 3). Some clones provided evidence for an adaptive response to predator cues by strongly modifying the expression of life-history traits across generations, while others did not (figure 3). Thus, adaptive interpretations of transgenerational plasticity will depend on the specific individuals or populations that are chosen for study. Future work should pay close attention to the amount of replication (i.e. genotypes, families, populations) that is included in studies and target populations that are most likely to display transgenerational plasticity in response to relevant environmental variables.

Opposing phenotypic responses to environmental stressors within- and across-generations has potentially broad implications (figure 3). Importantly, it shows that within- versus across-generation responses can be decoupled. Such genetic variation, in turn, presents the opportunity for selection to favour contrasting patterns of plasticity across environments. Evidence for a transgenerational influence of environmental cues [3] including cross-generational effects of predators on prey is growing [27,42–47]. Work has also shown that closely related species can differ in transgenerational plasticity [48] and that epigenetic markers can vary across populations [49]. The key next step is to determine, theoretically and empirically, the ecological conditions that favour within- versus across-generation responses. Future work needs to consider a greater array of traits including common fitness-related trade-offs (i.e. age versus size at maturation) to more fully describe connections between within- and across-generation responses to environmental cues. Improved understanding of the evolutionary drivers of transgenerational plasticity as well as the connection between transgenerational plasticity and ecological processes remains an important area of study.

Ethics statement. The protocols used in this paper were approved by IACUC protocol no. A13.014 at UTA.

Data accessibility. All data associated with this paper can be found at the designated Dryad depository (doi:10.5061/dryad.d38f2).

Acknowledgements. We thank Deirdre Whittington, Jennifer Nguyen, Mina Tricomi-Peugh, Ishrat Durdana for help in the laboratory. We thank several anonymous reviewers whose comments much improved this paper.

Funding statement. F.C. thanks the Louis Stokes Alliance for Minorities Program for funding. M.W. thanks UTA for funding.

References

1. Bossdorf O, Richards CL, Pigliucci M. 2008 Epigenetics for ecologists. *Ecol. Lett.* **11**, 106–115.
2. 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)
3. Bonduriansky R, Crean AJ, Day T. 2012 The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.* **5**, 192–201. (doi:10.1111/j.1752-4571.2011.00213.x)
4. Fox CW, Mousseau TA. 1998 Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. In *Maternal effects as adaptations* (eds TA Mousseau, CW Fox), pp. 159–177. New York, NY: Oxford University Press.
5. Salinas S, Munch SB. 2012 Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **15**, 159–163. (doi:10.1111/j.1461-0248.2011.01721.x)
6. Jablonka E, Lachmann M, Lamb MJ. 1992 Evidence, mechanisms and models for the inheritance of acquired traits. *J. Theor. Biol.* **158**, 245–268. (doi:10.1016/S0022-5193(05)80722-2)
7. Rossiter M. 1996 Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.* **27**, 451–476. (doi:10.1146/annurev.ecolsys.27.1.451)
8. Agrawal AA. 2001 Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *Am. Nat.* **157**, 555–569. (doi:10.1086/319932)
9. Richerson PJ, Boyd R. 2005 *Not by genes alone: how culture transforms human evolution*. Chicago, IL, USA: The University of Chicago Press.
10. Laland KN, Odling-Smee FJ, Myles S. 2010 How culture shaped the human genome: bringing genetics and the human sciences together. *Nat. Rev. Genet.* **11**, 137–148. (doi:10.1038/nrg2734)
11. Day T, Bonduriansky R. 2011 A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.* **178**, E18–E36. (doi:10.1086/660911)
12. Jablonka E, Raz G. 2009 Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* **84**, 131–176. (doi:10.1086/598822)
13. Uller T, Nakagawa S, English S. 2013 Weak evidence for anticipatory effects in plants and animals. *J. Evol. Biol.* **26**, 2161–2170. (doi:10.1111/jeb.12212)
14. Burgess SC, Marshall DJ. 2014 Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* **123**, 769–776. (doi:10.1111/oik.01235)
15. Levins R. 1968 *Evolution in changing environments*. Princeton, NJ: Princeton University Press.
16. Scheiner SM. 1993 Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Evol. S* **24**, 35–68. (doi:10.1146/annurev.es.24.110193.000343)
17. Kingsolver JG. 1995 Viability selection on seasonally polyphenic traits: wing dimorphism in western white butterflies. *Evolution* **49**, 932–941. (doi:10.2307/2410415)
18. Donohue K, Messiqua D, Hammond Pyle E, Heschel MS, Schmitt J. 2000 Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. *Evolution* **54**, 1956–1968. (doi:10.1111/j.0014-3820.2000.tb01240.x)
19. Hollander J. 2008 Testing the grain size model for the evolution of phenotypic plasticity. *Evolution* **62**, 1381–1389. (doi:10.1111/j.1558-5646.2008.00365.x)
20. Fischer B, Taborsky B, Kokko H. 2011 How to balance the offspring quality–quantity tradeoff when environmental cues are unreliable. *Oikos* **120**, 258–270. (doi:10.1111/j.1600-0706.2010.18642.x)
21. Shea N, Pen I, Uller T. 2011 Three epigenetic information channels and their different roles in evolution. *J. Evol. Biol.* **24**, 1178–1187. (doi:10.1111/j.1420-9101.2011.02235.x)
22. Kuijper B, Johnstone RA, Townley S. 2014 The evolution of multivariate maternal effects. *PLoS Comput. Biol.* **10**, e1003550. (doi:10.1371/journal.pcbi.1003550)
23. Hoyle RB, Ezard TH. 2012 The benefits of maternal effects in novel and in stable environments. *J. R. Soc. Interface* **9**, 2403–2413. (doi:10.1098/rsif.2012.0183)
24. Ezard TH, Prizak R, Hoyle RB. 2014 The fitness costs of adaptation via phenotypic plasticity and maternal effects. *Funct. Ecol.* **28**, 691–701. (doi:10.1111/1365-2435.12207)
25. Stibor H. 1992 Predator induced life history shifts in a freshwater cladoceran. *Oecologia* **92**, 162–165. (doi:10.1007/BF00317358)
26. Riessen HP. 1999 Predator-induced life history shifts in *Daphnia*: a synthesis of studies using meta-analysis. *Can. J. Fish. Aquat. Sci.* **56**, 2487–2494. (doi:10.1139/f99-155)
27. Agrawal AA, Laforsch C, Tollrian R. 1999 Transgenerational induction of defenses in plants

and animals. *Nature* **401**, 60–63. (doi:10.1038/43425)

28. Tollrian R. 1995 Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology* **76**, 1691–1705. (doi:10.2307/1940703)
29. Mikulski A, Pijanowska J. 2010 When and how can *Daphnia* prepare their offspring for the threat of predation? *Hydrobiologia* **643**, 21–26. (doi:10.1007/s10750-010-0131-0)
30. Post DM, Palkovacs EP, Schielke EG, Dodson SI. 2008 Intraspecific variation in a predator affects community structure and cascading trophic interactions. *Ecology* **89**, 2019–2032. (doi:10.1890/07-1216.1)
31. Palkovacs EP, Post DM. 2008 Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feedback to shape predator foraging traits? *Evol. Ecol. Res.* **10**, 699–720.
32. Walsh MR, Post DM. 2011 Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. *Proc. R. Soc. B* **278**, 2628–2637. (doi:10.1098/rspb.2010.2634)
33. Walsh MR, Post DM. 2012 The impact of intraspecific variation in a fish predator on the evolution of phenotypic plasticity and investment in sex in *Daphnia ambigua*. *J. Evol. Biol.* **25**, 80–89. (doi:10.1111/j.1420-9101.2011.02403.x)
34. Walsh MR, DeLong JP, Hanley TC, Post DM. 2012 A cascade of evolutionary change alters consumer resource dynamics and ecosystem function. *Proc. R. Soc. B* **279**, 3184–3192. (doi:10.1098/rspb.2012.0496)
35. Palkovacs EP, Dion KB, Post DM, Caccone A. 2008 Independent evolutionary origins of landlocked alewife populations and rapid parallel evolution of phenotypic traits. *Mol. Ecol.* **17**, 582–597. (doi:10.1111/j.1365-294X.2007.03593.x)
36. Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L. 1998 COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* **377**, 147–159. (doi:10.1023/A:1003231628456)
37. Laforsch C, Beccara L, Tollrian R. 2006 Inducible defenses: the relevance of chemical alarm cues in *Daphnia*. *Limnol. Oceanogr.* **51**, 1466–1472. (doi:10.4319/lo.2006.51.3.1466)
38. Brendelberger H. 1991 Filter mesh size of cladocerans predicts retention efficiency for bacteria. *Limnol. Oceanogr.* **36**, 884–894. (doi:10.4319/lo.1991.36.5.0884)
39. Winer BJ. 1971 *Statistical principles in experimental design*. New York, NY: McGraw-Hill.
40. Xu R. 2003 Measuring explained variation in linear mixed effects models. *Stat. Med.* **22**, 3527–3541. (doi:10.1002/sim.1572)
41. Galloway LF, Etterson JR. 2007 Transgenerational plasticity is adaptive in the wild. *Science* **318**, 1134–1136. (doi:10.1126/science.1148766)
42. Holeski LM, Jander G, Agrawal AA. 2012 Transgenerational defense induction and epigenetic inheritance in plants. *Trends Ecol. Evol.* **27**, 618–626. (doi:10.1016/j.tree.2012.07.011)
43. Mondor EB, Rosenheim JA, Addicott JF. 2005 Predator-induced trans-generational phenotypic plasticity in the cotton aphid. *Oecologia* **142**, 104–108. (doi:10.1007/s00442-004-1710-4)
44. Coslovsky M, Richner H. 2011 Predation risk affects offspring growth via maternal effects. *Funct. Ecol.* **25**, 878–888. (doi:10.1111/j.1365-2435.2011.01834.x)
45. Giesing ER, Suski CD, Warner RE, Bell AM. 2011 Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc. R. Soc. B* **278**, 1753–1759. (doi:10.1098/rspb.2010.1819)
46. Roche DP, McGhee KE, Bell AM. 2012 Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. *Biol. Lett.* **8**, 932–935. (doi:10.1098/rsbl.2012.0685)
47. Keiser CN, Mondor EB. 2013 Transgenerational behavioral plasticity in a parthenogenetic insect in response to increased predation risk. *J. Insect. Behav.* **26**, 603–613. (doi:10.1007/s10905-013-9376-6)
48. Sultan SE, Barton K, Wilczek AM. 2009 Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology* **90**, 1831–1839. (doi:10.1890/08-1064.1)
49. Herrera CM, Bazaga P. 2010 Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.* **187**, 867–876. (doi:10.1111/j.1469-8137.2010.03298.x)