ARTICLE

Quantifying multivariate genotype-by-environment interactions, evolutionary potential and its context-dependence in natural populations of the water flea, *Daphnia magna*.

Franziska S. Brunner, Alan Reynolds, Ian W. Wilson, Stephen Price, Steve Paterson, David Atkinson, Stewart J. Plaistow*

Department of Ecology, Evolution and Behaviour; IVES; University of Liverpool, UK

*corresponding author

Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown St., Liverpool L69 7ZB, U.K. Email: s.plaistow@liv.ac.uk

Corresponding author: s.plaistow@liv.ac.uk
Phone: +44 (0) 151 695 4556

Running title:

Words:
No. Figures: 5
Colour figure: 1:5
No. Tables: 3
Supplementary tables: 2

ABSTRACT

Genotype-by-environment interactions (G x E) underpin the evolution of plastic responses in natural populations. Theory assumes that G x E interactions exist but empirical evidence from natural populations is equivocal and difficult to interpret because G x E interactions are normally univariate plastic responses to a single environmental gradient. We compared multivariate plastic responses of 43 *Daphnia magna* clones from the same population in a factorial experiment that crossed temperature and food environments. Multivariate plastic responses explained more than 30% of the total phenotypic variation in each environment. G x E interactions were detected in most environment combinations irrespective of the methodology used. However, the nature of G x E interactions was context-dependent and led to environment-specific differences in additive genetic variation (G-matrices). Clones that deviated from the population average plastic response were not the same in each environmental context and there was no difference in whether clones varied in the nature (phenotypic integration) or magnitude of their plastic response in different environments. Plastic responses to food were aligned with additive genetic variation (gmax) at both temperatures, whereas plastic responses to temperature were not aligned with additive genetic variation (gmax) in either food environment. These results suggest that fundamental differences may exist in the potential for our population to evolve novel responses to food versus temperature changes, and challenges past interpretations of thermal adaptation based on univariate studies.

65 INTRODUCTION

66 A genotype-by-environment interaction ($G \times E$) occurs whenever genotypes differ in the way
67 that their trait values change across environments (Saltz *et al.*, 2018), or in other words, when
68 there is genetic variation in phenotypic plasticity (Gillespie and Turelli, 1989; Schlichting and
69 Pigliucci, 1993; Pigliucci and Preston, 2004). $G \times E$ interactions are critical for understanding
70 population responses to environmental change because they alter the expression of heritable
71 phenotypic variation between different environments (Hoffmann and Merila, 1999; Gibson and
72 Dworkin, 2004; Schlichting, 2008; Ledón-Rettig *et al.*, 2014; Wood and Brodie, 2016).
73 Moreover, they underpin the evolution of phenotypic plasticity in natural populations (Pigliucci,
74 2005; Levis and Pfennig, 2016; Oostra *et al.*, 2018; Fox *et al.*, 2019). When environments
75 change rapidly, plasticity may be the only response possible. Consequently, $G \times E$ interactions
76 and the potential to evolve adaptive plastic responses are crucial for population survival in the
77 face of climate change (Chevin *et al.*, 2010; Snell-Rood *et al.*, 2018).
78 There is increasing evidence that much of the phenotypic change associated with climate change
79 in wild populations is attributable to phenotypic plasticity (Chevin and Hoffmann, 2017).
80 Phenotypic variation explained by $G \times E$ interactions is expected to be larger in novel or extreme
81 environments because plastic responses will not yet have been exposed to selection, leading to
82 the release of what is termed cryptic genetic variation (Gibson and Dworkin, 2004; Schlichting,
83 2008; McGuigan and Sgrò, 2009; Ledón-Rettig *et al.*, 2014; Paaby and Rockman, 2014). But in
84 environments that have always been part of a population's evolutionary history, long-term
85 selection for an optimal plastic response may have depleted $G \times E$ interactions. For example, in
86 the African savannah butterfly, *Bicyclus anynana*, genetic variation for seasonal plasticity was
87 almost absent despite considerable additive genetic variation for trait means (Oostra *et al.*, 2018).
88 When $G \times E$ interactions are absent, traits can evolve but trait plasticity cannot, increasing a
89 population's susceptibility to changes in the frequency of extreme events such as heatwaves,
90 droughts and floods (Easterling *et al.*, 2000), or changes in the reliability of existing

environmental cues (Reed *et al.*, 2010; Oostra *et al.*, 2018; Bonamour *et al.*, 2019). Theory assumes that G x E interactions exist in natural populations (Via and Lande, 1985; Lande, 2009), and G x E interactions are frequently demonstrated in plant studies (Des Marais *et al.*, 2013) and laboratory studies (Vieira *et al.*, 2000; Valdar *et al.*, 2006; Ingleby *et al.*, 2010; Plaistow and Collin, 2014). But evidence for G x E interactions in wild animal populations is sparse (Nussey *et al.*, 2005a, b). Moreover, other recent studies haven't detected G x E interactions (Brommer *et al.*, 2008; Charmantier and Gienapp, 2014; Hayward *et al.*, 2018; Oostra *et al.*, 2018). G x E interactions are normally assessed in a single trait (Hayward *et al.*, 2018) but plastic responses typically involve coordinated shifts in many traits at the same time (Plaistow and Collin, 2014), and univariate studies do not distinguish differences in the magnitude of a plastic response from differences in the nature of a plastic response (Chun *et al.*, 2007; Collyer and Adams, 2007; Adams and Collyer, 2009; Plaistow and Collin, 2014). Figure 1 shows G x E interactions in two hypothetical populations that have the same average multivariate plastic response to an environmental contrast. Differences in the magnitude of multivariate plastic responses will create G x E interactions that generate most additive genetic variation (gmax) in alignment with the average plastic response (see Fig. 1A). But differences in the nature of a multivariate plastic response generate additive genetic variation (gmax) that is not aligned with the population average plastic response (see Fig 1B). Since populations are expected to have increased additive genetic variation along the axis of the average plastic response (Lande, 2009; Draghi and Whitlock, 2012; Noble *et al.*, 2019) the type of multivariate G x E interactions in a population may have important consequences for a population's ability to rapidly adapt to an environmental change.

Multivariate G x E interactions can be tested for using a character-state approach (Via and Lande, 1985) that uses Bayesian Markov chain Monte Carlo (MCMC) mixed models to compare the volume, shape and orientation of G-matrices estimated for the same genotypes reared in two or more different environments. The volume of the G-matrix characterises the amount of clonal

genetic variation (V_G) that selection can act on, whereas the shape defines whether the variation is attributable to few or many traits, and the orientation, defined by g_{max} , identifies the traits associated with the most clonal genetic variation (V_G) (Robinson and Beckerman, 2013; Lind *et al.*, 2015; Reger *et al.*, 2017). Alternatively, multivariate G x E interactions can be detected by testing for differences in the length or angle of reaction norms generated by measuring a multivariate phenotype of genotypes or families in two or more environments (Collyer and Adams, 2007; Plaistow and Collin, 2014). Irrespective of the approach used, multivariate G x E interactions are normally only quantified in response to a single environmental variable despite the fact that environments are often complex and have multiple dimensions that vary simultaneously (Westneat *et al.*, 2019). As a result, we do not yet know if multivariate G x E interactions are context-dependent, or if the evolved plastic response to one environmental variable has implications for a population's ability to evolve a response to a different environmental variable.

Clonal organisms such as *Daphnia* are ideal systems for studying G x E interactions because it is easy to separate genetic and non-genetic influences, and large numbers of genetically identical individuals can be reared simultaneously across different environments in parental and offspring generations (Harris *et al.*, 2012; Miner *et al.*, 2012). In order to better understand the evolutionary significance of multivariate G x E interactions in natural populations we isolated 43 genotypes (clones) of *Daphnia magna* from a single population and measured their multivariate plastic response to temperature in different food environments and their multivariate plastic response to food at different temperatures. We used a character-state approach and the tools developed in (Robinson and Beckerman, 2013) to test for genetic variation in multivariate plastic responses and its context-dependence and to compare the alignment of average plastic responses with g_{max} (Lind *et al.*, 2015; Noble *et al.*, 2019; Radersma *et al.*, 2020). We then used a reaction norm approach to determine the source of multivariate G x E interactions in each environmental context (differences in magnitude or phenotypic integration) and the number and identity of

clones that diverged from the average multivariate plastic response in each case (Collyer and Adams, 2007). We compared our results to two reference populations from a higher and a lower latitude in order to assess the generality of our findings.

METHODS

(a) *Experimental animals*

Daphnia magna clones from the UK were collected as resting eggs from Brown Moss, a shallow wetland in Shropshire (52°57'01"N 2°39'05"W, National Grid Reference SJ 562395) in July 2016. The eggs were stored in total darkness for a 3-month period at 4°C before being hatched out at 21°C on a 14:10 light: dark cycle. The 5 French clones were collected in 2007 from small pools in the Camargue in the South of France (van Doorslaer *et al.*, 2009a) and the 8 Danish clones were collected from Lake Ring in 2000 (Michels, 2007). The clones were maintained as laboratory stock cultures in a controlled temperature incubator at 21°C ± 1°C on a 14:10 light: dark cycle. Animals were kept in 200ml glass jars containing 150 ml of artificial pond water media (ASTM) enriched with additional organic extracts (AQ Xtract 30, Wilfrid Smith, UK) (Baird *et al.*, 1989). The jars were fed high food three times a week (200,000 cells ml⁻¹ of batch-cultured *Chlorella vulgaris*, quantified with a haemocytometer) and changed to fresh media once a fortnight.

(b) *Experimental set-up*

Prior to starting the experiment, three females from each clone were isolated from stocks and kept individually on *ad libitum* food (2x10⁵ cells ml⁻¹ day⁻¹ of batch-cultured *C. vulgaris*) until they produced a clutch. From that clutch, one offspring was randomly selected and reared individually in separate 200 ml jars fed for 2 generations to reduce possible maternal effects (Plaistow and Collin, 2014). In the 3rd generation, 3 - 8 second clutch neonates per clone were randomly allocated to 4 different experimental rearing treatments generated by crossing food levels (2x10⁵

and 4×10^4 *C. vulgaris* cells $\text{ml}^{-1} \text{day}^{-1}$) and temperature (24°C and 18°C). The jars were observed daily and transferred to a jar with fresh food and media every other day until they themselves dropped their second clutch. Experiments were performed in 5 temporal blocks between January 2017 and March 2018, each clone being assayed in multiple blocks.

(c) *Life history traits*

All 856 individuals in the study were photographed as neonates, upon reaching maturity (first eggs in the brood pouch) and upon releasing their second clutch, using a Canon EOS 350D digital camera connected to a Leica MZ6 dissecting microscope. The number of neonates each individual produced in their 1st and 2nd clutch were counted and 5 neonates in each clutch were photographed to obtain an estimate of offspring size. In all cases, body size was measured as the distance from the top of the head to the base of the tail spine using the image analysis software, ImageJ version 1.45s (Rasband, 1997). After mothers released their second clutch, their thermal tolerance (CT_{max}) was assayed as described by (Geerts *et al.*, 2015), allowing animals an acclimation period of 15 mins at 21°C followed by a ramping period of 40s/°C from 21°C to 50°C. So in total we measured thermal tolerance and 8 life-history traits for each individual: length at maturity, length at second clutch, age at maturity, age at second clutch, juvenile growth rate ((length at maturity - length as neonate)/ age at maturity), adult growth rate ((length at second clutch - length at maturity)/(age at second clutch-age at maturity)), average fecundity (across clutches 1 and 2), and average offspring size (across clutches 1 and 2).

(d) *Statistical analyses*

To test for multivariate GxE interactions and their context-dependence we estimated environment specific variance-covariance matrices (G) using a Bayesian MCMC multivariate mixed model (Hadfield, 2010). We then used the tools developed in (Robinson and Beckerman, 2013) to compare the volume, shape and orientation of the G-matrices generated by the two temperature

treatments when experiencing high food and low food, and the G-matrices generated by the food treatments when experiencing high and low temperatures. All trait variables were centred and scaled to s.d. = 1. We used parameter-expanded priors, and models were fitted with a burn-in of 50,000 and sampling that produced 1000 estimates of the joint posterior distribution from more than 500,000 iterations of the chains. All models were checked for autocorrelation in the chains.

To determine the source of multivariate G x E interactions in each environmental context (differences in magnitude or phenotypic integration) we analysed the 8 life history traits and the thermal tolerance of each individual using perMANOVAs with temperature, food level and clone and all their interactions fitted as fixed factors and temporal block fitted as a random factor. We used marginal R^2 values from the models to determine the proportion of phenotypic variance attributable to different model components. The multivariate plastic response of each clone to changes in food and temperature were quantified as phenotypic change vectors of scaled phenotypic data (9 traits: 8 life history variables and thermal tolerance CT_{max}) following (Collyer and Adams, 2007; Plaistow and Collin, 2014). Separate vectors were fitted for plastic responses to food and temperature in each environmental context, i.e. plasticity in response to temperature was quantified once in the low food environment and once in the high food environment and *vice versa*. The magnitude of the plastic response for each clone was calculated as the Euclidian length of the phenotypic change vector while the nature of the plastic response was calculated as the angle between a specific clone's plastic response and the mean response within the population (Collyer and Adams, 2007; Plaistow and Collin, 2014). The magnitude and nature of each clone's multivariate plastic response were then compared to the population mean response using a permutation procedure, where the actual deviation from the average response was compared to deviations generated by random vector pairs iteratively sampled from the data 9999 times (Collyer and Adams, 2007). The significance levels for these tests were adjusted by the Benjamini-Yekutieli method to $\alpha=0.024$ to correct for testing each clone in 4 separate tests (magnitude and phenotypic integration each in 2 environments). Differences in multivariate

phenotypes and reaction norms were visualised by projecting multivariate trait means onto the first two principal component axes of a PCA using all 8 life-history traits and thermal tolerance (Chun *et al.*, 2007; Plaistow and Collin, 2014). The same analyses described above were then also used to compare the reaction norm variation in the UK population with the reaction norms of 5 clones collected from a single population from the south of France (lower latitude) and 8 clones collected from a single population in Denmark (higher latitude). All of the analyses were conducted in R version 3.5.2 (Team, 2019) using the ‘vegan’ package (Oksanen, J. *et al.*, 2018).

RESULTS

(a) *A comparison of G-matrices in different environmental contexts*

Temperature had no effect on the volume or shape of the variance-covariance matrices (G) in either a high food or a low food environment (Table 1, Fig. 2 A,B). But temperature altered the orientation of the variance-covariance matrix (G) in both food environments. In the high food environment, additive genetic variation in clutch size explained g_{max} at 24°C but clones with larger clutches also developed at a faster rate at 18°C. Similarly, in the low food environment additive genetic variation in CT_{max} explained g_{max} at 24°C but clones with a lower CT_{max} matured faster at 18°C (Table 1, Fig. 2 A,B).

Food had no effect on the volume, shape or orientation of the variance-covariance matrices (G) at 24°C (Table 1, Fig. 2 C). But at 18°C, the volume of the high food G-matrix was significantly larger than the volume of the low food G-matrix (Table 1, Fig. 2C) demonstrating that there is more additive genetic variation in traits such as mean clutch size and development time in high food environments. Moreover, the orientation of the low food matrix was significantly rotated towards differences in age at maturity such that clones with fewer offspring per clutch also tended to have higher CT_{max} and slower development rates (Table 1, Fig. 2D). In order to explore the cause of the multivariate G x E's in more detail we compared clonal differences in the nature and magnitude of plastic responses to temperature and food.

247

248 (b) *Multivariate plastic responses to temperature in different food environments*

249 The multivariate plastic response to temperature explained just under 40% of the phenotypic
 250 variation in both the high food environment (36.9%) and the low food environment (35.8%) and
 251 was characterised by increased juvenile growth rates, earlier maturation and earlier production of
 252 the second clutch at higher temperatures (Table 2; Fig. 3A-C). Additive clonal variation
 253 explained slightly more life-history trait variation in the high food environment (23.2%)
 254 compared to the low food environment (18.5%). A significant G x E interaction explained 10.8%
 255 of phenotypic variation in the high food environment and 9.8% in the low food environment
 256 (Table 2; Fig. 3 B,C). Across both food environments, permutation tests revealed that 18 out of
 257 43 clones (41.8%) deviated significantly from the population average multivariate response to
 258 temperature in one way or another. In the high food environment 5 clones deviated in the
 259 magnitude of their plastic response, 4 clones deviated in the nature of their plastic response and 3
 260 clones deviated in both the magnitude and the nature of their plastic response. In the low food
 261 environment, 2 clones deviated in the magnitude of their plastic response, 5 clones deviated in the
 262 nature of their plastic response and 2 clones deviated in both the magnitude and the nature of
 263 their plastic response. Only 3 of the 18 clones deviated from the population average plastic
 264 response in some way in both environments (see Fig. 3D) highlighting the context-dependence of
 265 multivariate G x E interactions in response to temperature. Permutation test outcomes for each
 266 clone's plasticity values against population means can be found in the supplementary data.

267

268 (c) *Multivariate plastic responses to food in different temperatures*

269 The multivariate plastic response to food explained 40.1 % of the phenotypic variation at 24°C
 270 but only 33.8% of the phenotypic variation at 18°C. Individuals reared in high food environments
 271 matured at larger sizes, were larger at second clutch and had more offspring per clutch compared
 272 to individuals reared on low food at both temperatures (Table 3, Fig. 4A-C) but additive clonal

variation explained more phenotypic variation at 18°C (26.9%) than at 24°C (19.6%). Similarly, a significant G x E interaction (Table 2; Fig. 4 B,C) also explained more phenotypic variation at 18°C (9.6%) than at 24°C (6.7%). In total, 16 of the 43 clones (37.2%) had a multivariate plastic response to food that deviated significantly from the population average in one way or another (see Fig. 4D). At 18°C, 5 clones deviated in the magnitude of their plastic response, 5 clones deviated in the nature of their plastic response and 2 clones deviated in both the magnitude and the nature of their plastic response. In comparison, at 24°C only 2 clones each deviated in the magnitude and in the nature of their plastic response and no clones deviated in both the magnitude and the nature of their plastic response. None of the 16 clones deviated from the population average plastic response both at 18°C and 24°C (see Fig. 4D), highlighting the context-dependence of multivariate G x E interactions in response to food. Permutation test outcomes for each clone's plasticity values against population means can be found in the supplementary data.

(d) *Multivariate plasticity in different populations*

When we compared our UK population with a smaller subset of clones from a lower latitude population (South of France) and a higher latitude population (Denmark), we found no evidence that the average plastic response to temperature differed between populations despite their different latitudinal origins (Pop x Temp interaction, $F_{1,844}=0.0017$, $P=1$, FigS1, Table S1). When exposed to a higher temperature, individuals in all three populations grew faster and matured earlier (see Fig. 5). However, the average plastic response to food levels did differ between populations (Pop x Food interaction, $F_{2,844}=0.0025$, $P=0.016$). In high food, individuals in all populations matured at larger sizes and produced larger clutches of offspring but less so in the French population (see Fig. 5K). The proportion of phenotypic variation explained by different model components was analysed for each population separately. Average multivariate plastic responses to food and temperature explained between 39.5 - 57.6% of the phenotypic

variation in the French and Danish populations in different contexts. Clone-specific multivariate plastic responses to food and temperature were both context-dependent in the French population (see Table S2b) as in the UK population (see above). G x E interactions in response to temperature explained 12.4% of the total phenotypic variation in high food but only 6.5% in low food. Whereas G x E in response to food explained 13.2 % of the phenotypic variation in low temperature but only 6.5% of the phenotypic variation in the high temperature. In the Denmark population, plastic responses to temperature were indistinguishable between clones (see Table S2c?), whereas a significant G x E in response to food (see Table S2c) explained 8.4% of the phenotypic variation in low temperatures and 9.7% of the phenotypic variation in high temperatures.

DISCUSSION

Theory assumes that G x E interactions exist in natural populations (Via and Lande, 1985; Lande, 2009; Chevin and Hoffmann, 2017) but empirical evidence is equivocal (Hayward *et al.*, 2018) and not always easy to interpret when G x E interactions are measured as univariate responses to a single environmental gradient. We compared the multivariate plastic responses to food and temperature environments for 43 *Daphnia magna* clones collected from the same population. Average multivariate plastic responses to food-temperature environments explained three times more phenotypic variation than genetic variation in traits (G), or genetic variation in plasticity (G x E), supporting the idea that the phenotypic change attributable to plasticity in wild populations is higher than previously thought (Charmantier *et al.*, 2008; Gienapp *et al.*, 2008; Merilä and Hendry, 2014). Consequently, it is imperative that we understand how plasticity evolves and influences a population's ability to cope with environmental change (Chevin *et al.*, 2010). G x E interactions are critical as plasticity cannot evolve in their absence (Pigliucci, 2005; Hayward *et al.*, 2018; Oostra *et al.*, 2018; Fox *et al.*, 2019). Using a reaction norm approach we detected multivariate G x E interactions in all three of the

populations examined. Moreover, in our focal population we detected multivariate $G \times E$ interactions in almost all of the environmental contexts we examined irrespective of whether we used a character-state approach (Via and Lande, 1985; Robinson and Beckerman, 2013), or a reaction norm approach (Collyer and Adams, 2007). But it remains to be seen whether the 10% or less of phenotypic variation explained by $G \times E$ in a laboratory environment is sufficient to fuel the evolution of plastic responses in a natural environment. Recent studies have concluded that $G \times E$ interactions are not detectable in wild populations (Brommer *et al.*, 2008; Charmantier and Gienapp, 2014; Hayward *et al.*, 2018; Oostra *et al.*, 2018) but these studies only tested for univariate $G \times E$ interactions arising from differences in the magnitude of a plastic response. Our multivariate study also allowed us to test for differences in the nature of a plastic response, another potentially important source of $G \times E$ interaction (Plaistow and Collin, 2014). Food had no effect on the volume, shape or orientation of variance-covariance matrices (G) at high temperatures, suggesting that there was no genetic variation in the way that different clones responded to food at high temperatures. This is further supported by the reduced phenotypic variance explained by food plasticity $G \times E$ interactions at 24°C compared to 18°C, and fewer clones deviating from an average plastic response (Fig. 4). But in all other environmental contexts differences in the orientation of variance-covariance matrices (G) in different environments was observed, meaning that the combination of traits that expressed the most additive variation changed. For example, for temperature plasticity in a high food environment, most of the additive genetic variation (g_{max}) was attributable to a trade-off between the mean number of offspring in each clutch and CT max at 24°C, but at 18°C the trade-off also involved differences in age at maturity (see Fig. 2A,B). For food plasticity at 18°C, the orientation of the variance-covariance matrices (G) was significantly different, but there was also a significant reduction in the volume of variance-covariance matrix in the low food environment, meaning that the population's evolutionary potential was reduced in low food compared to high food. We hypothesised that context-dependent multivariate $G \times E$ interactions might be explained by

differences in the number and identity of clones whose multivariate reaction norms diverged in either magnitude or phenotypic integration from the average multivariate plastic response. But there was no pattern in the number of clones that differed in the magnitude or nature of their multivariate plastic response in different environments. Clones that deviated from the population average plastic response were not the same in each environment, suggesting that the outcome of selection in different environments must also be context-dependent, which may help explain why clonal variation is often maintained in natural populations (Hebert and Crease, 1980; Weeks and Hoffmann, 2008).

Studies normally only consider the evolution of plastic responses to a single environmental variable (Dennis *et al.*, 2011; Plaistow and Collin, 2014; Westneat *et al.*, 2019). The context-dependence of the G x E's observed in this study, and the effect that context-dependence had on evolutionary potential, suggests that it may be important to consider the evolution of plastic responses to more complex environmental cues if we want to understand how plasticity contributes to the demography and extinction risk of populations (Hoffmann and Sgrò, 2011; Merilä and Hendry, 2014; Chevin and Hoffmann, 2017). Context-dependent G x E interactions could be explained by plastic responses to one environmental variable altering plastic responses to another environmental variable. For example, in our study multivariate G x E interactions at 18°C but not at 24°C could arise because development is faster at higher temperatures and constrains the effect that food plasticity has on traits such as body size and clutch size (Atkinson, 1994; Geerts *et al.*, 2015). Similarly, a reduced evolutionary potential in low food at 18°C could be because low food constrains the expression of life-history traits, making G x E interactions less detectable. However, context-dependent evolutionary potential can also arise because the strength or nature of selection varies between environments. Selection-by-environment interactions are common and are widely reported (Wood and Brodie, 2016; Hayward *et al.*, 2018). Alternatively, some environments may be more common than others over a population's evolutionary history, allowing more occasions for selection to optimise multivariate responses to

that particular environment (Chevin and Hoffmann, 2017).

Quantifying G x E interactions is important for understanding the potential for plasticity to evolve in populations. But it is how the detected G x E influences heritable trait variation in each environment that will ultimately determine how the genotype – phenotype relationship interacts with selection (Draghi and Whitlock, 2012). The adaptive plastic responses that organisms have evolved can be viewed as a kind of developmental bias that converts environmental and genetic cues into variation in the plastic response (Draghi and Whitlock, 2012). As a result, the traits that contribute to an evolved plastic response are predicted to be the same traits that show the most additive genetic variation (g_{max}) in populations introduced to new environments (Draghi and Whitlock, 2012; Noble *et al.*, 2019; Radersma *et al.*, 2020). Interestingly, we found a close relationship between the average plastic response to food and g_{max} at 18°C and 24°C (Fig. 4) but no relationship between the average plastic response to temperature and g_{max} in either food environment (Fig. 3). This difference could arise if temperature has exerted a stronger selection pressure than food over evolutionary time and was therefore more effective at removing additive genetic variation. Alternatively, food environments may hide genetic variation from selection. Harsh environments are often assumed to exert the strongest selection pressures on a population (Hayward *et al.*, 2018), but harsh environments that reduce evolutionary potential, or have little demographic consequence, can also shield additive genetic variation from selection (Jong and Behera, 2002; Chevin and Hoffmann, 2017). In *Daphnia*, low food environments contribute little to demographic change (Heugens *et al.*, 2006) and reduce evolutionary potential (this study). This may explain why evolutionary responses to temperature manipulations were more likely in populations that were not food limited (van Doorslaer *et al.*, 2009b; De Meester *et al.*, 2011).

Irrespective of the causes of context-dependent differences in evolutionary potential, our results demonstrate that *Daphnia magna* populations have maintained genetic potential to evolve adaptive responses to resources compared to their capacity to evolve adaptive responses to

temperature. This observation is further supported by our finding that there was no difference in the average multivariate plastic response to temperature in our three populations, but there were differences in the population's average multivariate plastic response to food. This finding appears to contradict previous studies that used laboratory experiments, mesocosm experiments and resurrection ecology to demonstrate that in *D. magna* populations, thermal tolerance is genetically variable and can evolve rapidly in response to increased temperatures (Van Doorslaer *et al.*, 2007, 2009a, b, 2010; De Meester *et al.*, 2011; Geerts *et al.*, 2015). One explanation could be that in previous studies thermal tolerance evolved as an indirect consequence of selection for another trait (De Meester *et al.*, 2011). Geerts *et al.* (2017) demonstrated that CTmax was negatively genetically correlated with body size. Our study also found a negative genetic correlation between CTmax and the average number of offspring per clutch, a trait that is closely associated with body size (see Fig. 2, 3). So it is possible that selection for body size and/or faster demographic rates (Bruijning *et al.*, 2018) explained the evolution of thermal tolerance in previous studies. This interpretation might also explain why temperature manipulations that are still in many cases at least 10°C below CTmax values are capable of generating rapid adaptation in experimental populations (van Doorslaer *et al.*, 2009b). Our finding that increases in resource availability generate plastic decreases in CTmax (Fig. 4) but changes in temperature do not induce plastic shifts in CTmax (Fig. 3), also support the idea that thermal tolerance evolves indirectly. We therefore suggest that a multivariate understanding of rapid adaptation to thermal environments is required before we can determine whether the rapid evolution of thermal tolerance reported in numerous studies is a direct result of selection for thermal tolerance, or an indirect consequence of the effect that temperature has on the evolutionary potential of traits such as body size and population growth rate.

In summary, we have demonstrated that multivariate plastic responses to food and temperature explained three times more phenotypic variation than genetic variation in traits or trait plasticities. G x E interactions exist in natural populations of *Daphnia magna* but they are

typically context-dependent. For temperature plasticity, the context-dependence manifests as a shift in the suite of traits that explain the most additive genetic variance in different food environments. But for food plasticity, the context-dependence also resulted in a reduction in evolutionary potential in low food at 18°C. This reduced evolutionary potential may explain why the population still harbours additive genetic variation in traits related to adaptive plastic responses to food, but little additive genetic variation in traits involved in adaptive plastic responses to temperature.

ACKNOWLEDGEMENTS

We thank Luc De Meester for the provision of *Daphnia* clones from France and Denmark and Aurora Geerts for advice on the heat tolerance assay. The study was supported by NERC Highlight grant NE/N016017/1 to SJP, DA & SPa.

DATA ACCESSIBILITY

Data will be archived in Dryad upon acceptance.

Table 1. Matrix comparison statistics for

Plasticity (context)	Metric	Mode	Lower 95% CI	Upper 95% CI	Probability
Temperature (high food)	Var gmax Diff	-0.040	-0.292	0.247	NA
	Angle between gmax	39.683	22.104	69.414	<0.05
	Prob-Vol diff	0.000	0.000	0.000	NA
	Sum-Vol diff	0.711	-17.000	9.602	NA
Temperature (Low food)	Var gmax Diff	-0.070	-0.327	0.203	NA
	Angle between gmax	41.122	23.731	63.229	<0.05
	Prob-Vol diff	0.000	0.000	0.000	NA
	Sum-Vol diff	0.107	-6.499	6.803	NA
Food (24°C)	Var gmax Diff	-0.059	-0.283	0.233	NA
	Angle between gmax	42.746	18.054	75.971	0.158
	Prob-Vol diff	0.000	0.000	0.000	NA
	Sum-Vol diff	5.950	-0.403	18.220	NA
Food (18°C)	Var gmax Diff	-0.090	-0.300	0.221	NA
	Angle between gmax	56.184	36.123	78.765	<0.05
	Prob-Vol diff	0.000	0.000	0.000	NA
	Sum-Vol diff	8.730	0.068	21.674	<0.05

Table 2. – Multivariate plastic responses to temperature in high and low food environments.

	Df	SSQ	MSQ	F-value	R2	P
(a) High food						
Temp	1	3.012	3.012	308.456	0.369	<0.001
Clone	42	1.900	0.045	4.630	0.233	<0.001
Temp x Clone	42	0.878	0.021	2.57	0.108	<0.001
Residuals	243	2.373	0.010		0.291	
(b) Low food						
Temp	1	2.870	2.870	260.991	0.358	<0.001
Clone	42	1.525	0.036	3.302	0.190	<0.001
Temp x Clone	42	0.783	0.019	1.696	0.098	<0.001
Residuals	258				0.354	

Table 3. – Multivariate plastic response to food at high and low temperatures.

	Df	SSQ	MSQ	F-value	R2	P
(a) High temperature						
Food	1	3.081	3.081	303.041	0.401	<0.001
Clone	42	1.499	0.036	3.510	0.195	<0.001
Food x Clone	42	0.514	0.012	1.203	0.067	<0.001
Residuals	255	2.592	0.010		0.337	
(b) Low temperature						
Food	1	3.428	3.428	297.379	0.338	<0.001
Clone	42	2.907	0.069	6.005	0.287	<0.001
Food x Clone	42	0.969	0.023	2.001	0.096	<0.001
Residuals	246				0.280	

FIGURE LEGENDS

Figure 1. G x E interactions in two hypothetical populations that have the same average plastic response. Differences in the magnitude of plastic responses (A) generate additive genetic variation that is aligned with the average plastic response whereas differences in the nature of plastic responses (B) generate additive genetic variation that is not aligned with the average plastic response.

Figure 2. Genetic variance–covariance matrix visualizations for each treatment within each environmental context. The volume of the three-dimensional hull represents the amount of additive genetic variance whereas the shape and rotation reflect changes in covariance between traits. The loadings for PC1 represent the contributions of traits towards gmax in each environmental context.

Figure 3: Variable plastic responses to temperature within the UK population.

(A) The first two Principal Component Axes summarize the multivariate trait space as shown by PCA biplot. agemat = age at maturity, age2cl = age at second clutch, LMat = length at maturity, L2cl = length at second clutch, aveclNo = average fecundity across clutches 1 and 2, aveoffsize = average offspring size, grjuv = juvenile growth rate, grad = adult growth rate, CTmax = temperature tolerance. Reaction norms in response to temperature for each clone are shown in the low food context (B) and in the high food context (C) within the same multivariate space. Average clonal phenotypes in each environment are indicated in dark red/light red for 24°C and dark blue/light blue for 18°C. Insets show components of variation estimated from marginal R^2 in perMANOVA models. E= (temperature) environment, G = genotype, GxE=plasticity variation, Res= Residuals. (D) Differences from population means for each clone are summarised in a Venn diagram to show the overlap in clones for different outlier tests. Black and grey outlines indicate high food and low food environment, respectively; solid and dashed lines indicate differences from population mean response in magnitude or phenotypic integration, respectively.

496

497

498 **Figure 4. Variable plastic responses to resources within the UK population.**

499 (A) The first two Principal Component Axes summarize the multivariate trait space as shown
 500 by PCA biplot. agemat = age at maturity, age2cl = age at second clutch, LMat = length at
 501 maturity, L2cl = length at second clutch, aveclNo = average fecundity across clutches 1 and
 502 2, aveoffsize = average offspring size, grjuv = juvenile growth rate, grad = adult growth rate,
 503 CTmax = temperature tolerance. Reaction norms in response to resources for each clone are
 504 shown in 18°C context (B) and in the 24°C context (C) within the same multivariate space.
 505 Average clonal phenotypes in each environment are indicated in dark red/dark blue for high
 506 food and light red/light blue for low food. Insets show components of variation estimated
 507 from marginal R^2 in perMANOVA models. E= (temperature) environment, G = genotype,
 508 GxE=plasticity variation, Res= Residuals. (D) Differences from population means for each
 509 clone are summarised in a Venn diagram to show the overlap in clones for different outlier
 510 tests. Black and grey outlines indicate high food and low food environment, respectively;
 511 solid and dashed lines indicate differences from population mean response in magnitude or
 512 phenotypic integration, respectively.

513

514 **Figure 5. Plastic responses to temperature and resources within two reference populations**

515 (A, D, G, J) The first two Principal Component Axes summarize the multivariate trait space
 516 as shown by PCA biplot. agemat = age at maturity, age2cl = age at second clutch, LMat =
 517 length at maturity, L2cl = length at second clutch, aveclNo = average fecundity across
 518 clutches 1 and 2, aveoffsize = average offspring size, grjuv = juvenile growth rate, grad =
 519 adult growth rate, CTmax = temperature tolerance. Reaction norms in response to
 520 temperature for each clone in the Danish (B) and French (E) populations are shown in high
 521 food context (dark red/ dark blue) in low food context (light red/light blue) within the same
 522 multivariate plot. Reaction norms in response to food for each clone in the Danish (H) and
 523 French (K) populations are shown in high temperature context (dark red/ light red) and in low
 524 temperature context (dark blue/light blue) within the same plot. Population average plastic
 525 responses are shown by solid black lines. Insets show components of variation estimated
 526 from marginal R^2 in perMANOVA models. E= (temperature) environment, G = genotype,
 527 GxE=plasticity variation, Res= Residuals. (C, F, I, L) Differences from population means for

each clone are summarised in a Venn diagram to show the overlap in clones for different outlier tests.

Fig. 1

Fig. 2

Fig. 3

Fig. 5

571

572 REFERENCES

- 573 Adams DC, Collyer ML (2009) A General Framework For The Analysis Of Phenotypic
574 Trajectories In Evolutionary Studies. *Evolution*, **63**: 1143–1154
- 575 Atkinson D (1994) Temperature and organism size: a biological law for ectotherms. *Adv Ecol*
576 *Res*, **25**: 1–58
- 577 Baird D, Barber I, Bradley M, Calow P, Soares AMVM (1989) The Daphnia Bioassay - a
578 Critique. **188**: 403–406
- 579 Bonamour S, Chevin LM, Charmantier A, Teplitsky C (2019) Phenotypic plasticity in
580 response to climate change: the importance of cue variation. *Philos Trans R Soc Lond B*
581 *Biol Sci*, **374**: 20180178
- 582 Brommer JE, Rattiste K, Wilson AJ (2008) Exploring plasticity in the wild: laying date-
583 temperature reaction norms in the common gull *Larus canus*. *Proc Biol Sci*, **275**: 687–
584 693
- 585 Bruijning M, ten Berge ACM, Jongejans E (2018) Population-level responses to temperature,
586 density and clonal differences in *Daphnia magna* as revealed by integral projection
587 modelling. *Funct Ecol*, **32**: 2407–2422
- 588 Charmantier A, Gienapp P (2014) Climate change and timing of avian breeding and
589 migration: evolutionary versus plastic changes. *Evolutionary Applications*, **7**: 15–28
- 590 Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC (2008)
591 Adaptive phenotypic plasticity in response to climate change in a wild bird population.
592 *Science*, **320**: 800–803
- 593 Chevin LM, Hoffmann AA (2017) Evolution of phenotypic plasticity in extreme
594 environments. *Philos Trans R Soc Lond B Biol Sci*, **372**:
- 595 Chevin L-M, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing
596 environment: Towards a predictive theory. *PLoS Biology*, **8**: e1000357
- 597 Chun YJ, Collyer ML, Moloney KA, Nason JD (2007) Phenotypic plasticity of native vs.
598 invasive purple loosestrife: A two-state multivariate approach. *Ecology*, **88**: 1499–1512
- 599 Collyer ML, Adams DC (2007) Analysis of two-state multivariate phenotypic change in
600 ecological studies. *Ecology*, **88**: 683–692
- 601 De Meester L, van Doorslaer W, Geerts A, Orsini L, Stoks R (2011) Thermal genetic
602 adaptation in the water flea *Daphnia* and its impact: an evolving metacommunity
603 approach. *Integr Comp Biol*, **51**: 703–718
- 604 Dennis SR, Carter MJ, Hentley WT, Beckerman AP (2011) Phenotypic convergence along a
605 gradient of predation risk. *Proceedings Of The Royal Society B-Biological Sciences*,
606 **278**: 1687–1696
- 607 Des Marais DL, Hernandez KM, Juenger TE (2013) Genotype-by-environment interaction
608 and plasticity: exploring genomic responses of plants to the abiotic environment.
609 *Annual Review of Ecology, Evolution, and Systematics*, **44**: 5–29
- 610 Draghi JA, Whitlock MC (2012) Phenotypic Plasticity Facilitates Mutational Variance,
611 Genetic Variance, And Evolvability Along The Major Axis Of Environmental Variation.
612 *Evolution*, **66**: 2891–2902
- 613 Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate
614 extremes: observations, modeling, and impacts. *Science*, **289**: 2068–2074
- 615 Fox RJ, Donelson JM, Schunter C, Ravasi T, Gaitán-Espitia JD (2019) Beyond buying time:
616 the role of plasticity in phenotypic adaptation to rapid environmental change.
- 617 Geerts AN, Vanoverbeke J, Vanschoenwinkel B, Van Doorslaer W, Feuchtmayr H, Atkinson
618 D, Moss B, Davidson TA, Sayer CD, De Meester L (2015) Rapid evolution of thermal

- tolerance in the water flea *Daphnia*. *Nature Climate Change*, **5**: 665–668
- Gibson G, Dworkin I (2004) Uncovering cryptic genetic variation. *Nat Rev Genet*, **5**: 681–690
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Mol Ecol*, **17**: 167–178
- Gillespie JH, Turelli M (1989) Genotype-environment interactions and the maintenance of polygenic variation. *Genetics*,
- Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, **33**: 1–22
- Harris KDM, Bartlett NJ, Lloyd VK (2012) *Daphnia* as an Emerging Epigenetic Model Organism. *Genetics Research International*, **2012**: 1–8
- Hayward AD, Pemberton JM, Berenos C, Wilson AJ, Pilkington JG, Kruuk LEB (2018) Evidence for selection-by-environment but not genotype-by-environment interactions for fitness-related traits in a wild mammal population. *Genetics*, **208**: 349–364
- Hebert PDN, Crease TJ (1980) Clonal coexistence in *Daphnia pulex* (Leydig): another planktonic paradox. *Science*, 1363–1365
- Heugens EHW, Tokkie LTB, Kraak MHS, Hendriks AJ, van Straalen NM, Admiraal W (2006) POPULATION GROWTH OF *DAPHNIA MAGNA* UNDER MULTIPLE STRESS CONDITIONS: JOINT EFFECTS OF TEMPERATURE, FOOD, AND CADMIUM. *Environ Toxicol Chem*, **25**: 1399
- Hoffmann AA, Merila J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol Evol*, **14**: 96–101
- Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**: 479–485
- Ingleby FC, Hunt J, Hosken DJ (2010) The role of genotype-by-environment interactions in sexual selection. *J Evol Biol*, **23**: 2031–2045
- Jong GD, Behera N (2002) The influence of life-history differences on the evolution of reaction norms. *Evol Ecol Res*,
- Lande R (2009) Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J Evol Biol*, **22**: 1435–1446
- Ledón-Rettig CC, Pfennig DW, Chunco AJ, Dworkin I (2014) Cryptic genetic variation in natural populations: a predictive framework. *Integr Comp Biol*, **54**: 783–793
- Levis NA, Pfennig DW (2016) Evaluating ‘Plasticity-First’ Evolution in Nature: Key Criteria and Empirical Approaches. *Trends in ecology & evolution (Personal edition)*, **31**: 563–574
- Lind MI, Yarleth K, Reger J, Carter MJ, Beckerman AP (2015) The alignment between phenotypic plasticity, the major axis of genetic variation and the response to selection. *Proceedings Biological sciences / The Royal Society*, **282**: 20151651
- Mcguigan K, Sgrò CM (2009) Evolutionary consequences of cryptic genetic variation. *Trends Ecol Evol*, **24**: 305–311
- Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary applications*, **7**: 1–14
- Michels H (2007) Micro-evolutionary response of *Daphnia magna* to changes in biotic stress associated with habitat degradation and restoration of a shallow lake.
- Miner BE, De Meester L, Pfender ME, Lampert W, Hairston NG (2012) Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings Of The Royal Society B-Biological Sciences*, **279**: 1873–1882
- Noble DWA, Radersma R, Uller T (2019) Plastic responses to novel environments are biased towards phenotype dimensions with high additive genetic variation. *Proc Natl Acad Sci U S A*, **116**: 13452–13461

- 669 Nussey DH, Clutton-Brock TH, Elston DA, Albon SD, Kruuk LEB (2005a) Phenotypic
670 plasticity in a maternal trait in red deer. *J Anim Ecol*, **74**: 387–396
- 671 Nussey DH, Postma E, Gienapp P, Visser ME (2005b) Selection on heritable phenotypic
672 plasticity in a wild bird population. *Science*, **310**: 304–306
- 673 Oksanen, J., Blanchet, F.G., Friendly M, Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R.,
674 O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner H
675 (2018) vegan: Community Ecology Package.
- 676 Oostra V, Saastamoinen M, Zwaan BJ, Wheat CW (2018) Strong phenotypic plasticity limits
677 potential for evolutionary responses to climate change. *Nature communications*, **9**: 1005
- 678 Paaby AB, Rockman MV (2014) Cryptic genetic variation: evolution’s hidden substrate. *Nat*
679 *Rev Genet*, **15**: 247–258
- 680 Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now. *Trends Ecol*
681 *Evol*, **20**: 481–486
- 682 Pigliucci M, Preston K. (2004) *Phenotypic Integration: Studying the Ecology and Evolution*
683 *of Complex Phenotypes*. Oxford University Press,
- 684 Plaistow SJ, Collin H (2014) Phenotypic integration plasticity in *Daphnia magna*: an integral
685 facet of G × E interactions. *J Evol Biol*, **27**: 1913–1920
- 686 Radersma R, Noble DWA, Uller T (2020) Plasticity leaves a phenotypic signature during
687 local adaptation. *Evolution Letters*,
- 688 Rasband WS (1997) ImageJ, U.S.
- 689 Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010) Phenotypic plasticity and
690 population viability: the importance of environmental predictability. *Proc Biol Sci*, **277**:
691 3391–3400
- 692 Reger J, Lind MI, Robinson MR, Beckerman AP (2017) Predation drives local adaptation of
693 phenotypic plasticity. *Nature Ecology & Evolution*, 1–8
- 694 Robinson MR, Beckerman AP (2013) Quantifying multivariate plasticity: genetic variation in
695 resource acquisition drives plasticity in resource allocation to components of life history.
696 *Ecol Lett*, **16**: 281–290
- 697 Saltz JB, Bell AM, Flint J, Gomulkiewicz R, Hughes KA, Keagy J (2018) Why does the
698 magnitude of genotype-by-environment interaction vary. *Ecol Evol*, **8**: 6342–6353
- 699 Schlichting CD, Pigliucci M (1993) Control of Phenotypic Plasticity Via Regulatory Genes.
700 *Am Nat*, **142**: 366–370
- 701 Schlichting CD (2008) Hidden reaction norms, cryptic genetic variation, and evolvability.
702 *Ann N Y Acad Sci*, **1133**: 187–203
- 703 Snell-Rood EC, Kobiela ME, Sikkink KL, Shephard AM (2018) Mechanisms of Plastic
704 Rescue in Novel Environments. *Annual Review of Ecology, Evolution, and Systematics*,
705 **49**: 331–354
- 706 Valdar W, Solberg LC, Gauguier D, Cookson WO, Rawlins JNP, Mott R, Flint J (2006)
707 Genetic and environmental effects on complex traits in mice. *Genetics*, **174**: 959–984
- 708 Van Doorslaer W, Stoks R, Jeppesen E (2007) Adaptive microevolutionary responses to
709 simulated global warming in *Simocephalus vetulus*: a mesocosm study - VAN
710 DOORSLAER - 2007 - Global Change Biology - Wiley Online Library. *Global Change*
711 ...,
- 712 Van Doorslaer W, Stoks R, Swillen I, Feuchtmayr H, Atkinson D, Moss B, De Meester L
713 (2010) Experimental thermal microevolution in community-embedded *Daphnia*
714 populations. *Clim Res*, **43**: 81–89
- 715 van Doorslaer W, Vanoverbeke J, Duvivier C, Rousseaux S, Jansen M, Jansen B,
716 Feuchtmayer H, Atkinson D, Moss B, Stoks R, De Meester L (2009a) Local adaptation
717 to higher temperatures reduces immigration success of genotypes from a warmer region
718 in the water flea *Daphnia*. **15**: 3046–3055

719 van Doorslaer W, Stoks R, Duvivier C, Bednarska A, De Meester L (2009b) Population
720 dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution*, **63**: 1867–
721 1878
722 Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic
723 plasticity. *Evolution*, **39**: 505–522
724 Vieira C, Pasyukova EG, Zeng Z-B, Hackett JB, Lyman RF, Mackay TFC (2000) Genotype-
725 environment interaction for quantitative trait loci affecting life span in *Drosophila*
726 *melanogaster*. *Genetics*, **154**: 213–227
727 Weeks AR, Hoffmann AA (2008) Frequency-dependent selection maintains clonal diversity
728 in an asexual organism. *Proceedings of the National Academy of Sciences*, **105**: 17872–
729 17877
730 Westneat DF, Potts LJ, Sasser KL, Shaffer JD (2019) Causes and consequences of
731 phenotypic plasticity in complex environments. *Trends Ecol Evol*,
732 Wood CW, Brodie ED (2016) Evolutionary response when selection and genetic variation
733 covary across environments. *Ecol Lett*, **19**: 1189–1200
734