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Revealing hidden evolutionary capacity to cope with global change

EVATT CHIRGWIN, KEYNE MONRO, CARLA M. SGRO and DUSTIN J. MARSHALL

School of Biological Sciences, Monash University, Melbourne Vic. 3800, Australia

Abstract

The extent to which global change will impact the long-term persistence of species depends on their evolutionary potential to adapt to future conditions. While the number of studies that estimate the standing levels of adaptive genetic variation in populations under predicted global change scenarios is growing all the time, few studies have considered multiple environments simultaneously and even fewer have considered evolutionary potential in multivariate context. Because conditions will not be constant, adaptation to climate change is fundamentally a multivariate process so viewing genetic variances and covariances over multivariate space will always be more informative than relying on bivariate genetic correlations between traits. A multivariate approach to understanding the evolutionary capacity to cope with global change is necessary to avoid misestimating adaptive genetic variation in the dimensions in which selection will act. We assessed the evolutionary capacity of the larval stage of the marine polychaete *Galeolaria caespitosa* to adapt to warmer water temperatures. *Galeolaria* is an important habitat-forming species in Australia, and its earlier life-history stages tend to be more susceptible to stress. We used a powerful quantitative genetics design that assessed the impacts of three temperatures on subsequent survival across over 30 000 embryos across 204 unique families. We found adaptive genetic variation in the two cooler temperatures in our study, but none in the warmest temperature. Based on these results, we would have concluded that this species has very little capacity to evolve to the warmest temperature. However, when we explored genetic variation in multivariate space, we found evidence that larval survival has the potential to evolve even in the warmest temperatures via correlated responses to selection across thermal environments. Future studies should take a multivariate approach to estimating evolutionary capacity to cope with global change lest they misestimate a species' true adaptive potential.

Keywords: adaptation, evolutionary potential, evolutionary rescue, genetic variance

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Introduction

Global change will impose new, and alter existing, selection pressures on natural populations (Holt, 1990; Hughes, 2000; Davis *et al*., 2005). The extent to which global change will impact the long-term persistence of populations depends on a population's ability to respond using one or a combination of three mechanisms: migration to favorable habitats, phenotypic plasticity, and adaptive evolution (Holt, 1990; Davis *et al*., 2005; Williams *et al*., 2008; Dam, 2013). Although all three mechanisms will be important in the initial stages of change (Williams *et al*., 2008), the relative importance of each of the three mechanisms will vary among species and is likely to change over time (Gienapp *et al*., 2008). For instance, only organisms with both sufficient dispersal capability and suitable alternative habitat will be able to successfully respond to global change through migration (Hughes, 2000). In the short term, phenotypic plasticity will play a major role in determining the tolerance of species that are unable to disperse from habitats that degrade in their suitability. While there has been a large focus on the likely role of migration (Walther *et al*., 2002; Thomas *et al*., 2004; Guisan & Thuiller, 2005), and a growing body of studies considering the role and limitations of phenotypic plasticity (Przybylo *et al*., 2000; Réale *et al*., 2003; Charmantier *et al*., 2008), an understanding of the evolutionary potential of most species to global change remains elusive (Hoffmann & Sgro, 2011). The uncertainty around evolutionary potential is concerning as most species will have to undergo adaptive evolution to some extent to avoid extinction from the pressures of long-term global change (Gienapp *et al*., 2008).

Adaptive evolution in response to global change can occur quickly, particularly for species with short lifecycles (Huey *et al*., 2000; Franks *et al*., 2007; Lohbeck *et al*., 2012). However, a population's ability to undergo adaptive evolution depends on the presence of heritable genetic variation (Lynch & Walsh, 1998; Conner & Hartl, 2004; Bell, 2013). As such, a population that possesses little heritable variation in a trait(s) under selection will have a limited capacity to respond via

Correspondence: Evatt Chirgwin, tel. +61 3 9902 4449, fax +61 3 9905 5613, e-mail: evatt.chirgwin@monash.edu

evolutionary change (Lynch & Walsh, 1998; Hoffmann *et al*., 2003a; Kellermann *et al*., 2006). Even when adaptive genetic variance is present in the traits under selection, evolutionary adaptation to global change may be constrained by genetic covariances among traits (Dam, 2013). This is important because the ability of a population to respond to selection will be determined by the patterns of genetic variation and covariation in the traits under selection (Blows & Hoffmann, 2005). Thus, adaptation is likely to occur via evolutionary changes in several traits at any one time, a multivariate process (Lande, 1979; Phillips & Arnold, 1989; Blows, 2007b). Surprisingly, we still lack an understanding of how multivariate genetic constraints will influence the evolutionary trajectory of species in response to global change (Hellmann & Pineda-Krch, 2007; Hoffmann & Sgro, 2011). Indeed, a recent review of adaptation to global change emphasized the need for more studies exploring multiple traits simultaneously (Dam, 2013).

Genetic covariances have the capacity to constrain or facilitate evolution depending on the type of covariance between traits and how selection is operating on each trait (Lande, 1979; Lande & Arnold, 1983; Blows & Hoffmann, 2005). Multivariate constraints have traditionally been inferred through interpretation of pairwise genetic correlations, particularly in a life-history context (Houle, 1991; Roff & Fairbairn, 2007; Poissant *et al*., 2010). However, pairwise correlations cannot account for the influence of the broader context of the multivariate phenotype (Pease & Bull, 1988; Fry, 1993). The effect of genetic correlations on evolution will always depend on trait variances *and* covariances because correlations can only be considered in truly multivariate space through genetic covariances (i.e., $r_{12} = G_{12}/\sqrt{G_{11}G_{22}}$ where r_{12} = genetic correlation between traits 1 and 2, G_{12} = genetic covariance between traits 1 and 2, G_{11} and G_{22} are the additive genetic variance in traits 1 and 2, respectively).

Although covariances are unlikely to prevent evolution completely (Schluter, 1996; Blows & Hoffmann, 2005), they may slow the rate of adaptation (Arnold, 1992; Walsh & Blows, 2009), which is of particular concern given the relatively rapid rates of evolution that may be necessary for species to cope with anthropogenic global change. For instance, despite substantial additive genetic variance in two individual leaf traits in the plant *Chamaecrista fasciculata*, negative covariances between the two traits imply limited capacity to evolve in the direction of selection predicted due to global change (Etterson & Shaw, 2001). Alternatively, genetic covariances among traits may facilitate and increase the rate of adaptive evolution if two or more traits are positively correlated with fitness (Lande & Arnold, 1983; Marshall & Morgan, 2011). If the evolution of one trait causes an indirect evolutionary change in the correlated trait, then both traits will achieve a higher fitness simultaneously (Agrawal & Stinchcombe, 2009). To illustrate, consider the multivariate breeders equation:

$$\Delta z_1 = (G_{1,1} \times \beta_1) + (G_{1,2} \times \beta_2)$$

where z_1 represents the change in the value of trait 1 from one generation to the next, $G_{1,1}$ is the additive genetic variance in trait one, β_1 is selection on trait 1, $G_{1,2}$ is the genetic covariance between traits one and two, and β_2 is selection on trait 2.

If we consider only genetic variation in the trait of interest and ignore genetic covariation with other traits, then for a given condition where $G_{1,1} = 0.5$ and $\beta_1 = 0.5$:

$$\Delta z_1 = (0.5 \times 0.5) = 0.25$$

We would predict an evolutionary change of 0.25 in the population mean after one generation. If, however, we include a positive genetic covariance between the traits and selection on trait 2, then for a given condition where $G_{1,2} = 0.2$ and $\beta_2 = 0.5$:

$$\Delta z_1 = (0.5 \times 0.5) + (0.2 \times 0.5) = 0.35$$

The predicted evolutionary response would be 0.35. Consequently, if genetic covariances are ignored, we may either over- or underestimate the evolutionary capacity of a given trait (Lynch & Walsh, 1998; Marshall & Morgan, 2011).

Importantly, multivariate evolutionary constraints may not only arise from genetic covariances between different traits within the same environments, they may also arise from genetic covariances between the same traits in different environments (Falconer, 1952). Falconer's insight is of key importance given that abiotic factors such as temperature are expected to become more extreme and are also expected to become more variable with ongoing global change (Schar *et al*., 2004; IPCC, 2013). Thus, organisms must adapt to survive under different, but also more variable, conditions. Accordingly, if traits such as thermal tolerance are genetically correlated across multiple thermal environments (which seems likely), then fitness in one thermal environment may influence the fitness across all other thermal environments (Lynch & Walsh, 1998; Sgro & Hoffmann, 2004; Galletly *et al*., 2007). To illustrate, consider the example using the multivariate breeders equation presented earlier: If one exchanges 'trait' for 'environment', then the same misestimation of evolutionary responses will occur if one considers only a single environment and ignores natural variability in time. Consequently, even if a species can adapt to the

long-term directional selection imposed by increased temperature, it may still become extinct if adaptation to higher temperatures results in a correlated reduction in fitness at other temperatures that are still encountered by the species.

An important next step for evolutionary studies of global change is to move beyond bivariate genetic covariances and correlations to a truly multivariate characterization of evolutionary potential over multiple environments. While some studies have estimated the evolutionary potential of species to cope with global change using a **G**-matrix approach across multiple environments (Etterson, 2004; Foo *et al*., 2012; Ketola *et al*., 2012b), these studies considered only bivariate genetic covariances and correlations, such that both positive (Foo *et al*., 2012; Ketola *et al*., 2012b; Clark *et al*., 2013) and negative (Etterson, 2004) genetic correlations have been detected across environments. To the best of our knowledge, none have used an analysis of the **G**-matrix to visualize how multivariate genetic variances and covariances might influence evolution in response to global change. Where more than two traits are either genetically correlated or expected to come under selection, viewing genetic variances and covariances over multivariate space will always be more informative than relying on bivariate genetic correlations between traits (Blows, 2007a). A reliance on bivariate correlations may be misleading, as multivariate evolutionary constraints can be present even in the absence of negative bivariate genetic correlation (Blows & Hoffmann, 2005; Walsh & Blows, 2009).

In an attempt to address the concerns discussed above, we assessed the evolutionary capacity of the larval stage of the marine polychaete, *G. caespitosa*, to adapt to warmer and more variable water temperatures. *Galeolaria* has a typical marine invertebrate lifecycle with a free swimming larval stage and a sessile adult stage (Jackson & Strathmann, 1981). The adult stage plays an important ecological role as a habitat-forming species in intertidal areas across South Eastern Australia, forming high-density colonies that support unique endemic communities (Edgar, 2000; Bulleri *et al*., 2005). Here, we focus on the thermal tolerances of the larval stage because this larval stage is typically more sensitive and vulnerable to environmental stressors than the adult stage in marine organisms (Jackson & Strathmann, 1981; Marshall & Morgan, 2011; Byrne, 2012). As such, the early larval stage is likely to be an important bottleneck in the persistence of future populations. Using a quantitative genetic breeding design that allowed the estimation of additive genetic variances and covariances, the survival of larvae was measured across three thermal environments. These temperatures, which ranged from the mean temperature currently experienced by the population in nature to the maximum sea surface temperature experienced by the study population over the previous year, are likely to reflect higher temperatures becoming more common with continuing global change. Treating larval survival in each thermal environment as a separate trait, this study estimated the additive genetic variance within, and additive genetic covariance between, the three traits (environments) to predict how additive genetic variance and covariances will affect *Galeolaria*'s evolutionary response to global change. Finally, we applied an eigenanalysis to assess the multivariate nature of adaptive genetic variation in thermal tolerance among temperature environments. Importantly, our study does not estimate adaptation, but like all quantitative genetics breeding designs, it provides estimates of the potential for evolutionary adaptation given specific selection regimes that are likely to vary in space and time.

Materials and methods

Field and laboratory procedures

Study site and study species. *Galeolaria caespitosa* (henceforth described by its genus name) is an intertidal tubeworm common to South Eastern Australia. Typical of marine invertebrates, *Galeolaria* has a free swimming larval stage and sessile adult stage (Marshall & Evans, 2005a). The lifecycle of *Galeolaria* includes a developmental phase (~2 days), from a fertilized egg to a feeding larval stage. The feeding larval phase lasts ~4 days, before larvae settle and metamorphose into juveniles.

A population of *Galeolaria* was sampled regularly across two main periods (winter of 2013 and summer of 2014) by detaching adults from pier pylons in the intertidal zone at Brighton marina, Victoria (37°54′S 144°59′E). *Galeolaria* spawns year-round, and our study population experiences temperatures as low as 8 °C and as high as 25 °C. Typically, maximum temperatures are around 22 °C – in the past 15 years, temperatures have exceeded 24.5 °C for only 6 days, 3 days each in January 2013 and 2014. The mean water temperature at our site is 17 °C. As an intertidal species, this organism and its larvae may also experience even higher temperatures when retained in smaller rockpools at low tide.

Collected *Galeolaria* were transported in insulated aquaria to a controlled temperature room at Monash University, Clayton. *Galeolaria* were then housed in separate tanks according to date collected. To reduce the effect of variation in the parental environment among field collections, all adults were acclimatized for 2–3 weeks at ~16 °C before gametes were extracted.

Gamete collection and fertilization. Each sexually mature individual was removed from their calcareous external tube with forceps and placed into a petri dish of fresh seawater. All

seawater used during gamete collection and fertilization was filtered to 0.22 μm and pasteurized. Individuals began spawning eggs or sperm within 10 s of being removed from their tube, at which point gametes were collected.

Once sperm and eggs had been collected, we diluted sperm to a concentration of ~4 $\times$ 10^6 sperm mL^{-1} solution with seawater (a concentration shown to achieve highest fertilization success during pilot studies). Egg concentration has a very minor influence on fertilization success (Levitan *et al*., 1991), so we simply extracted the maximum number of eggs possible from each female and then diluted the eggs into a 1.2-mL solution using seawater. We went on to add 0.1 mL of the diluted sperm solution to 0.1 mL of the egg solution three times at 10 min intervals. The gradual addition of sperm reduced the likelihood of multiple sperm fertilizing the same egg (polyspermy), increasing total fertilization success of each male–female cross (Styan, 1998). Following sperm addition to the egg solution, the resulting gamete solution was left for 1 h before being rinsed through a 0.25 μm Nitex mesh twice to remove excess sperm.

Experimental design. Male and female gametes of *Galeolaria* larvae were crossed in accordance with the North Carolina II breeding design (NCII). NCII is a factorial breeding design, where within each block, each father (sire) is crossed with each mother (dam) (Lynch & Walsh, 1998). NCII was selected for two reasons: First it allowed us to estimate how much phenotypic variance in the traits examined among larval offspring was due to the sire, the dam, the interaction between sire and dam inheritance, and nongenetic environmental variation (Lynch & Walsh, 1998). As the sire is assumed to contribute essentially only genes to the offspring (Conner & Hartl, 2004; see below for important caveats to this assumption), variation across offspring due to the sire allowed us to estimate heritable additive genetic variance within and between traits. Second, due to the free spawning nature of *Galeolaria*, this species is well suited to the NCII design as sperm and eggs from individual adults can be split such that the sperm of one sire can fertilize multiple females at the same time (and *vice versa* for dams; Munday *et al*., 2013).

We did 51 experimental blocks, and in each block, we crossed gametes from two sires and two dams to yield four parent combinations for each block (Fig. 1). We replicated each sire–dam combination six times within a block, with each of the six replicates individually fertilized using the fertilization methods described above (creating 24 fertilizations per block) (Fig. 1). Approximately 2 h after fertilization, a subsample of fertilized eggs ($n \sim 25$) was collected from each of the 24 fertilization replicates and placed in individual 1.5-mL capped test tubes with sterilized filtered seawater. Eggs were classified as fertilized if they displayed regular symmetrical cleavage, which is a standard approach for assessing fertilization success in this species (Marshall & Evans, 2005b). The replicate test tubes containing ~25 fertilized eggs were each randomly assigned to one of three thermal treatments (17, 21, and 25 °C), such that each parental combination was replicated twice for each temperature (Fig. 1). Overall, our study yielded and scored over 30 000 fertilized embryos from 204 families. Importantly, statistical power in such designs stems from the number of sires included in the sire as this is where the variance of interest is estimated (Munday *et al*., 2013),and the replicate vials represent subsamples that provide better precision only.

Thermal temperatures were chosen as they represented the approximate yearly average sea surface temperature at our field site (17 °C), a low-moderate rise from the yearly average sea surface temperature (21 °C), and the highest recorded sea surface temperature within the preceding 12-month period (25 °C) at the site of collection (CSIRO, 2014). More specifically, our scenarios represent typical temperatures for summer or extremely warm temperatures for winter (21 °C), and a temperature that is currently exceedingly rare, but is likely to be much more common in the future (25 °C). As such, our thermal treatments are likely to be a good reflection of near future stressors for our study population. For the two treatments above 17 °C, we gradually warmed seawater within the test tubes to the desired temperature over ~20 min from when the fertilized eggs were inserted in their test tubes using a major science md-mini dry bath heater and maintained within 0.2 °C throughout the 48 h incubation period. A 48-h incubation period was used as it was sufficient time to allow the visual determination of whether larvae had survived and successfully developed into the trochophore stage, and ecotoxicological studies indicate that this is the most sensitive and

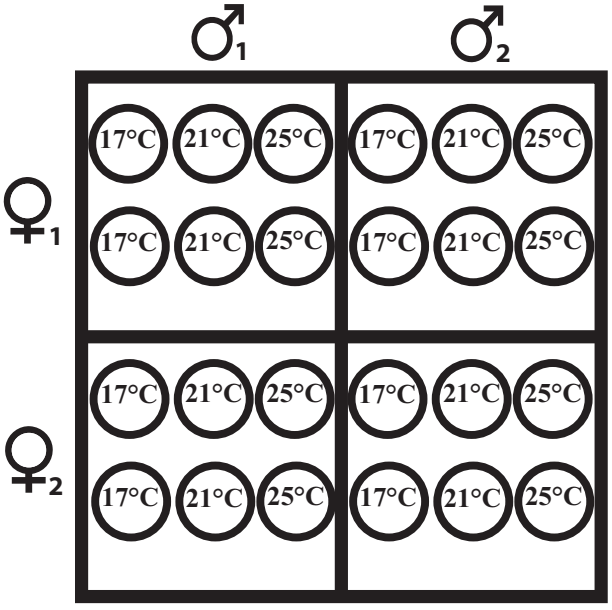

Fig. 1 Schematic depicting the North Carolina II block design used in the present study. For each experimental block, eggs from two individual dams were crossed with sperm from two individual sires. Each cross was replicated by six separate fertilizations. Fertilized eggs were then assigned to one of three different temperature treatments (17, 21, or 25 °C) so that each sire–dam combination was replicated twice per thermal environment.

reliable stage for examining larval tolerance to stress (Ross & Bidwell, 2001). Following the 48-h incubation period, larval survival was quantified by determining the number of larvae that were present and showed normal larval development.

Statistical analyses

*Estimating the additive genetic (co)variance matrix, **G**.* The additive genetic variance covariance (**G**) matrix was estimated in a multivariate linear mixed model, fitted with restricted estimation maximum likelihood (REML) in the MIXED procedure of SAS 9.3 (SAS version 8; SAS Institute, Cary, NC, USA, 1998). This model estimated the additive genetic variance for, and additive genetic covariance between, survival in each of the three thermal environments. Sire, dam, and the sire x dam interaction were nested within block and treated as random factors, while environment (thermal treatments) and block (to account for differences between blocks) were treated as fixed factors. While this analysis allows for the exploration of dam and dam × sire effects, conventional quantitative genetics analyses focus on sire effects because these effects best estimate additive genetic variance – this variance responds most straightforwardly to selection (Lynch & Walsh, 1998).

To account for any effects of main sampling period (winter vs. summer), the model also included this term as an additional fixed factor crossed with thermal treatment, as well as a separate residual variance for each period (in essence, block was nested in sampling period). Preliminary analyses detected no significant effects of sampling period on the expression of additive genetic variance or covariance within or across thermal treatments, so such effects were not considered further at this level – excluding these effects had no qualitative effect on our results.

To determine whether levels of additive genetic variance for each trait (survival in each thermal environment) were significantly different from zero, log-likelihood ratio tests were performed, where the final model for each trait was compared to a model specifying the sire-level variance for that trait to be zero (Littell *et al.*, 2006). The same approach was used to test the significance of dam and dam x sire effects. The additive genetic covariances across the thermal treatments were also individually tested for significance from zero by performing log-likelihood ratio tests where the final models for each trait were compared to models specifying the sire-level covariances (COV_S) to be zero (Littell *et al.*, 1996; McGuigan *et al.*, 2011; Simonsen & Stinchcombe, 2010). All *P*-values were adjusted for multiple testing by holding the false discovery rate to 5% (Benjamini & Hochberg, 1995).

Note that this approach assumes that all of the variance attributable to sires can be considered additive genetic variance. While this assumption reflects convention in quantitative genetics studies, there is increasing evidence that some sire-level variance in offspring performance may indeed be environmentally induced (i.e., paternal effects; Crean & Bonduriansky, 2014; Marshall, 2015). We cannot rule this possibility out, but in our study, males were sourced in identical conditions and temporal environmental variation was partitioned via blocking effects so we suspect the contribution of paternal effects to our results is minimal. As such, our estimates are likely to represent the upper range of genetic variation and remain the most common approach for assessing evolutionary potential.

*Eigenanalysis of **G**.* While the **G**-matrix estimated above provides an estimate of how bivariate covariances between traits might influence evolutionary responses to changes in temperature, a multivariate approach was necessary to determine how all three traits (thermal environments) contributed simultaneously to the **G**-matrix to obtain a more accurate picture of the evolutionary relationships among the three traits (Phillips & Arnold, 1989; Blows & Hoffmann, 2005; Walsh & Blows, 2009). An eigenanalysis of **G** was performed to determine how many genetically independent traits (eigenvectors – linear combinations of all of the traits reflecting patterns of covariance between traits) were represented by the original three traits measured, and how much additive genetic variance (eigenvalues) was associated with each independent set of eigenvectors (traits). The eigenvector with the largest eigenvalue (g_{max}, Schluter, 1996) is the vector explaining most of the additive genetic variance in the **G**-matrix. The contribution of each trait to each eigenvector is demonstrated by the individual trait loadings from trait eigenvalues onto the eigenvector (Blows, 2007b; Galletly *et al.*, 2007).

The first two eigenvectors that accounted for the greatest amount of additive genetic variance in *G* (g_{max} & g_2) were then displayed in a biplot to visualize the multivariate genetic relationships between the three traits (Cooper & Delacy, 1994). In a biplot, the cosine of the angle between vectors represents the genetic correlation between them in the two-dimensional space (Smith *et al.*, 2001). Therefore, vectors orientated in the same direction have a high correlation. As the angle between vectors increases, the genetic correlation decreases; the closer the angle of two vectors is to 90° the lower the genetic correlation, the closer the angle is to 0° the greater the positive genetic correlation, and the closer the angle is to 180° the greater the negative genetic correlation (Yan & Tinker, 2005).

Results

Effect of thermal treatments on survival and variance partitioning

Thermal environment had a significant effect on larval survival ($F_{2,1129} = 349.4$, $P < 0.001$). *Post hoc* tests confirmed that survival at each temperature was significantly different (Fig. 2). While survival varied among families, survival in the highest temperature was around 66% of that in the lowest temperature. Most of the variance in survival was attributable to dams although this varied among temperatures. Dams explained the most variance at the highest temperatures while sires explained the least variance at the highest temperatures (Table 1).

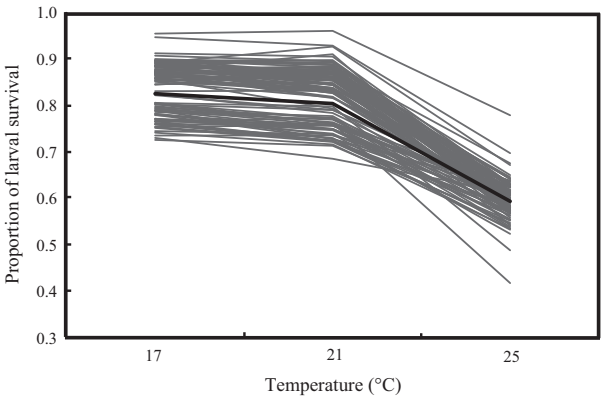

Fig. 2 Additive genetic variation (represented by variation among sires) in the proportions of larval survival after 48 h of exposure to different thermal environments (17, 21, and 25 °C). Each gray line connects a single sire's breeding values (obtained from linear mixed model output; see details in main text), while the black line shows the mean survival across all sires.

Table 1 Variance in developmental success explained by sire, dam, and sire × dam interactions across three different temperatures in the tubeworm *Galeolaria caespitosa*. Significant variance components, based on log-likelihood ratio tests, are shown in bold

Temperature	Component	Variance (%)	χ^2	*P*
All	Sire	**5**	21	<0.001
	Dam	**15**	55	<0.001
	Interaction	5	2	0.157
	Error	75		
17°	Sire	**25**	54	<0.001
	Dam	**12.5**	34	<0.001
	Interaction	**25**	15	<0.001
	Error	37.5		
21°	Sire	**18**	32	<0.001
	Dam	**27**	52	<0.001
	Interaction	9	1	0.317
	Error	45		
25°	Sire	<0.01	<0.01	1
	Dam	**41**	93	<0.001
	Interaction	**27**	24	<0.001
	Error	52		

*Estimating the additive genetic (co)variance matrix, **G***

We detected significant levels of additive genetic variance for larval survival at 17 and 21 °C (Table 2), with the highest amount of additive genetic variance being detected for survival in the 21 °C environment (Table 2). However, additive genetic variance for larval survival at 25 °C was not significantly different from zero (Table 2). The additive genetic covariances between survival at 17 and 21 °C and between survival at 17 and 25 °C were positive and significantly different from zero (Table 2). The covariance between survival at 21 and 25 °C was not significantly different from zero (Table 3).

Table 2 Additive genetic (co)variance matrix (**G**) estimated from the model with the unconstrained sire-level variances and covariances and additive genetic correlations. Additive genetic variances on the diagonal in bold, additive genetic covariances below the diagonal in italics, additive genetic correlations above the diagonal. 17 °C = larval survival at 17 °C; 21 °C = larval survival at 21 °C; 25 °C = larval survival at 25 °C. *$P < 0.05$ for log likelihood ratio test of significant difference from zero

	17 °C	21 °C	25 °C
17 °C	**0.00138***	0.802	1.000
21 °C	*0.00125**	**0.00176***	0.551
25 °C	*0.00202**	*0.00056*	**0.00058**

*Eigenanalysis of **G***

The genetic relationship between larval survival across the three temperatures was investigated by examining the additive genetic (co)variance matrix **G**. An eigenanalysis of **G** was performed to determine how many genetically independent traits (eigenvectors) were represented by the original three traits, and how much genetic variance (eigenvalues) was associated with each eigenvector. The eigenanalysis of **G** revealed that the genetic variance in **G** was distributed across two dimensions, with the third eigenvector (g_3) explaining 0% of the total additive genetic variance; subsequently, g_3 loadings are not shown (Table 3). This means that the **G**-matrix was of reduced rank. Considering the two eigenvectors that explained all of the additive genetic variance across the three traits, g_{max} and g_2 accounted for 78.3% and 21.8% of total additive genetic variance in **G,** respectively. Survival at all three temperatures loaded positively on $g_{\max}$ with relatively equal contributions (Table 3). Survival at 21°C made a large and positive contribution to the second eigenvector, g_2, with small and moderate negative contributions from survival at 17 and 25 °C, respectively.

The first two dimensions of **G**, $g_{\max}$, and g_2, were displayed in a biplot (Fig. 3) to visualize the genetic relationships across the three temperatures. As outlined earlier, the squared length of a vector in the biplot is the variance explained by the two dimensions, while the cosine angle between vectors is the genetic correlation between them in this two-dimensional space (Smith *et al*., 2001). Therefore, vectors orientated in the same direction have a high correlation. As the angle between vectors increases, the genetic correlation decreases. Therefore, larval survival at 17 and 25 °C

Table 3 Eigen analysis of genetic variation for all traits examined. Trait loadings on eigenvectors of the unconstrained sire-level additive genetic variance covariance matrix (**G**), the additive genetic variance, V_A, (eigenvalue) associated with each eigenvector, and the percentage of the total additive genetic ($\%V_A$) variance explained by each eigenvector

Eigenvector	Eigenvalue (V_A)	Percentage of total additive genetic variance ($\%V_A$)	17 °C	21 °C	25 °C
g_{max}	0.004197	78.2	0.5986	0.4935	0.6309
g_2	0.001171	21.8	−0.0427	0.8062	−0.5901

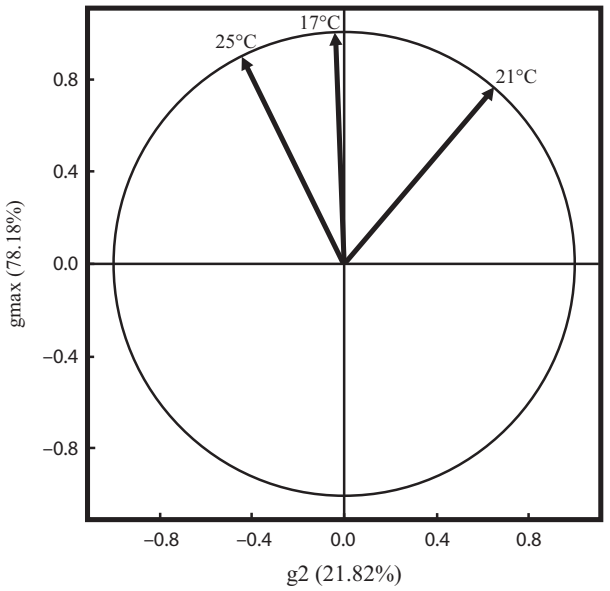

Fig. 3 Biplot visualizing the two statistically supported dimensions (g_{max} and g_2) of additive genetic variation in larval survival across thermal environments (17, 21, and 25 °C). Vectors are based on the loadings of each of the three environments on each multivariate dimension in Table 2, scaled so that the angles between them represent crossenvironment genetic correlations (i.e., angles of 0°, 90°, and 180° between vectors would indicate crossenvironment correlations of 1, 0, and -1, respectively).

and survival at 17 and 21 °C have vectors of similar direction and magnitude, reflecting the high pairwise genetic correlations between them (Table 2). In contrast, the angle between the vectors for survival at 21 and 25 °C reflects the weaker genetic correlation between the two traits (Fig. 3, Table 2).

Discussion

Anthropogenic global change is predicted to create warmer and more variable water temperatures, but the adaptive evolutionary capacity of populations to respond to these changes is poorly understood (Hoffmann & Sgro, 2011; IPCC, 2013). In particular, the extent to which adaptation to a warmer world will be constrained or facilitated by levels of additive genetic variance for, and covariances between, the traits under selection remains largely unknown. Indeed, most studies that have looked at the potential for evolutionary responses to global change have taken a largely univariate or bivariate approach (but see Williams *et al.*, 2012; van Heerwaarden & Sgro, 2013; Blackburn *et al.*, 2014 who looked at multiple traits in a single environment) and have not considered additive genetic variances and covariances across multiple (more than 2) environments in a multivariate framework. Our findings suggest that our study population will have some evolutionary capacity to adapt to increases in the frequency of temperatures of 25 °C. However, this capacity was only revealed by a multivariate analysis of genetic variances and covariances across multiple temperatures (importantly, we can say nothing about the evolutionary capacity of this population for temperatures beyond 25 °C). Yet, a broad understanding of how multivariate genetic interactions among traits and environments will affect the adaptive evolution of global population to global change is presently lacking. Attempts to understand the capacity of populations to adaptively evolve in response to global change must approach the issue as a multivariate problem.

The motivation of our study was to take a multivariate approach to determine the extent to which adaptation to a warmer world might be constrained by additive genetic variances within, and covariances between, different thermal environments. Our focal species was an important habitat-forming species of marine tubeworm, *G. caespitosa*. We first showed significant additive genetic variance for survival in two of the three thermal environments considered; additive genetic variance for larval survival at 17° and 21 °C was significantly different from zero, while it was not different from zero at the warmest temperature of 25 °C. We then showed that the additive genetic (co) variance matrix was of reduced rank; two eigenvectors explained all of the additive genetic variance in *G*, with g_{max} accounting for 78% and g_2 accounting for 22% of the total genetic variance. Importantly, all three traits made equally large contributions to the leading eigenvector of **G** (g_{max}), suggesting that selection for increased larval survival across different thermal envi-

ronments (in the direction of g_{max}) should result in an evolutionary increase in survival across all three thermal environments.

We found that larval survival has the potential to evolve via correlated responses to selection across thermal environments, as revealed by significant and positive additive genetic covariances between survival at 17° and 21 °C and between survival at 17° and 25 °C. These genetic covariances across thermal environments are therefore likely to promote, rather than constrain, adaptive evolution in response to warming temperatures. Given that our study population will likely have to endure selection under each of the three thermal environments considered here in the future, we would expect adaptive evolution for survival at any of the three temperatures to occur at a faster rate than in the absence of genetic covariances across thermal environments. Importantly, the rate of adaptive evolution for survival at 25 °C appears likely to be accelerated by selection on survival at the two cooler temperatures. In essence, evolutionary adaptation in response to exposure to 17° and 21 °C will 'warm up' *Galeolaria*'s genome for adaptive responses to even higher temperatures. Importantly, we only measured one life-history trait (albeit in multiple temperatures). Recent studies suggest that genetic correlations across the life history (Aguirre *et al*., 2014) will further alter the evolutionary trajectories of populations and exploring this possibility is an important next step (Dam, 2013).

Our findings highlight the importance of a multivariate approach to studies of the evolutionary capacity to cope with global change. While the reduced larval survival at 25 °C makes future environmental warming concerning, the presence of significant additive genetic covariance between larval survival at 17° and 25 °C and the contribution of survival at 25 °C to g_{max} indicate that our study population has the evolutionary potential to adapt to future, warmer, conditions. That is, even in the absence of univariate additive genetic variance for survival at 25ºC, adaptation in response to increasing temperatures will still be possible because of the presence of additive genetic variance in multivariate space (survival at 25 °C contributes to g_{max}), and because of the significant genetic covariance across thermal environments. Adaptive evolution in the face of future warming will be important, because even small changes in larval survival can drive large fluctuations in marine population dynamics (Houde, 1987; Johnson, 2007). Were we to have tested the evolutionary potential of this species to cope with 25 °C alone, we would have erroneously concluded that this species lacked all capacity to cope with future temperature increases.

There is increasing recognition of the fact that additive genetic variance (and thus adaptive capacity) may change with environmental conditions (e.g., Sgro & Hoffmann, 1998; Hoffmann & Merilä, 1999; McGuigan & Sgrò, 2009; Husby *et al.,* 2011). We detected higher levels of additive genetic variance under the moderately warm (21 °C) and most benign (17 °C) thermal environments, while no additive genetic variance was detected in the most stressful thermal environment (25 °C), despite a relatively powerful experimental design (204 families). It has been suggested that additive genetic variance could increase in novel, stressful environments because selection will act to increase the frequency of rare alleles unselected in the ancestral environment, thereby causing an increase in the standing genetic variance (Holloway *et al.,* 1990; Falconer & Mackay, 1996; Guntrip *et al.,* 1997; Holloway *et al.,* 1997). Some studies have indeed shown that additive genetic variance increases with environmental stress (Sgro & Hoffmann, 1998; Imasheva *et al*., 1999; Sisodia & Singh, 2009). However, other studies have observed no increase in additive genetic variance under warmer temperatures (e.g., Scheiner & Lyman, 1989; Bubliy *et al*., 2000; Garant *et al.,* 2007), and in some instances, decreases in additive genetic variance or heritability have been found (e.g., Ketola *et al.,* 2012a,b, Bennington & Mcgraw, 1996; Merila, 1997; Rumbaugh *et al*., 1984).

An alternative hypothesis often used to explain the differences in additive genetic variance across environments is that the amount of additive genetic variance present under a specific level of environmental stress reflects the result of historical selection (Hoffmann & Parsons, 1991). Given that selection should remove alleles of low fitness, depleting additive genetic variance, the lowest levels of additive genetic variance should occur in environments that match historical regimes (Hoffmann & Merilä, 1999). However, neither explanation can account for the higher additive genetic variance we observed at both 17 and 21 °C. Our study population has experienced temperatures of both 17 and 21 °C over the previous 14 years, while 25 °C has been experienced only twice (2013 and 2014) in the past 14 years (CSIRO, 2014). A third possibility is that the occasional strong selection imposed by temperatures >21 °C has purged certain alleles, reducing genetic variance at the highest temperatures.

Our study has a number of important limitations. First, we exposed zygotes to higher temperatures whereas in nature, both parents and gametes will first be exposed to these temperatures and so our design was somewhat artificial. Previous studies show that the experience of both parents and gametes will affect their subsequent performance as offspring (Ritchie &

Marshall, 2013; Jensen *et al.*, 2014). We chose to exclude parental and gamete experience from out study here as a necessary first step so that no systematic differences in the phenotype of gametes occurred, thereby obscuring the genetic effects of interest. Further studies that combine parental exposure and quantitative genetics, while logistically challenging, represent an exciting next step. Second, we considered only one environmental stressor (albeit at multiple levels) – temperature. Global change will bring not only changes to temperature but also ocean chemistry (Byrne, 2012), and multistressor studies are an important next step in using this approach. Third, recent studies show that genetic variation in one life-history stage may not reflect variation in others (Aguirre *et al.*, 2014). As such, the genetic correlations we observed during early development may not continue throughout the life history. Nevertheless, given that earlier life-history stages tend to be the most sensitive to climate stressors (Byrne, 2012), we suspect that the genetic variation observed during this phase of key importance for the adaptive capacity of this species.

It should be noted that the capacity to undergo adaptive evolutionary shifts via direct and correlated responses to selection imposed by increasing temperatures alone is insufficient to avoid extinction in response to global change. Populations are likely to be better adapted to previously encountered conditions, so any changes in selection will cause the difference between the mean population phenotype and the optimum phenotype, commonly referred to as the lag load, to increase (Hellmann & Pineda-Krch, 2007; Kopp & Matuszewski, 2014). In simple terms, the ability of a population to overcome the lag load will depend on both the genetics and demography of the population. First, a population must have the evolutionary potential to change; some individuals must carry an allele that conveys high fitness under the changed selection pressures – this is what our quantitative genetic analysis has provided. Second, the allele conferring high fitness under the altered conditions must be able to increase in frequency in the population (Bell, 2013). While the genetic requirement is dependent on the presence of additive genetic variance in the direction of selection, the demographic requirement is dependent on the cost of selection. Thus, even if a population contains the required alleles to survive under future selection pressures, it may still go extinct if the cost of selection causes extinction before the allele can spread throughout the population (Bell, 2013). Our results suggest that larval survival may decline during warmer summer temperatures during the initial stages of evolutionary adaptation. The extent to which this decline will be reversed in subsequent generations remains unknown.

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References

Agrawal AF, Stinchcombe JR (2009) How much do genetic covariances alter the rate of adaptation? *Proceedings of the Royal Society B-Biological Sciences*, **276**, 1183–1191.

Aguirre JD, Blows MW, Marshall DJ (2014) The genetic covariance between life cycle stages separated by metamorphosis. *Proceedings of the Royal Society B-Biological Sciences*, **281**, 1–8.

Arnold SJ (1992) Constraints on phenotypic evolution. *American Naturalist*, **140**, S85–S107.

Bell G (2013) Evolutionary rescue and the limits of adaptation. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **368**, e20120080.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B*, **57**, 289–300.

Bennington CC, Mcgraw JB (1996) Environment-dependence of quantitative genetic parameters in *Impatiens pallida*. *Evolution*, **50**, 1083–1097.

Blackburn S, van Heerwaarden B, Kellermann V, Sgro CM (2014) Evolutionary capacity of upper thermal limits: beyond single trait assessments. *Journal of Experimental Biology*, **217**, 1918–1924.

Blows MW (2007a) Complexity for complexity's sake? *Journal of Evolutionary Biology*, **20**, 39–44.

Blows MW (2007b) A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology*, **20**, 1–8.

Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. *Ecology*, **86**, 1371–1384.

Bubliy OA, Loeschcke V, Imasheva AG (2000) Effect of stressful and nonstressful growth temperatures on variation of sternopleural bristle number in *Drosophila melanogaster*. *Evolution*, **54**, 1444–1449.

Bulleri F, Chapman MG, Underwood AJ (2005) Intertidal assemblages on seawalls and vertical rocky shores in Sydney Harbour, Australia. *Austral Ecology*, **30**, 655–667.

Byrne M (2012) Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Marine Environmental Research*, **76**, 3–15.

Charmantier A, Mccleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**, 800–803.

Clark JS, Poore AGB, Ralph PJ, Doblin MA (2013) Potential for adaptation in response to thermal stress in an intertidal macroalga. *Journal of Phycology*, **49**, 630–639.

Conner JK, Hartl DL (2004) *A Primer of Ecological Genetics*. Sinauer Associates, Sunderland, MA.

Cooper M, Delacy IH (1994) Relationships among analytical methods used to study genotypic variation and genotype-by-environment interaction in plant breeding multi-environment experiments. *Theoretical and Applied Genetics*, **88**, 561–572.

Crean AJ, Bonduriansky R (2014) What is a paternal effect? *Trends in Ecology & Evolution*, **29**, 554–559.

CSIRO (2014) CSIRO Marine and Atmopsheric Research. Available at: http://www.cmar.csiro.au (accessed 28 July 2014).

Dam HG (2013) Evolutionary adaptation of marine zooplankton to global change. *Annual Review of Marine Science*, **5**, 349–370.

Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. *Ecology*, **86**, 1704–1714.

Edgar GJ (2000) *Australian Marine Life*. Reed New Holland, Sydney, NSW.

Etterson JR (2004) Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the great plains. *Evolution*, **58**, 1459–1471.

Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to global warming. *Science*, **294**, 151–154.

Falconer DS (1952) The problem of environment and selection. *American Naturalist*, **86**, 293–298.

Falconer DS, Mackay FC (1996) *Introduction to Quantitative Genetics*, 4th edn. Longmans Green, Harlow, Essex, UK.

Foo SA, Dworjanyn SA, Poore AGB, Byrne M (2012) Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. *PLoS ONE*, **7**, e42497.

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1278–1282.

Fry JD (1993) The "general vigor" problem: can antagonistic pleiotropy be detected when genetic covariances are positive? *Evolution*, **47**, 327–333.

Galletly BC, Blows MW, Marshall DJ (2007) Genetic mechanisms of pollution resistance in a marine invertebrate. *Ecological Applications*, **17**, 2290–2297.

Garant D, Hadfield JD, Kruuk LEB, Sheldon BC (2007) Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population. *Molecular Ecology*, **17**, 179–188.

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merila J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology*, **17**, 167–178.

Guntrip J, Sibly RM, Holloway GJ (1997) The effect of novel environment and sex on the additive genetic variation and covariation in and between emergence body weight and development period in the cowpea weevil, *Callosobruchus maculatus* (*Coleoptera*, *Bruchidae*). *Heredity*, **78**, 158–165.

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993–1009.

Haldane JBS (1957) The cost of natural selection. *Journal of Genetics*, **55**, 511–524.

van Heerwaarden B, Sgro CM (2013) Multivariate analysis of adaptive capacity for upper thermal limits in *Drosophila simulans*. *Journal of Evolutionary Biology*, **26**, 800–809.

Hellmann JJ, Pineda-Krch M (2007) Constraints and reinforcement on adaptation under climate change: selection of genetically correlated traits. *Biological Conservation*, **137**, 599–609.

Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution*, **14**, 96–101.

Hoffmann AA, Parsons PA (1991) *Evolutionary Genetics and Environmental Stress*. Oxford University Press, New York.

Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.

Hoffmann AA, Hallas RJ, Dean JA, Schiffer M (2003a) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science*, **301**, 100–102.

Holloway GJ, Povey SR, Sibly RM (1990) The effect of new environment on adapted genetic architecture. *Heredity*, **64**, 323–330.

Holloway GJ, Crocker HJ, Callaghan A (1997) The effects of novel and stressful environments on trait distribution. *Functional Ecology*, **11**, 579–584.

Holt RD (1990) The microevolutionary consequences of climate change. *Trends in Ecology & Evolution*, **5**, 311–315.

Houde ED (1987) Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*, **2**, 17–29.

Houle D (1991) Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution*, **45**, 630–648.

Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science*, **287**, 308–309.

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution*, **15**, 56–61.

Husby A, Visser ME, Kruuk LEB (2011) Speeding up microevolution: the effects of increasing temperature on selection and genetic variance in a wild bird population. *Plos Biology*, **9**, 1–9.

Imasheva AG, Bosenko DV, Bubli OA (1999) Variation in morphological traits of *Drosophila melanogaster* (fruit fly) under nutritional stress. *Heredity*, **82**, 187–192.

IPCC (2013) Working Group I Contribution to the IPCC Fifth Assessment Report Climate Change 2013: The Physical Science Basis Summary for Policymakers. Available at: http://www.ipcc.ch (accessed 20 June 2014).

Jackson GA, Strathmann RR (1981) Larval mortality from offshore mixing as a link between pre-competent and competent periods of development. *American Naturalist*, **118**, 16–26.

Jensen N, Allen RM, Marshall DJ (2014) Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. *Functional Ecology*, **28**, 724–733.

Johnson DW (2007) Habitat complexity modifies post-settlement mortality and recruitment dynamics of a marine fish. *Ecology*, **88**, 1716–1725.

Kellermann VM, Van Heerwaarden B, Hoffmann AA, Sgro CM (2006) Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. *Evolution*, **60**, 1104–1108.

Ketola T, Kristensen T, Kellermann V, Loeschcke V (2012a) Can evolution of sexual dimorphism be triggered by developmental temperatures? *Journal of Evolutionary Biology*, **25**, 847–855.

Ketola T, Kellermann V, Kristensen TN, Loeschcke V (2012b) Constant, cycling, hot and cold thermal environments: strong effects on mean viability but not on genetic estimates. *Journal of Evolutionary Biology*, **25**, 1209–1215.

Kopp M, Matuszewski S (2014) Rapid evolution of quantitative traits: theoretical perspectives. *Evolutionary Applications*, **7**, 169–191.

Kupriyanova EK, Havenhand JN (2005) Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete, *Galeolaria caespitosa* (Annelida: Serpulidae). *Invertebrate Reproduction & Development*, **48**, 7–17.

Lande R (1979) Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution*, **33**, 402–416.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.

Levitan DR, Sewell MA, Chia FS (1991) Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interaction of gamete dilution, age, and contact time. *Biological Bulletin*, **181**, 371–378.

Littell RC, Milliken GA, Stroup WW, Wolfinge RD (1996) *SAS System for Mixed Models*, 2nd edn. SAS Institute Inc., Cary, NC.

Littell RC, Milliken GA, Stroup WW, Wolfinge RD (2006) *SAS System for Mixed Models*, 2nd edn. SAS Institute Inc., Cary, NC.

Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. *Nature Geoscience*, **5**, 346–351.

Lynch M, Walsh B (1998) *Genetics and the Analysis of Quantitative Traits*. Sunderland Sinauer, Sunderland, MA.

Marshall DJ (2015) Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. *Journal of Experimental Biology*, **218**, 107–113.

Marshall DJ, Evans JP (2005a) The benefits of polyandry in the free-spawning polychaete *Galeolaria caespitosa*. *Journal of Evolutionary Biology*, **18**, 735–741.

Marshall DJ, Evans JP (2005b) Does egg competition occur in marine broadcast-spawners? *Journal of Evolutionary Biology*, **18**, 1244–1252.

Marshall DJ, Morgan SG (2011) Ecological and evolutionary consequences of linked life-history stages in the sea. *Current Biology*, **21**, R718–R725.

McGuigan K, Sgrò CM (2009) Evolutionary consequences of cryptic genetic variation. *Trends in Ecology & Evolution*, **24**, 305–311.

McGuigan K, Nishimura N, Currey M, Hurwit D, Cresko WA (2011) Cryptic genetic variation and body size evolution in threespine stickleback. *Evolution*, **65**, 1203–1211.

Merila J (1997) Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution*, **51**, 526–536.

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013) Predicting evolutionary responses to climate change in the sea. *Ecology Letters*, **16**, 1488–1500.

Pease CM, Bull JJ (1988) A critique of methods for measuring life history trade-offs. *Journal of Evolutionary Biology*, **1**, 293–303.

Phillips PC, Arnold SJ (1989) Visualizing multivariate selection. *Evolution*, **43**, 1209–1222.

Poissant J, Wilson AJ, Coltman DW (2010) Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution*, **64**, 97–107.

Przybylo R, Sheldon BC, Merila J (2000) Climatic effects on breeding and morphology: evidence for phenotypic plasticity. *Journal of Animal Ecology*, **69**, 395–403.

Réale D, Mcadam AG, Boutin S, Berteaux D (2003) Genetic and plastic responses of a northern mammal to climate change. Proceedings: biological. *Sciences*, **270**, 591–596.

Ritchie H, Marshall DJ (2013) Fertilisation is not a new beginning: sperm environment affects offspring developmental success. *Journal of Experimental Biology*, **216**, 3104–3109.

Roff DA, Fairbairn DJ (2007) The evolution of trade-offs: where are we? *Journal of Evolutionary Biology*, **20**, 433–447.

Ross KE, Bidwell JR (2001) A 48-h larval development toxicity test using the marine polychaete *Galeolaria caespitosa lamarck* (fam. serpulidae). *Archives of Environmental Contamination and Toxicology*, **40**, 489–496.

Rumbaugh MD, Asay KH, Johnson DA (1984) Influence of drought stress on genetic variances of alfalfa and wheatgrass seedlings. *Crop Science*, **24**, 297–303.

Schar C, Vidale PL, Luthi D, Frei C, Haberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. *Nature*, **427**, 332–336.

Scheiner SM, Lyman RF (1989) The genetics of phenotypic plasticity.1. Heritability. *Journal of Evolutionary Biology*, **2**, 95–107.

Schluter D (1996) Adaptive radiation along genetic lines of least resistance. *Evolution*, **50**, 1766–1774.

Sgro CM, Hoffmann AA (1998) Effects of stress combinations on the expression of additive genetic variation for fecundity in *Drosophila melanogaster*. *Genetical Research*, **72**, 13–18.

Sgro CM, Hoffmann AA (2004) Genetic correlations, tradeoffs and environmental variation. *Heredity*, **93**, 241–248.

Simonsen AK, Stinchcombe JR (2010) Quantifying evolutionary genetic constraints in the Ivyleaf Morning Glory, *Ipomoea Hederacea*. *International Journal of Plant Sciences*, **9**, 972–982.

Sisodia S, Singh BN (2009) Variations in morphological and life-history traits under extreme temperatures in *Drosophila ananassae*. *Journal of Biosciences*, **34**, 263–274.

Smith A, Cullis B, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. *Biometrics*, **57**, 1138–1147.

Styan CA (1998) Polyspermy, egg size, and the fertilization kinetics of free-spawning marine invertebrates. *American Naturalist*, **152**, 290–297.

Thomas CD, Cameron A, Green RE *et al.* (2004) Extinction risk from climate change. *Nature*, **427**, 145–148.

Walsh B, Blows MW (2009) Abundant genetic variation plus strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology Evolution and Systematics*, **40**, 41–59.

Walther GR, Post E, Convey P *et al.* (2002) Ecological responses to recent climate change. *Nature*, **416**, 389–395.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA, Langham G (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. *Plos Biology*, **6**, 2621–2626.

Williams BR, van Heerwaarden B, Dowling DK, Sgrò CM (2012) A multivariate test of evolutionary constraints for thermal tolerance in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, **25**, 1415–1426.

Yan WK, Tinker NA (2005) An integrated biplot analysis system for displaying, interpreting, and exploring genotype x environment interaction. *Crop Science*, **45**, 1004–1016.