Genetic variation underlies temperature tolerance of embryos in the sea urchin *Heliocidaris erythrogramma armigera*

ROWAN A. LYMBERY & JONATHAN P. EVANS

Centre for Evolutionary Biology, School of Animal Biology, University of Western Australia, Crawley 6009, WA, Australia

Running head: Genetic variation in sea urchin embryo viability

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Abstract

Ocean warming can alter natural selection on marine systems and in many cases the long-term persistence of affected populations will depend on genetic adaptation. In this study we assess the potential for adaptation in the sea urchin *Heliocidaris erythrogramma armigera*, an Australian endemic that is experiencing unprecedented increases in ocean temperatures. We used a factorial breeding design to assess the level of heritable variation in larval hatching success at two temperatures. Fertilized eggs from each full-sibling family were tested at 22°C (current spawning temperature) and 25°C (upper limit of predicted warming this century). Hatching success was significantly lower at higher temperatures, confirming that ocean warming is likely to exert selection on this life-history stage. Our analyses revealed significant additive genetic variance and genotype-by-environment interactions underlying hatching success. Consistent with prior work, we detected significant non-additive (sire-by-dam) variance in hatching success, but additionally found that these interactions were modified by temperature. Although these findings suggest the potential for genetic adaptation, any evolutionary responses are likely to be influenced (and possibly constrained) by complex genotype-by-environment and sire-by-dam interactions and will additionally depend on patterns of genetic covariation with other fitness traits.

Keywords**:** broadcast spawning, climate change, genetic variation, genetic compatibility, phenotypic plasticity, evolvability.

Introduction

The Earth's climate is changing at an unprecedented and accelerating rate, and there is a clear scientific consensus that this change has been induced by anthropogenic greenhouse gas emissions (Oreskes, 2004). The biological impacts of these changes on natural populations have been recorded worldwide across most major taxonomic groups (Parmesan, 2006; Rosenzweig *et al.*, 2008). In light of such widespread and continuing impacts of climate change, a major challenge facing evolutionary biologists is to determine the prospects for long-term persistence of natural populations. Broadly, there are three ways in which populations can respond to new selection pressures: distribution shifts, phenotypic plasticity, and genetic adaptation (Gienapp *et al.*, 2008; Hoffmann & Sgrò, 2011; Hansen *et al.*, 2012). Distribution shifts can allow species to track favourable climatic conditions through space. For example, pole-ward range shifts have been observed in several species over the past hundred years, particularly in birds and butterflies (Parmesan *et al.*, 1999; Thomas & Lennon, 1999), but also in a number of marine groups such as fish and plankton (Perry *et al.*, 2005; Parmesan, 2006). Many species, however, are likely to have restricted abilities to shift their distributions because of intrinsic or extrinsic constraints to dispersal (Hansen *et al.*, 2012). Some sessile species, for example, may have intrinsically limited long-distance dispersal, while those that are capable of moving long distances may be limited by physical barriers or habitat fragmentation (Pimm & Raven, 2000; Kinlan & Gaines, 2003; Hansen *et al.*, 2012). Because of such constraints, many species are unable to shift their distributions in response to changing climatic conditions.

Phenotypic plasticity, or the ability of the same genotype to produce different phenotypes in different environments, is a further way in which organisms can persist in

changing environmental conditions (Scheiner, 1993; Via *et al.*, 1995; Pigliucci, 2001). In many of the observed responses to climate change, such as the widely reported shifts in bird breeding times in response to warmer winters (e.g. Crick *et al.*, 1997; Brown *et al.*, 1999; Koike & Higuchi, 2002; Møller *et al.*, 2006), there is increasing evidence that the changes can be accounted for by plasticity (Przybylo *et al.*, 2000; Gienapp *et al.*, 2008). The fact that phenotypic plasticity is neither infinite nor ubiquitous in nature, however, suggests that there may be costs and limits to plasticity (DeWitt *et al.*, 1998). Costs of plasticity would result in reduced fitness of individuals with plastic genotypes even when the optimum phenotype is expressed, whereas limits would prevent the expression of the optimum phenotype (DeWitt *et al.*, 1998; Pigliucci, 2005). Although the evidence for such costs and limits is currently equivocal (Relyea, 2002; Auld *et al.*, 2010; Snell-Rood *et al.*, 2010), it is clear that genotypes cannot produce the optimum phenotype in all possible environments (DeWitt *et al.*, 1998; Gienapp *et al.*, 2008). This means that under continued directional environmental change, such as that expected under climate change, the limits of genotypes to produce the optimum phenotype through plasticity will probably be exceeded, unless the plastic response itself can evolve (Gienapp *et al.*, 2008; Visser, 2008). For many populations, therefore, long-term persistence under climate change will require genetic adaptation.

While some studies have found evidence for adaptive genetic changes in natural populations in response to climate change (e.g. Bradshaw & Holzapfel, 2001; Balanya *et al.*, 2006), such evidence is rare and limited to a few taxonomic groups (Gienapp *et al.*, 2008; Merilä, 2012). Instead, many studies have used quantitative genetic approaches to determine whether natural populations exhibit sufficient standing additive genetic variance (V_A) to permit evolutionary responses to selection (Blows & Hoffmann, 2005). Among studies

that have applied such approaches to natural populations impacted by climate change, several have revealed very low levels of V_A in a range of key fitness traits. For example, two recent studies on frogs revealed statistically non-significant levels of V_A for a number of larval traits when tested at different levels of desiccation stress (Laurila *et al.*, 2002; Eads *et al.*, 2012). Similarly, selection experiments on rainforest-specialist species of *Drosophila* have revealed limited additive genetic variance underlying desiccation tolerance and cold resistance (Hoffmann *et al.*, 2003; Kellermann *et al.*, 2006; Kellermann *et al.*, 2009), while Kelly *et al.* (2012) found no response to selection for increased thermal tolerance in the marine isopod *Tigriopus californicus*. Although these examples suggest that low levels of heritable variation may limit the potential of some natural populations to adapt to climate change, studies on a wider range of taxa are required before drawing any general conclusions.

Heliocidaris erythrogramma is a common sea urchin in southern Australian waters that plays a key ecological role in shallow sub-tidal reef communities (Keesing, 2001; Vanderklift *et al.*, 2006; Ling *et al.*, 2010). This species is likely to be impacted by rising sea surface temperatures (SSTs), which around Australia have increased by 0.7°C in the last 100 years and are predicted to increase by a further 3°C by the end of this century (Lough, 2009). For example, recent experimental work revealed that temperature increases of between 4 and 6°C sharply impacted embryonic and larval stages of *H. erythrogramma*, including gastrulation and embryonic development (Byrne *et al.*, 2009). Unlike its congeners and most other sea urchins (Ebert, 1982), *H. erythrogramma* exhibits a highly reduced larval stage in which embryos spend just 3-5 days in the water column before undergoing metamorphosis and settlement (Williams & Anderson, 1975). The ability for long-range dispersal is therefore

limited in this species (Binks *et al.*, 2011). *Heliocidaris erythrogramma armigera*, the dominant subspecies of *H. erythrogramma* in Western Australia, is likely to be especially impacted by ocean warming given the recent and unprecedented marine heat wave in 2011 in which SSTs rose 2-4°C above average for more than ten weeks (Pearce *et al.*, 2011; Wernberg *et al.*, 2012).

In the present study we apply an experimental quantitative genetic design to reveal sources of genetic and environmental variance underlying the ability of embryos to tolerate increased temperatures within the range predicted by the year 2100. As an external fertilizer, *H. e. armigera* offers a highly tractable model for applying the North Carolina II (NCII) breeding design (Comstock and Robinson 1948; Lynch and Walsh 1998; see also Evans and Marshall 2005; Evans et al. 2007), which crosses parental males (sires) and females (dams) in all combinations, allowing sources of genetic variation to be partitioned among sires, dams and their interacting effects. Our primary focus was to determine whether there is significant additive genetic variance underlying temperature tolerance by developing embryos. Mortality rates are very high in pre-settlement life history stages of broadcast spawning marine invertebrates, as these stages are most vulnerable to physical and biological stresses (Gosselin & Qian, 1997). Indeed, early juvenile traits in these species are considered to be more evolutionarily responsive to environmental changes than traits in later life-history stages (Gosselin & Qian, 1997). Additive genetic variance in embryonic temperature tolerance is therefore likely to be a key prerequisite for genetic adaptation to ocean warming in *H. e. armigera*. We therefore reared first generation full- and half-sibling offspring from the NCII design at two temperature treatments to assess hatching success under current and near-future predicted temperature levels, thus revealing patterns of

additive and non-additive genetic variance, and genotype-by-environment interactions (GEIs) underlying juvenile survival.

Materials and Methods

Study species and sampling

Heliocidaris erythrogramma (Valenciennes 1846) consists of two morphologically and genetically distinct subspecies (Binks *et al.*, 2011). There is a broad-scale geographic separation of these subspecies, with *Heliocidaris erythrogramma armigera* occurring on the west coast of Australia and *Heliocidaris erythrogramma erythrogramma* confined mostly to the east coast, although *H. e. erythrogramma* is also found in low abundance on the west coast (Binks *et al.*, 2011). *Heliocidaris e. armigera* spawns from March to June, after SSTs have dropped to 22°C (Binks *et al.*, 2011). Both subspecies are broadcast spawners, meaning that eggs and sperm are shed into the water column where fertilization occurs. Uniquely among echinoids, larval development in both subspecies is lecithotrophic, with larvae surviving on egg yolk rather than actively feeding. As a result, the larvae spend a relatively short time in the water column, with embryonic development and hatching from the fertilization membrane complete by two days post-fertilization (Williams & Anderson, 1975; McMillan *et al.*, 1992). Metamorphosis into the adult form and settlement typically occur from 3-5 days after fertilization (Williams & Anderson, 1975; McMillan *et al.*, 1992).

Adults of *H. e. armigera* of reproductive age were collected from South Mole Jetty in Fremantle (32°03.355'S, 115°44.075'E), Western Australia during April and May 2012. Urchins were held in aerated aquaria of recirculating seawater at the University of Western Australia until required (within 1-2 weeks of collection).

Experimental design

A cross-classified NCII block breeding design was used to cross sperm and eggs from males and females in all combinations within each block of the design. In each block, sperm from three sires were crossed with eggs from three dams in all nine combinations using *in vitro* fertilization, with two replicate crosses performed for each male-female pair (i.e. 18 crosses in total per block; Fig. 1). Six blocks were established, comprising a total of 18 sires and 18 dams, thus yielding 108 crosses from 54 unique combinations of sires and dams. This design yields full-sib, paternal half-sib and maternal half-sib offspring, thus making it possible to partition sources of genetic variation into additive effects, maternal effects and non-additive effects (Comstock & Robinson, 1948; Lynch & Walsh, 1998). The sire variance, equivalent to the covariance among paternal-half sibs, was used to estimate additive genetic variance (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Dam variance (equivalent to the covariance among maternal half-sibs), on the other hand, includes additive genetic effects plus other maternal effects such as egg provisioning or inheritance of organelle genomes (Mousseau & Fox, 1998). The sire×dam interaction variance was used to estimate the importance of non-additive genetic effects that arise from interactions among alleles at the same locus (dominance effects) or at different loci (epistatic effects) (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

We randomly assigned offspring from each full-sibling family to one of two temperature treatments, thus enabling us to also evaluate genetic variation in plasticity (Scheiner & Goodnight, 1984; Scheiner, 1993). The treatment effect (temperature) indicates whether there is a plastic response of hatching success to different temperatures. The interaction

effect of sire×treatment indicates whether the phenotypic effect of temperature varies among sire groups (i.e. genotypes). Under the assumption that sires contribute no common environmental effects, which is likely to be satisfied in externally fertilizing broadcast spawners with no parental care, the sire-by-treatment interaction can be used to determine whether there is significant additive genetic variation in plasticity (i.e. GEIs). The interaction effect of dam×treatment indicates whether the phenotypic effect of temperature varies among dam groups due to maternal additive genetic and/or environmental effects. The interaction of sire×dam×treatment indicates whether the phenotypic effect of temperature varies among particular sire×dam combinations, i.e. due to non-additive genetic effects (Nystrand *et al.*, 2011; Eads *et al.*, 2012). Put differently, a significant sire×dam×treatment effect would indicate that temperature modifies the way maternal and paternal haplotypes combine to produce genetically compatible offspring.

***In vitro* fertilization**

Adults of both sexes were induced to spawn using 5 mL injections of 3% KCl into the coelomic cavity (Evans *et al.*, 2007). For each of the six blocks, gametes were collected from three males and three females and adjusted to the appropriate concentrations for fertilizations. Sperm concentrations were measured using an improved Neubauer haemocytometer (Hirschmann Laborgeräte, Eberstadt, Germany), and egg concentrations measured by counting the number of eggs in 0.5 mL of solution under a dissecting microscope. Previous studies that have partitioned variance in fertilization rates of *H. e. armigera* found that sperm concentrations of 7.0×10^5 sperm/mL and egg concentrations of 50 eggs/mL yielded fertilization rates within the natural range (Evans & Marshall, 2005;

Evans *et al.*, 2007). However, our preliminary trials yielded relatively low fertilization rates at these concentrations, making it difficult to collect sufficient numbers of fertilized eggs for the assessment of survival to hatching (see below). Sperm and egg concentrations were therefore adjusted to 1.4×10^6 sperm/mL and 100 eggs/mL, respectively, ensuring that there were sufficient fertilized eggs for our needs.

Crosses were performed for each block in the layout depicted in Fig. 1, using 10 mL of sperm solution and 10 mL of egg solution for each cross. The resultant sperm-egg solutions were left for 2 h (with aeration) to allow sufficient time for all fertilized eggs to divide into the two cell stage (Williams & Anderson, 1975). The samples were then gently mixed to homogenize fertilized and unfertilized eggs, and fertilization rates were estimated by counting the first 50 eggs encountered in each sample and scoring them as fertilized (1) or unfertilized (0). Eggs were only scored as fertilized if regular cell division had occurred (Marshall *et al.*, 2000). All fertilization trials took place in an air-conditioned laboratory set at 22°C.

Hatching success assays

Prior to applying the treatments, we ensured that only fertilized eggs with clear cell division were collected to avoid confounding differences in fertilization success among groups with differences in hatching success (Evans & Marshall, 2005; Evans *et al.*, 2007). Sixty fertilized eggs were collected from each cross and 30 assigned at random to one of two temperature treatments: ambient (22°C) and elevated (25°C), hereafter referred to as T1 and T2, respectively (see Fig. 1). The T1 level was chosen as the approximate SST after which *H. e. armigera* begins to spawn in the field (Binks, 2011), and matched the SST at the sites from

which these samples were taken. The T2 treatment was set at 3°C above T1, which reflects the upper extremes of predicted warming in southwestern Australian waters by the end of the 21st century (Lough, 2009). For two crosses, 60 fertilized eggs could not be found. In the first, 54 fertilized eggs were collected and 27 allocated to each treatment, while in the second, 58 were collected, with 30 allocated to T1 and 28 to T2. Each batch of embryos was placed in an aerated vial with 30 mL of seawater submerged in a temperature-controlled water bath set at the appropriate temperatures. As there were two replicate crosses per full-sib family, there were also two replicate water baths per temperature. The water baths were kept in a controlled temperature room, with the ambient air temperature set at 18°C and water baths heated to the required temperatures. The water baths were aligned in a row with high and low temperature baths interspersed (low, high, low, high) to avoid confounding any spatial effects (i.e. position of tanks on shelves) with treatment effects. Temperatures in each water bath were measured using a Radio Spares 2063738 thermocouple reader calibrated at 2°C intervals over a range of 20–30°C. Deviations from the set temperatures did not exceed 0.5°C for any water bath throughout the experiment.

Hatching success, defined as emergence of free-swimming larvae from egg membranes, was assayed two days (40-48 h) after fertilizations took place, when all survivors had hatched but prior to the beginning of metamorphosis and juvenile settlement (Williams & Anderson, 1975). Free-swimming gastrula were easily identifiable at this stage by movement, their elongated shape (often with one or more constrictions) and red pigmentation (Williams & Anderson, 1975). Embryos were scored as successful (1) if they had emerged from egg membranes and as unsuccessful (0) if they had died prior to reaching this stage.

Data analyses

Both fertilization and hatching success data were analysed as binomial response traits using generalized linear mixed-effects models (GLMMs) with logit link functions in the lme4 package of R version 2.14.1 (R Development Core Team, 2011). These models were fit and the effects parameters estimated by maximum likelihood using the Laplace approximation of the log-likelihood (Raudenbush *et al.*, 2000). As fertilizations were performed at a uniform temperature, the model for this trait included only the random effects sire, dam, and block, and the sire-by-dam interaction term. The model for hatching success included the same random effects but also included the fixed effect of treatment, and the interactions sire×treatment, dam×treatment and sire×dam×treatment. All interactions between fixed and random effects were treated as random. The significance of the fixed treatment effect was evaluated using a Wald Z test, which calculates a test statistic as the fixed effect parameter estimate divided by its asymptotic standard error, and compares the test statistic to a standard Z distribution (Agresti, 2002). The significance of random effects was evaluated using log-likelihood ratio tests (Lynch & Walsh, 1998). These tests exclude each random effect in turn, and compare the fit of the full model to the models without the random factors (Quinn & Keough, 2002). These methods of model fitting and hypothesis testing are considered appropriate for data with up to three main random effects (Bolker *et al.*, 2009), as in our study.

Estimates of additive genetic variance (V_A) and total phenotypic variance (V_P) were calculated for each temperature treatment. Additive genetic variance was calculated as four times the sire variance component from the mixed-effects models. Estimates for V_P were

calculated by summing the variance components of random effects derived from the GLMM model and adding this value to an estimate of residual variance calculated according to Nakagawa & Schielzeth (2010) (residual variance = $\omega^*[\pi 2/3]$, where ω = multiplicative dispersion parameter for models with binomial error structures).

Results

Fertilization rates

Fertilization rates of crosses ranged between 4% and 95%, with a mean (± standard error) of 44.6 ± 2.5. Sire and dam effects both had a significant influence on fertilization, as did the sire×dam interaction, indicating that fertilization rates varied significantly among the specific combinations of parents (Table 1). Fertilization rates did not differ significantly among blocks (Table 1).

Hatching success

The percentage of embryos that survived to hatch and reach the free-swimming larval stage ranged from 0% to 100%, with a mean of 41.9 ± 3.7 SE per cross at 22°C and 34.1 ± 3.5 SE per cross at 25°C. There was a significant decrease in hatching success at the higher temperature treatment (Wald $Z = -3.51$, $p < 0.001$; see Fig. 2). The effect of sire on hatching success was also significant, as were the interactions of sire×dam, sire×treatment, and sire×dam×treatment (Table 2). There was no significant effect of block, dam, or dam×treatment (Table 2).

The sire×treatment interaction indicates that the effect of temperature varied among sire (paternal half-sib) families, shown by the different slopes of the reaction norms for the

proportion of successful hatchings for sire families at each temperature (Fig. 2a). The temperature effect averaged across families was evident by a decrease in hatching success in T2, but for some families temperature had no effect (slopes of reaction norms were zero), and a few had increased success at T2 (slopes of reaction norms were positive). Crossing of reaction norms resulted in a change in phenotypic rank of families from T1 to T2 (Fig. 2a). There was variation among sire families at both temperatures and at the midpoints (averages) of the reaction norms, representing the overall sire effect. Estimates of V_A and V_P at each temperature are reported in Table 3.

The sire×dam×treatment interaction indicates that the temperature effect varied among specific parental combinations. The reaction norms for full-sib families, similar to those for paternal half-sib families, had different slopes, with crossing of reaction norms and a change of phenotypic rank from T1 to T2 (Fig. 2b). While most families had decreased hatching success in T2 (slopes of reaction norms were negative), there were also many that were relatively unaffected (slopes of zero) or had increased success at the high temperature (positive slopes). There was variation among full-sib families at both temperatures and at the midpoints, reflecting the significant overall effect of sire×dam combinations.

Four sire groups had less than 5% of offspring hatch successfully, which could have had a disproportionate effect on the results. The model analysis was therefore re-run following the removal of these sire groups from the data. The sire effect became marginally non-significant ($G^2_1 = 3.45$, $P = 0.063$), but none of the other conclusions changed, with a significant temperature effect (Wald Z = -3.10, $P = 0.002$) and significant sire×dam, sire×treatment, and sire×dam×treatment interactions (Table 4).

Discussion

Climate change represents a major threat to largely sedentary marine organisms such as sea urchins, and this study is one of only a few to address the potential for populations to adapt genetically to increasing sea temperatures (e.g. see Meyer *et al.*, 2009; Foo *et al.*, 2012; Pespeni *et al*., 2013). Our analyses generated four main findings. First, we found that hatching success in *H. e. armigera* is adversely affected by increasing temperatures, thus supporting our prediction that rising sea temperatures will generate an important source of selection on this species. Second, our analyses revealed substantial additive genetic variance and GEIs underlying hatching success in different thermal environments. Third, we found significant non-additive genetic variance for hatching success, confirming previous findings that male-by-female interactions (genetic compatibility) constitute an important source of variation in larval fitness in this species (Evans *et al.*, 2007). Interestingly, these interacting effects of males and females at hatching were modified by temperature, thus providing additional evidence for the instability of genetic compatibility effects across environments (see Nystrand *et al.*, 2011; Eads *et al.*, 2012). Fourth, we found significant effects of males and females on fertilization rates, and a significant male-by-female interaction at fertilization (the latter effect was previously detected for the eastern congener but not *H. e. armigera*; see Evans and Marshall 2005; Evans *et al.* 2007). We discuss each of these findings in turn and infer their implications for the likely response of *H. e. armigera* to sustained increases in temperature.

The effect of temperature on hatching success

Increased temperature had a significant deleterious effect on hatching success in *H. e. armigera*. The high temperature treatment of 25°C, at 3°C above the average spawning season temperature for *H. e. armigera*, matches the predicted increase of SSTs over the species' distribution by the end of this century (Lough, 2009). Temperatures within this range are already being reached in extreme events such as the 2011 marine heat wave experienced by *H. e. armigera*, with such events predicted to increase in frequency under climate change (Solomon *et al.*, 2007; Wernberg *et al.*, 2012). The detrimental effect of increased temperature on larval survival reported in this study is consistent with previous work on the eastern subspecies (*Heliocidaris e. erythrogramma*), which showed a marked decline in the success of gastrulation at 4-6°C above ambient SSTs (Byrne *et al.*, 2009). Predicted levels of ocean warming may therefore exert selection through embryo mortality in both subspecies of *H. erythrogramma*.

Distribution shifts that track favourable climatic conditions are likely to be an important ecological response to climate change for some species and, as we note in the introduction, pole-ward range shifts have been recorded in a range of taxa (Parmesan, 2006). For example, the eastern Australian sea urchin *Centrostephanus rodgersii* has recently increased its range south, facilitated by southern warming and strengthening of the southward-flowing East Australian Current (EAC) (Ling *et al.*, 2009). The higher thermal tolerance of northern compared to southern populations of the east Australian subspecies of *H. erythrogramma* also suggests the possibility that thermally tolerant propagules may move south with warming oceans (Byrne *et al.*, 2011). A potential limitation to long-distance dispersal for this species, however, is its short larval dispersive stage of only 3-5 days, compared to around 100 days in *C. rodgersii* (Williams & Anderson, 1975; Huggett *et al.*, 2005; Binks *et al.*, 2011).

Additionally, *Heliocidaris erythrogramma* is already distributed along the southern Australian coast (Keesing, 2001; Byrne *et al.*, 2011), and, in Western Australia, the southward-flowing Leeuwin Current is weakening under climate change (Feng *et al.*, 2009). Together, these factors suggest that rather than a range shift, *H. e. armigera* may undergo a contraction of the northern margins of its range, unless it can adapt genetically to climate change.

Additive genetic variance in hatching success

We detected significant levels of additive genetic variance and sire-by-environment interaction (GEI) underlying hatching success. Interestingly, the strength of the sire effect far exceeded the dam effect in the model, which raises the question of whether non-genetic sources of variance contributed to the sire component, as found by Hallsson and Björklund (2012) for life history traits in the seed beetle *Callosobruchus maculatus*. However, the reproductive biology of broadcast spawners, coupled with our carefully controlled experimental design, should have minimized non-genetic sire contributions to offspring fitness (the male contribution to reproduction is limited to sperm). Furthermore, our design ensured that only fertilized eggs were used to assay offspring fitness, thus minimizing the possibility that pre-zygotic (possibly non-genetic) mechanisms of incompatibility avoidance influenced subsequent measures of survival. Nevertheless, it is possible that the inflated V_A estimates, which exceeded the theoretical maximum set by total phenotypic variance (see Table 3 and accompanying footnote), may have arisen due to a violation of the assumption of autosomal inheritance that underlie our estimates of additive genetic variance in larval fitness (Lynch & Walsh 1998). Unfortunately, the NCII breeding design is not sufficient for partitioning the sex-linked component from other sources of variance, and thus the extent

to which our estimates of V_A may have been inflated by sex-linkage and other sources of genetic (or indeed environmental) variance has yet to be determined.

We also detected significant sire×treatment interaction underlying larval survival in *H. e. armigera*, suggesting that there is additive genetic variance in plasticity for hatching success (GEI). A common form of GEI is a reduction in additive genetic variance across environments, which results when reaction norms showing variation in phenotypic responses of genotypes to one environment converge on the same response in another environment (Conner & Hartl, 2004). Reviews of quantitative genetic studies have found both an increase and a decrease in heritabilities under more 'stressful' conditions (Hoffmann & Merilä, 1999; Charmantier & Garant, 2005). In this study, however, the variation among sire groups (genotypes) was not obviously less at either temperature, suggesting the GEI effect resulted from the crossing of reaction norms rather than a reduction in additive genetic variance in one temperature. There was overall a lower hatching success at the higher temperature, but the severity of the temperature effect differed among genotypes, with the crossing of reaction norms indicating that the most successful genotypes at the lower temperature were not necessarily the most successful at the higher temperature.

The significant GEI for *H. e. armigera* is consistent with a recent study on the sea urchin *Centrostephanus rodgersii* by Foo *et al.* (2012), who using a similar NCII design found a significant effect of sire×temperature interaction on the percentage of normal gastrulation in embryos. It is still unclear, however, whether genetic variation in the tolerance of traits to changing conditions is common in natural populations, partly due to the difficulty of directly estimating genetic sources of variance in wild animals (Visser, 2008). With a few notable exceptions (e.g. Nussey *et al.*, 2005; Pistevos *et al.*, 2011; Foo *et al.*, 2012), the majority of

studies have revealed limited additive variance in the tolerance of traits to stressful environments (e.g. Laurila *et al.*, 2002; Hoffmann *et al.*, 2003; Kellermann *et al.*, 2006; Eads *et al.*, 2012; Kelly *et al.*, 2012). Marine broadcast spawners (and other external fertilizers) are ideal model systems for quantitative genetic studies, and our findings, together with those of Foo *et al.* (2012) and Pespeni *et al.* (2013), suggest that future studies on these systems may further illuminate the potential of natural populations to exhibit adaptive responses to climate change.

Even if adaptive responses are possible, genetic adaptation needs to proceed sufficiently quickly to track the unprecedented speed of current climate change. Ideal tests of this potential come from selection experiments (Hill & Caballero, 1992; Scheiner, 2002), where studies on model species such as *Drosophila melanogaster* have shown that responses to selection on traits such as thermal tolerance and desiccation resistance can be rapid (e.g. Hoffmann & Parsons, 1989; Huey *et al.*, 1991). However, it has been argued that species with longer generation times may not be able to respond sufficiently rapidly to the current rate of climate change (Hoffmann & Sgrò, 2011). Some broadcast spawners may provide tractable systems for selection experiments. Mussels and other bivalves, for example, are bred extensively in aquaculture (Gosling, 2003), suggesting they could readily be used in selection experiments to test the ability of marine broadcast spawners to respond genetically to experimentally manipulated environmental conditions (Hill & Caballero, 1992; Falconer & Mackay, 1996; Scheiner, 2002). Molecular genetic approaches may also provide insights into the adaptive potential of these systems. Accordingly, recent work on the sea urchin *Strongylocentrotus purpuratus* has revealed striking patterns of genome-wide selection on larvae cultured under realistic future carbon dioxide levels, thus revealing the

potential for rapid evolution in the face of environmental change in this species (Pespeni *et al*., 2013).

Non-additive genetic variance for hatching success

The significant sire×dam interaction for hatching success can be interpreted as non-additive genetic variance, arising from dominance or epistatic effects (i.e. variation in the compatibility of parental haplotypes). The finding of significant non-additive genetic effects on hatching success in *H. e. armigera* is consistent with results for the same population presented by Evans *et al.* (2007), and contributes to a growing body of evidence from quantitative genetic studies that genetic incompatibilities can limit offspring viability (Ivy, 2007; Pitcher & Neff, 2007; Dziminski *et al.*, 2008; Evans *et al.*, 2010). There have been several mechanisms proposed for genetic incompatibilities between males and females, including selfish genetic elements, which promote their own transmission to the detriment of other genes, and inbreeding depression, where mating between related individuals can increase the frequency of homozygotes and reduce fitness by exposing deleterious recessive alleles or because of intrinsic disadvantages of homozygosity (Zeh & Zeh, 1996; Tregenza & Wedell, 2000). Direct examination of the mechanisms responsible for genetic incompatibilities could be a promising area for future research in this system.

The sire×dam×treatment interaction indicates that the effect of different parental combinations was modified by temperature. Specifically, some parental combinations were more affected by the increased temperature than others, and combinations that performed best in terms of hatching success at the low temperature did not necessarily perform best at the high temperature. It is possible, therefore, that such genotype-by-genotype-by-

environment interactions could constrain the evolution of offspring temperature tolerance despite the presence of additive genetic variation. Offspring with genes with an additive effect of higher temperature tolerance may still be selected against if these effects are confounded by incompatibilities among parental genotypes that reduce phenotypic fitness.

A possible explanation for the three-way interaction involving sires, dams and temperature is that the detrimental effects of incompatible genotypes only become fully exposed under stressful conditions. For example, experimental work on *Drosophila* has shown that inbreeding depression can increase in more stressful environments, including under thermal stress (Miller, 1994, Bijlsma *et al.*, 1999; Joubert & Bijlsma, 2010). Such interactions between genetic and environmental stresses could have detrimental effects on inbred populations under climate change (Joubert & Bijlsma, 2010). Generalizations about inbreeding cannot be made for *H. e. armigera* from the current study, as the mechanisms of genetic incompatibilities were not examined. The results, however, reflect recent studies that have detected interactions of non-additive genetic effects with thermal stress in field crickets (Nystrand *et al.*, 2011), and desiccation stress in frogs (Eads *et al.*, 2012), and together these findings suggest interactions between parental genotypes should be interpreted cautiously if a range of environmental conditions are not considered.

Variance in fertilization rate

Fertilization rate is influenced by gamete (egg and sperm) quality and by the compatibility of gametes from particular combinations of males and females, and these may all be influenced by both genetic and environmental factors. The significant main effects of male and female in this study are consistent with previous work on this species (Evans & Marshall, 2005;

Evans *et al.*, 2007), and suggest that variation in both sperm and egg traits can affect fertilization rates in *H. e. armigera*. These effects are difficult to interpret, however, as there was also significant variation in compatibility of particular sperm-egg combinations. This male×female interaction, although not found at fertilization by Evans *et al.* (2007), reflects previous findings on *H. e. armigera*'s east coast congener *H. e. erythrogramma* (Evans & Marshall, 2005). Differences in gamete compatibility for broadcast spawners have been linked to gamete recognition proteins that influence sperm-to-egg attachment, such as the sperm protein bindin in sea urchins (Vacquier & Moy, 1977; Vacquier *et al.*, 1995; Zigler, 2008; reviewed by Evans & Sherman 2013). Although the evolution of bindin has been widely implicated as a mechanism of reproductive isolation between sea urchin species (Palumbi & Metz, 1991; Zigler *et al.*, 2003), there is also evidence for intraspecific variation in sperm-egg compatibility in sea urchins (Palumbi, 1999; Levitan & Ferrell, 2006; Zigler, 2008).

Conclusions

In conclusion, we found that embryo development, as measured by hatching success, is vulnerable to increased temperatures in *H. e. armigera*, and consequently that predicted ocean warming may exert selection pressure on early life history stages of this marine broadcast spawner. Our analyses also revealed significant additive genetic variation underlying hatching success, thus suggesting the possibility that affected populations may have the potential to adapt genetically to climate change. However, caution is needed before drawing firm conclusions on the potential for adaptation as we have considered only a small subset of potential life-history traits, and patterns of genetic covariance with unmeasured traits may yet constrain evolutionary responses. There has been relatively little

empirical research to date on genetic covariances in the context of genetic adaptation to climate change (Merilä, 2012). The few studies that have addressed this topic suggest that genetic covariances could constrain adaptation to climate change for some traits (e.g. Etterson & Shaw, 2001; Teplitsky *et al.*, 2011), and facilitate adaptation for others (Bradshaw & Holzapfel, 2008; Carlson & Seamons, 2008). Thus, further research focusing on patterns of genetic variation and covariation of a broad range of traits and life history stages is needed in order to gain a fuller understanding of the potential for adaptive responses to climate change in these systems. Non-additive genetic effects also appear to underlie hatching success in *H. e. armigera*, and as with additive effects are modified by temperature. It is possible, therefore, that genetic incompatibilities that are not expressed under current environmental conditions will be exposed in embryos by stressful climatic changes (Eads *et al.*, 2012), thus limiting adaptive response to climate change in this species.

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Figure legends

Figure 1. A single block from the experimental North Carolina II breeding design. In each block (six blocks in total), three sires (S1-S3) were crossed to three dams (D1-D3) in all nine combinations. Two replicate fertilizations were performed for each of the nine combinations (shown by the replicate in the background), yielding 18 crosses in total. Following assessment of fertilization rates, 60 fertilized eggs were collected from each of the 18 crosses, and 30 assigned to each temperature treatment. Hatching success was assessed two days after fertilization.

Figure 2. Reaction norms for (a) each of the 18 paternal half-sib (sire) and (b) each of the 54 full-sib families, showing the proportion of successfully hatching offspring at each temperature treatment (T1 = 22°C, T2 = 25°C). In panel (a), success for each sire was calculated as the mean proportion of all crosses (6) that sire was involved in, while in (b), success was calculated as the mean proportion of the two replicate crosses for each family. In both panels, the overall mean proportion of successful hatchings at each temperature (with standard error bars) is shown by closed circles.

Table 1. Results of log-likelihood ratio tests for each of the random effects on fertilization rate. A generalized linear mixed-effects model with a logit-link function was fit for fertilization rate, with the listed random effects. Model fit is measured by log-likelihood. Each random effect was excluded in turn, and the fit of each reduced model compared to the full model. The likelihood ratio (G^2) is calculated as -2×difference in log-likelihood, and compared to a χ^2 distribution with one degree of freedom (Lynch & Walsh, 1998).

Model	Log-likelihood	G^2	P-value
Full	-3043.9		
(-Sire)	-3050.9	14.15	< 0.001
(-Dam)	-3048.9	10.00	0.002
(-S×D)	-3091.6	95.36	< 0.001
(-Block)	-3044.0	0.22	0.637

Table 2. Results of likelihood ratio tests for each of the random effects on hatching success. A generalized linear mixed-effects model with a logit-link function was fit for hatching success with a fixed treatment effect (T) and the listed random effects. Model fit is measured by log-likelihood. Each random effect was excluded in turn, and the fit of each reduced model compared to the full model. The likelihood ratio (G^2) is calculated as -2×difference in log-likelihood, and compared to a χ^2 distribution with one degree of freedom (Lynch & Walsh, 1998).

Model	Log-likelihood	G^2	P-value
Full	-2329.1	-	-
(-Sire)	-2338.2	18.14	<0.001
(-Dam)	-2329.2	0.20	0.654
(-S×D)	-2347.0	35.75	<0.001
(-S×T)	-2331.7	5.14	0.023
(-D×T)	-2329.1	0	1
(-S×D×T)	-2333.2	8.18	0.004
(-Block)	-2329.5	0.667	0.414

Table 3. Mean proportion of offspring surviving, additive genetic variance ($V_A = 4 \times$ sire variance component, estimated using generalized linear-mixed effects models; GLMMs) and total phenotypic variance (V_P) for hatching success at each temperature treatment. Phenotypic variance was estimated following Nakagawa & Schielzeth (2010).

Treatment	Mean (±SE)	V_A	V_P*
T1 (22°C)	0.42 (0.04)	20.77	12.36
T2 (25°C)	0.34 (0.03)	32.99	16.47

* Note that violations of the assumptions of autosomal inheritance and/or possible non-genetic effects may account for inflated estimates of V_A that exceed V_P in each treatment (see main text).

Table 4. Results of likelihood ratio tests for each of the random effects on hatching success, with 4 sire families that had <5% successful hatchings removed. A generalized linear mixed-effects model with a logit-link function was fit for hatching success with a fixed treatment effect (T) and the listed random effects. Model fit is measured by log-likelihood. Each random effect was excluded in turn, and the fit of each reduced model compared to the full model. The likelihood ratio (G^2) is calculated as -2×difference in log-likelihood, and compared to a χ^2 distribution with one degree of freedom (Lynch & Walsh, 1998).

Model	Log-likelihood	G^2	P-value
Full	-2266.2	-	-
(-Sire)	-2267.9	3.4537	0.063
(-Dam)	-2266.4	0.475	0.491
(-S×D)	-2282.2	32.023	<0.001
(-S×T)	-2268.4	4.349	0.037
(-D×T)	-2266.2	0	1
(-S×D×T)	-2270.4	8.5143	0.004
(-Block)	-2266.2	0.0412	0.839

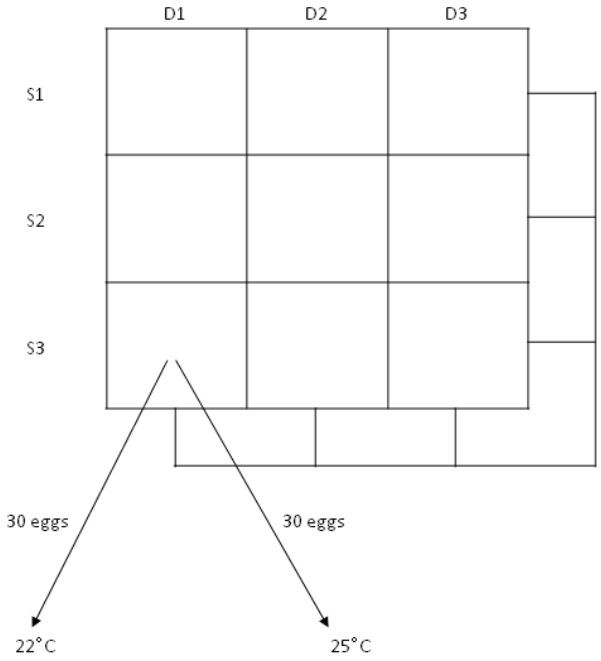

Figure 2. A single block from the experimental North Carolina II breeding design. In each block (six blocks in total), three sires (S1-S3) were crossed to three dams (D1-D3) in all nine combinations. Two replicate fertilizations were performed for each of the nine combinations (shown by the replicate in the background), yielding 18 crosses in total. Following assessment of fertilization rates, 60 fertilized eggs were collected from each of the 18 crosses, and 30 assigned to each temperature treatment. Hatching success was assessed two days after fertilization.

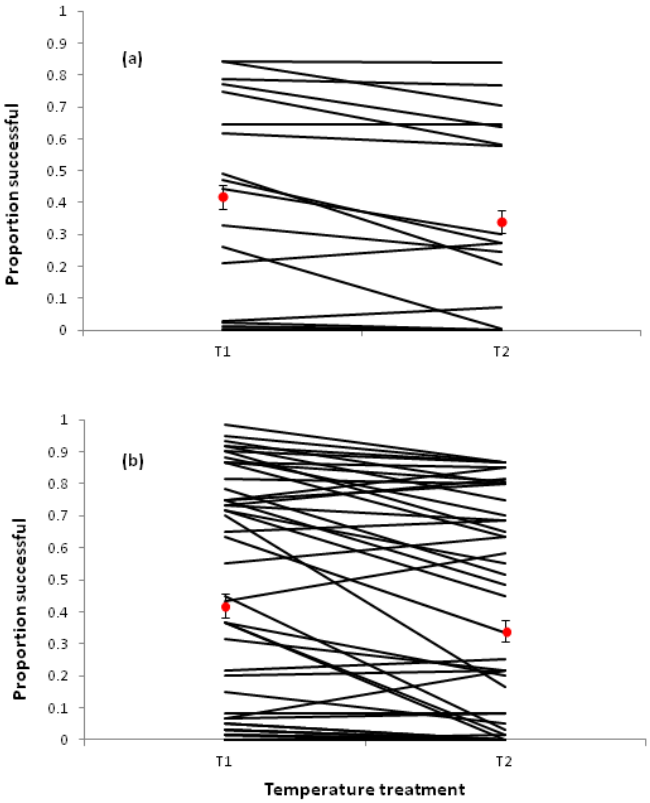

Figure 2. Reaction norms for (a) each of the 18 paternal half-sib (sire) and (b) each of the 54 full-sib families, showing the proportion of successfully hatching offspring at each temperature treatment (T1 = 22°C, T2 = 25°C). In panel (a), success for each sire was calculated as the mean proportion of all crosses (6) that sire was involved in, while in (b), success was calculated as the mean proportion of the two replicate crosses for each family. In both panels, the overall mean proportion of successful hatchings at each temperature (with standard error bars) is shown by closed circles.