Ecology, 94(11), 2013, pp. 2575–2582

Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance

Angela J. Crean,[1,2,4] John M. Dwyer,[1] and Dustin J. Marshall[1,3]

[1]*School of Biological Sciences, University of Queensland, Brisbane QLD 4072 Australia*
[2]*Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052 Australia*
[3]*School of Biological Sciences, Monash University, Melbourne VIC 3800 Australia*

Abstract. The ability of females to adaptively influence offspring phenotype via maternal effects is widely acknowledged, but corresponding nongenetic paternal effects remain unexplored. Males can adjust sperm phenotype in response to local conditions, but the transgenerational consequences of this plasticity are unknown. We manipulated paternal density of a broadcast spawner (*Styela plicata*, a solitary ascidean) using methods shown previously to alter sperm phenotype in the field, then conducted in vitro fertilizations that excluded maternal effects and estimated offspring performance under natural conditions. Offspring sired by males from low-density experimental populations developed faster and had a higher hatching success than offspring sired by males living in high densities. In the field, offspring survived relatively better when their environment matched their father's, raising the possibility that fathers can adaptively influence the phenotype of their offspring according to local conditions. As the only difference between offspring is whether they were artificially fertilized by sperm from males kept in high- vs. low-density cages, we can unequivocally attribute any differences in offspring performance to an environmentally induced paternal effect. Males of many species manipulate the phenotype of their sperm in response to sperm competition: our results show this plasticity can influence offspring fitness, potentially in adaptive ways, raising the possibility that adaptive nongenetic paternal effects may be more common than previously thought.

Key words: external fertilization; nongenetic inheritance; parental effect; paternal effect; sperm phenotype; sperm plasticity; Styela plicata.

Introduction

Populations are linked across generations both demographically (numerically) and phenotypically. While the demographic links among generations are obvious and well studied (Caley et al. 1996), the population consequences of phenotypic links between generations are more subtle and have received less attention (Marshall and Morgan 2011). The best known sources of nongenetic phenotypic links between generations are maternal effects, whereby the environment and/or phenotype of the mother affects the phenotype of the offspring (Mousseau and Fox 1998). Maternal effects can take a variety of forms ranging from hormonally induced morphological and behavioral changes, to transference of resistance to pollution and pathogens, to epigenetic modification of gene expression (Uller 2008, Jablonka and Raz 2009). They can buffer offspring from environmental change, or act as conduits whereby stress in one generation reduces productivity in the next (Mousseau and Fox 1998). Surprisingly, variation in offspring phenotype can have a greater influence on population structure than variation in offspring number (Burgess and Marshall 2011*a*), and impacts of maternal effects on population dynamics can persist for up to three subsequent generations (Benton et al. 2005). Moreover, mothers can adaptively adjust offspring phenotype to match local conditions, and hence maternal effects can initiate and direct adaptive evolution (Galloway and Etterson 2007, Uller 2008).

While the ecological role of maternal effects is increasingly well recognized, other sources of nongenetic phenotypic links among generations have largely been ignored. In particular, the ecological consequences of nongenetic paternal effects are poorly understood. Traditionally, paternal effects (with the obvious exceptions of paternal care and paternal genetic effects) have been assumed to be absent or negligible (Mousseau and Fox 1998); but this assumption has been challenged by a recent series of studies showing transgenerational epigenetic effects transferred down the male line (reviewed in Curley et al. 2011, Jenkins and Carrell 2012), and transmission of compounds in the sperm cytoplasm and accessory gland products (Avila et al. 2011). In humans, for example, early paternal smoking has been linked to an increased body mass index in sons, and paternal grandfather's food supply has been linked

Manuscript received 30 January 2013; revised 6 May 2013; accepted 7 May 2013. Corresponding Editor: A. L. Shanks.
[4] E-mail: a.crean@unsw.edu.au

to the mortality risk of grandsons (Pembrey et al. 2006). In mice, paternal obesity reduces sperm function (Bakos et al. 2011), impairs embryo development and viability (Mitchell et al. 2011), and diminishes the reproductive health of offspring for two generations (Fullston et al. 2012). To date, studies of nongenetic paternal effects have focused on the transgenerational effects of paternal behavior, nutrition, toxins, and age (Curley et al. 2011). Thus, with the exception of cases of paternal care, it is unclear whether nongenetic paternal effects can be adaptive, whether they are vehicles for the transfer of pathological effects, or whether they are simply physiological inevitabilities (Marshall and Uller 2007).

Concurrently, evidence is accumulating that males can adjust their sperm phenotype in response to their social environment and perceived risk of sperm competition (e.g., Cornwallis and Birkhead 2007, Simmons et al. 2007, Crean and Marshall 2008, Immler et al. 2010). The functional consequences of this plasticity in sperm phenotype have only been considered in terms of their effects on fertilization success (Pizzari and Parker 2009), and thus this potentially important source of paternal effects remains unexplored. The recent evidence that sperm can transfer more than just the paternal genome raises the possibility that plasticity in sperm phenotype may have transgenerational consequences for offspring performance, and thus population dynamics (Zeh and Zeh 2008). In biomedical studies of humans, there has been increasing speculation regarding the existence of "adaptive" paternal effects (e.g., the "thrifty telomere hypothesis"; Eisenberg 2011), whereby males adjust the phenotype of their sperm in response to their own environment in order to increase offspring fitness. While the potential for such effects is clearly exciting, it has not been explored empirically.

External fertilizers are an ideal system in which to empirically examine paternal effects, as eggs are released before fertilization, precluding the possibility of females biasing their investment in response to sperm quantity or quality (Cunningham and Russell 2000, Sheldon 2000). In addition, sessile organisms cannot move after settlement to cope with environmental heterogeneity, and therefore developmental and transgenerational plasticity may be particularly important mechanisms to ensure offspring success in these species (Galloway and Etterson 2007, Uller 2008). However, unlike plants, as both gametes are released prior to fertilization in broadcast spawners, paternal effects may be equally as likely as maternal effects to develop in these systems.

Variation in conspecific density is an important ecological driver in marine sessile communities, directly influencing the availability of food and space, with strong effects on post-settlement survival and growth (Allen et al. 2008). We have previously demonstrated that the broadcast-spawning ascidian *Styela plicata* adjusts the phenotype of their sperm (size, motility, and longevity) in response to changes in the density of conspecifics (Crean and Marshall 2008). Furthermore, significant spatial genetic structure has been detected among *S. plicata* individuals at the same location as the present study that were 0–5 m apart (David et al. 2010), suggesting that dispersal distances are short and thus the paternal environment is likely to be a good predictor of the offspring environment: a requirement for adaptive transgenerational plasticity (Galloway and Etterson 2007). Here, we manipulate the paternal environment using the same field manipulation previously shown to induce plasticity in sperm phenotype (Crean and Marshall 2008). We use sperm collected from males in these high- and low-density treatments to in vitro fertilize a common pool of eggs, and track the performance of offspring. Offspring developmental performance is measured in the laboratory (due to logistical constraints), and post-metamorphic performance is measured in the field in both low- and high-density conditions to test for adaptive paternal effects.

Materials and Methods

Study species and field location

We used the solitary ascidian, *Styela plicata*, as a model system to examine environmentally induced paternal effects, as previous work has shown that they exhibit plasticity in sperm size, motility, longevity, and fertilization potential (Crean and Marshall 2008). *S. plicata* is a hermaphroditic broadcast spawner commonly found in disturbed habitats such as marinas and harbors throughout eastern Australia (Kott 1972). We completed all field work at the East Coast Marina (Manly, Brisbane, Australia; 27.467° E, 153.183° S), a sheltered private-access marina consisting of floating pontoons. At this site, *S. plicata* naturally occur in densities ranging from isolated individuals to large clumps (e.g., >50 adults on 1 m length of rope; A. J. Crean, *personal observation*). Reproductively mature individuals are present at this site most of the year, excluding winter months (July to September; A. J. Crean, *personal observation*).

Manipulation of the paternal habitat

The paternal environment was manipulated using the same density manipulation methods that were shown previously to induce adaptive plasticity in sperm phenotype (Crean and Marshall 2008). Briefly, reproductive adults were collected from the field site and randomly allocated to either a high-density (15 individuals) or a low-density (single individual) treatment cage (cage dimensions, $18 \times 18 \times 18$ cm; mesh size, 1 cm^2). Treatment cages were suspended from the pontoons and maintained in the field for one month, and then a randomly selected high-density individual from each cage and all low-density individuals were transported to the laboratory at the University of Queensland. Importantly, no mortality of treatment animals occurred in any of the high- or low-density

cages, and thus differences in offspring performance cannot be explained by selection acting on fathers in each treatment group. We also collected randomly selected non-treatment individuals from our field site to be used as mothers in each assay. Data were collected from three experimental runs beginning in January, April, and June 2009, respectively. Measurements of time to hatching, hatching success, and larval size were collected in run one, cell cleavage rate in run two, and post-metamorphic survival in runs two and three. Within each run, multiple trials were conducted (run one, six trials; run two, five trials; run three, five trials), with each trial using a paired, split-clutch design: a common pool of eggs was split into two groups and then each group fertilized with sperm from either a high-density or low-density treatment male. Trials were initiated consecutively over two days to minimize temporal differences.

In vitro fertilization

Experimental in vitro fertilization roughly approximates natural reproduction for external fertilizers, and can be tightly controlled such that all offspring are reared under standardized conditions from fertilization through to deployment in the field (Marshall et al. 2008). As we were interested in the nongenetic effects of the paternal environment on offspring performance, the sperm from males in density treatments was used in vitro to fertilize eggs from non-treatment animals in standardized conditions. Gametes were extracted using standard strip-spawning techniques (Crean and Marshall 2008). Eggs were harvested from four randomly selected non-treatment animals, combined together, and 5 mL of this combined egg solution was transferred into each of two 35 mm diameter petri dishes, one for each paternal treatment. Eggs from multiple females were used in each trial to reduce maternal effects and male-by-female interactions that could confound the interpretation of results. A 5-mL control egg sample was also put aside and not exposed to sperm to check for any self-fertilization of eggs ($<1\%$ in all trials). We then collected 5 mL of sperm solution from one high-density and one low-density treatment animal (order of treatments randomized), and added the sperm to its assigned petri dish, gently shaking each sample every two minutes to mix the sperm and eggs. We washed eggs free of sperm after 15 minutes (so exposure time was constant between treatments) by gently rinsing each sample through a 100-μm filter, and left the eggs to develop in 10 mL of filtered seawater in a covered petri dish. The concentration of sperm solution collected from each male was estimated using a Neubauer improved hemocytometer under 400× magnification (three replicate counts per sample). There was no difference in the average concentration of sperm collected from males in high- and low-density treatments (high-density males, $1.719 \times 10^8 \pm 1.861 \times 10^7$ sperm/mL [mean ± SE]; low-density males, $1.608 \times 10^8 \pm 1.627 \times 10^7$; paired $t = 0.075$; df = 15; $P = 0.941$; Appendix: Fig. A1).

Cell cleavage rate

To examine the effect of paternal environment on embryonic cell cleavage rates, we placed each petri dish with the developing eggs under a dissecting microscope at 20× magnification, and used time-lapse photography to record a digital image every 30 s with PixeLINK Capture SE software (PixeLINK, Ottawa, California, USA). As we did not know the exact time of fertilization, cell development rate was estimated from the time of the first cleavage to the four-cell stage (Rius et al. 2010). The size of fertilized eggs was measured from images before the first cleavage using Image-Pro Express (version 5.1; Media Cybernetics, Silver Spring, Maryland, USA). We estimated total egg area by digitally tracing around the perimeter of the follicle cells, and ovicell area by tracing around the intersection of the follicle cells and ovicell (Crean and Marshall 2008). We measured 15 eggs per paternal treatment per trial.

Time to hatching and hatching success

To measure development time and calculate hatching success, fertilized eggs were individually collected with a micro-pipette and transferred into individual 10 mm diameter wells with 2 mL of filtered seawater in a 24-well plate (one plate per replicate = 24 subsamples). These plates were left in a constant temperature (CT) cabinet at 22°C overnight. Developing embryos were observed under a microscope (30× magnification) every 15 minutes (starting 10 hours post-fertilization) to measure time to hatching. Eggs that had not hatched after 15 hours, and hatched larvae with highly abnormal morphology, were classed as unsuccessful. Digital images of successfully hatched larvae were recorded under a microscope (45× magnification), and larval area was measured by tracing around the perimeter of each larva using Image-Pro Express. Larval area was calculated from the mean of at least three traces from different images of the same individual to minimize the effects of measurement error.

Post-metamorphic survival

To measure the post-metamorphic performance of offspring sired by fathers that experienced different environments, we settled larvae sired by low- and high-density fathers on to 35 mm diameter petri dishes and then deployed them into the field for two weeks. Although this may seem to be a short time frame to measure offspring performance, a previous study on *Styela plicata* at the same field site showed that 99% of all post-settlement mortality occurred within the first two weeks after settlement (Rius et al. 2009). We also examined whether the effects of paternal environment were context dependent by varying the density of settlers in the field. Our analysis therefore included treatments

applied at two scales: the paternal density treatment was applied at the scale of trial, and the offspring density treatment was applied at the scale of petri dish.

We fertilized eggs with sperm from males in low and high densities, transferred the embryos from each treatment into separate 250-mL beakers filled with filtered seawater, and left them covered overnight in the CT cabinet at 22°C. Larvae were collected with a pipette the following morning (approximately 11 hours after fertilization), and transferred to pre-roughened and bio-filmed petri dishes in a drop of water. For low-density offspring treatments, we transferred five larvae to each petri dish (six low-density offspring replicates per paternal treatment per trial), to ensure that at least one larva settled on the base of the dish. For offspring allocated to the high-density treatment, we transferred between twenty and thirty larvae to each petri dish (depending on how many larvae successfully hatched), with four high-density offspring replicates per paternal treatment per trial. We first ensured larvae were free swimming and not stuck in the water surface layer, and then placed the covered petri dishes in the CT cabinet for 24 hours to allow larvae to settle.

In low-density offspring treatments, we marked the position of a haphazardly selected settler, and removed all other settlers so that low-density treatments had a single individual to mirror paternal density manipulations. In high-density offspring treatments, all settlers were marked and numbered (number of settlers per dish ranged from 11 to 26; mean = 20 individuals; mean density = 0.02 individuals/mm^2). All replicates were then transported to the marina in an insulated container filled with seawater, and deployed in the field that afternoon. Petri dishes were suspended vertically within a rigid plastic mesh cage (dimensions: $44 \times 28 \times 18$ cm length $\times$ width $\times$ height; mesh size 1 cm^2), as the majority of *S. plicata* were observed growing on vertical surfaces at the field site. Replicates were enclosed within cages as pilot studies showed that fish are attracted to field equipment and preferentially eat *S. plicata* settlers (A. J. Crean, *personal observation*). These cages were hung from the pontoons in the marina, approximately 2 m below the water surface, and left in the field for two weeks. After this time, all settlers were transported back to the laboratory and examined under a microscope (30$\times$ magnification) to calculate survival.

Statistical analyses

Hatching success was modeled as a binary response using mixed-effects logistic regression (logit-link function and binomial errors), with treatment (high or low paternal density) included as a fixed effect and trial included as a random effect. Eggs that did not hatch or those that hatched with visible lethal deformities were considered unsuccessful and given a value of zero in this analysis. Time to hatching was analyzed using mixed-effects Cox proportional hazards models, again with paternal density as a fixed effect and trial included as a random effect. Exploratory Kaplan-Meier plots indicated that these data met the assumption of proportionality. We ran the Cox models using two censoring approaches that make different assumptions about the fate of unhatched eggs. First, we only included individuals that hatched successfully and no individuals were censored. This approach ignores the possibility that some unhatched eggs could have hatched once monitoring ceased. Second, we included all individuals that hatched successfully and those that did not hatch within the monitoring period. The individuals that did not hatch were censored. This approach assumes that all unhatched eggs will eventually hatch, despite the prospect that many of the unhatched eggs were nonviable. Average estimates of sperm concentration, cell development time, egg ovicell and total area, and larval area were calculated for each trial, and paired *t* tests used to test for consistent differences between low and high-density paternal treatments.

Post-metamorphic survival was modeled as a binary response using mixed-effects logistic regression (logit-link function and binomial errors), with trial and dish included as random effects. The number of larvae that settled in high-density offspring treatments ranged from 11 to 26 individuals per dish, and for this reason we chose to treat density as a continuous variable rather than categorical to retain more of the information contained within the dataset. Hence, fixed effects were paternal density (high or low), offspring density (continuous) and their interaction. As survival data was collected in runs two and three, we checked for a possible run effect by fitting models that allowed slopes and intercepts to vary by run (as random effects). All random-effect terms for run were not significant (Appendix: Table A1) and the fixed-effect estimates were very similar to those from models that excluded run. We therefore present data pooled across both runs for clarity. The significance of fixed-effect terms was assessed using likelihood ratio tests (LRTs) by comparing nested models with and without the variable of interest. All analyses were completed using the R statistical program (R Development Core Team 2013), and the LME4 package (Bates et al. 2013) used to fit all mixed effects logistic models.

Results

Offspring developmental performance

Embryos fertilized by sperm from fathers in low-density environments were more likely to successfully complete development and hatch into larvae (Fig. 1a), with 69% (95% CI 55.2–79.5) of eggs fertilized by high-density males successfully hatching into larvae, compared with an 80% (95% CI 71.1–87.6) hatching success rate of eggs fertilized by low-density males. Paternal environment also influenced the time offspring took to hatch into larvae, with offspring from high-density males taking longer to complete development than offspring from low-density males (Fig. 1b). This result

was confirmed by the mixed-effects Cox models, although the effect of paternal density on hatching time was more pronounced in the model that excluded all unhatched eggs (low paternal density coefficient = 0.79, SE = 0.154, $\chi^2_1 = 26.4$, $P < 0.0001$), compared to the model that censored unhatched eggs (low paternal density coefficient = 0.50, SE = 0.14, $\chi^2_1 = 12.9$, $P = 0.0003$). These coefficients can be interpreted as hazard ratios to assist interpretation of relative treatment differences. Using the latter model as an example, the hazard ratio was $e^{0.5} = 1.65$. Thus, by the time that 50% of the eggs hatched in the high-paternal-density treatment, 68% ($1 - 0.5^{1.65}$) had already hatched in the low-paternal-density treatment.

To determine what drove this difference in time to hatching, we measured the cleavage rate of eggs fertilized by males in each environment. Eggs fertilized by sperm from high-density males took 6% longer on average to develop from the two-cell to the four-cell stage than eggs fertilized with the sperm of low-density males (mean time from two- to four-cell stage: high-density father = 882 s, low-density father = 830 s; paired $t = 3.104$, df = 4, $P = 0.036$; Appendix: Fig. A1b). Thus, it appears that differences in time to hatching are driven by a faster developmental rate of offspring sired by males in low densities. There was no significant difference between paternal density treatments in the size of eggs that were fertilized (total egg area, paired $t = -0.558$, df = 4, $P = 0.607$; ovicell, paired $t = -0.623$, df = 4, $P = 0.567$; Figs. A1b and c), and therefore differences in developmental rate between paternal treatments are unlikely to be driven by differences in egg size.

Offspring post-metamorphic survival

The effect of the paternal environment on the post-metamorphic survival of offspring was context-dependent, indicated by a significant interaction between paternal and offspring densities (LRT, $\chi^2_1 = 5.1$, $P = 0.020$; Table 2; Fig. 2a). To investigate this interaction further, we fitted two sets of post-hoc models; essentially, simple main effects tests for exploring significant interactions (see Quinn and Keough 2002). First, we ran mixed-effects logistic regressions for each of the paternal environments to assess the significance of slopes with offspring density. This revealed that offspring density had a significant negative effect on survival of offspring of low-density males (LRT, $\chi^2_1 = 15.2$, $P < 0.001$), but that offspring from high-density males survived equally well at all densities (LRT, $\chi^2_1 = 1.03$, $P = 0.310$). Second, we categorized offspring density into their original low- and high-density treatments and analyzed the relative survival of offspring within each treatment; as the *relative* fitness of offspring from each paternal treatment within each offspring treatment can provide a more evolutionarily informative estimate of fitness (Kawecki and Ebert 2004, Stanton and Thiede 2005, Burgess and Marshall 2011*b*). In low-density offspring environments, offspring relative survival was higher for individuals from low-density males, although this difference was not statistically significant (LRT, $\chi^2_1 = 2.25$, $P = 0.134$; Fig. 2b). In high-density offspring environments, the relative survival was lower for individuals from low-density males (LRT, $\chi^2_1 = 10.41$, $P = 0.001$; Fig. 2b). As there was no difference in larval size between paternal-density treatments (paired $t = 1.397$, df = 5, $P = 0.221$), these observed differences in post-metamorphic survival cannot be explained by differences in offspring size.

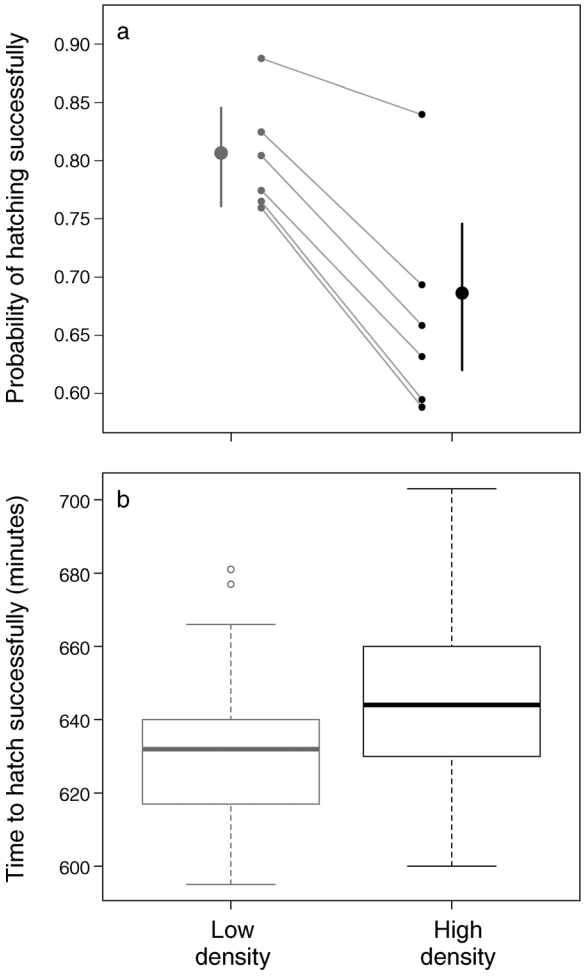

FIG. 1. Effect of manipulating paternal density on offspring developmental traits. Offspring sired from high-density males are shown in black; offspring sired by low-density males are shown in gray. Large points in panel (a) show the probability (mean ± SE) of hatching into a larva with a normal phenotype. The small points linked by lines are the hatching probabilities for each trial, as estimated by the mixed-effects logistic model. The box and whisker plots in panel (b) represent raw values of time from fertilization to hatching. The thick lines are medians, boxes are 0.25 and 0.75 quantiles, and whiskers indicate maximum and minimum values accounting for outliers (circles).

DISCUSSION

Conspecific density in the paternal environment has transgenerational consequences for offspring perfor-

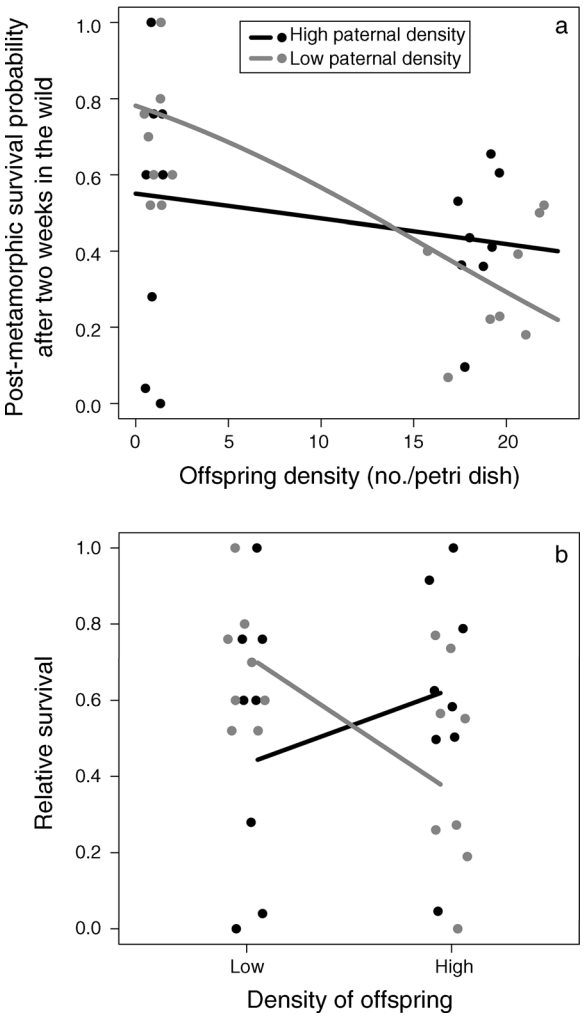

FIG. 2. Effect of manipulating paternal density on offspring post-metamorphic survival across low- and high-density offspring environments. Offspring sired from high-density males are shown in black; offspring sired by low-density males are shown in gray. (a) Absolute survival. Points are mean survival proportions for each set of six replicates per treatment combination per trial. Low offspring density values have been jittered slightly so that each point can be seen clearly. High offspring density values are mean densities for each set of six replicates. (b) Relative survival. Points are relative survival, calculated by dividing each point by the average survival within each environment.

mance across multiple life-history stages in the broadcast spawning ascidian *Styela plicata*. Eggs fertilized in vitro with sperm from individuals in experimentally manipulated high-density cages took longer to develop and hatch into larvae, and had a lower hatching success rate than offspring of low-density males. However, offspring survival in the field was context dependent: offspring from low-density males survived better than offspring from high-density males in low-density environments, but offspring from high-density males survived better than offspring from low-density males in high-density environments. In other words, offspring were more likely to survive in environmental conditions that matched the environment their father experienced. Across a range of taxa, mothers exposed to more intense intraspecific competition will adaptively manipulate the phenotype of their offspring to increase the competitive ability of their offspring (Bashey 2006, Galloway and Etterson 2007, Allen et al. 2008). Such adaptive maternal effects are known as anticipatory maternal effects (sensu Marshall and Uller 2007) because mothers anticipate the likely environment of their offspring and adjust the phenotype of their offspring accordingly. Our results suggest that fathers, like mothers, may show adaptive, anticipatory paternal effects, an idea that has been speculated upon (e.g., Eisenberg 2011) but never shown.

Interestingly, in a previous study we showed that *S. plicata* alter their sperm phenotype (sperm size, motility, and longevity) in response to the same density manipulations used in this study (Crean and Marshall 2008). Sperm plasticity was found to be adaptive at the fertilization stage, as sperm from high-density males achieve higher fertilization success at high sperm concentrations (Crean and Marshall 2008). In the present study, we used moderate sperm concentrations to equalize fertilization success among treatments, and therefore minimize any effects from differential fertilization. Using this approach, we found that offspring sired by high-density males had a lower hatching success than offspring from low-density fathers. This suggests that males in high population densities may increase their fertilization success by altering the phenotype of their sperm, but that these gains carry a cost to offspring developmental success (Bilde et al. 2009). Parents are expected to maximize their own fitness rather than that of any individual offspring, and hence selection may favor paternal effects that maximize a male's fertilization success at a cost to both offspring and mothers (Parker et al. 2002, Marshall and Uller 2007, Uller 2008). However, the extent of the conflict in this system is unclear, as although offspring of high-density males have a lower hatching success and slower developmental rate, their offspring are more likely to survive after settlement under competitive conditions. Increased mortality and slower growth early in life may facilitate the acquisition of robustness by survivors (Levitis 2011), potentially explaining the opposite fitness patterns pre- and post-settlement. In addition, alternative post-settlement fitness measures (e.g., growth and reproduction) may also vary significantly, and will therefore be required to characterize total fitness effects on offspring in future studies. These differences in effects observed across life-history stages highlight the complexities associated with interpreting the adaptive value of plasticity, and the importance of examining how selection acting on a trait varies across environments (Marshall and Morgan 2011).

In broadcast-spawning marine invertebrates (such as *S. plicata*), gametes are released before fertilization, and therefore both maternal and paternal provisioning and care can be excluded as possible explanations for the effects observed in this study. Moreover, there was no adult mortality observed over the duration of the paternal density manipulations, thus differences in offspring performance cannot be explained by selection acting on males in each treatment group. Hence, differences in offspring performance observed in this study can be unambiguously attributed to an environmentally induced paternal effect. A number of mechanisms could account for these observed paternal effects. For example, differences in the paternal environment may have imposed distinct selective regimes on the developing sperm (analogous to meiotic drive), causing genetically based, yet environmentally induced, paternal effects (Mazer and Gorchov 1996). Alternatively, the paternal environment may have altered gene expression in sperm, and these epigenetic changes may have been stably transmitted to, and expressed in, offspring (Curley et al. 2011). It is also possible that the observed decrease in developmental success of offspring sired by high-density males was caused by an increase in the germ-line mutation rate in stressful environments (Agrawal 2002); however, this explanation is not consistent with the observed differences in developmental rate or higher survival after metamorphosis. Although we cannot elucidate the specific mechanism of the paternal effects found in this study, it is clear that the paternal environment can influence offspring performance, even in species lacking any form of paternal care.

Clearly, selection pressures acting on broadcast spawners will be very different to those acting on internal fertilizers. Indeed, plasticity in *S. plicata* sperm size changed in the opposite direction to that predicted by classic sperm competition theory (Crean and Marshall 2008). However, given the diversity of taxa in which plasticity in sperm phenotype has already been demonstrated (Pizzari and Parker 2009), we believe that adaptive paternal effects are likely to be widespread (albeit more difficult to empirically demonstrate in internal fertilizers). While environmentally induced paternal effects are likely to be weaker, and therefore harder to detect, than maternal effects (Fox et al. 1995), they should not be ignored. Most quantitative genetics analyses currently assume that paternal contributions are restricted to nuclear genetic effects, and thus any paternal effects on offspring phenotype are attributed to additive genetic variation (Falconer and Mackey 1996). Our results suggest that, in some cases, this assumption may not be supported: paternal influences on offspring may also be modulated by the paternal environment. Maternal effects too were once dismissed as nuisance effects, but are now regarded as one of the most important determinants of offspring performance and, consequently, population dynamics (Wade 1998). The strength and prevalence of maternal effects has even led to calls to change fishery management practices, as ignoring maternal effects could lead to catastrophic overexploitation (Birkeland and Dayton 2005). Epigenetic inheritance has been predicted to be ubiquitous (Jablonka and Raz 2009), and therefore paternal effects may have a much greater influence on offspring performance than previously anticipated (Bonduriansky and Day 2009). Hence, like maternal effects, it is possible that environmentally induced paternal effects may emerge as an important driver of ecological and evolutionary change in the future.

Acknowledgments

We thank East Coast Marina for access to private docks. R. Bonduriansky, M. Adler, and two anonymous reviewers provided helpful comments on the manuscript. D. J. Marshall and A. J. Crean were supported by grants from the Australian Research Council.

Literature Cited

Agrawal, A. F. 2002. Genetic loads under fitness-dependent mutation rates. Journal of Evolutionary Biology 15:1004–1010.

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. American Naturalist 171:225–237.

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner. 2011. Insect seminal fluid proteins: identification and function. Annual Review of Entomology 56:21–40.

Bakos, H. W., M. Mitchell, B. P. Setchell, and M. Lane. 2011. The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. International Journal of Andrology 34:402–410.

Bashey, F. 2006. Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulata*. Evolution 60:348–361.

Bates, D., M. Maechler, and B. Bolker. 2013. lme4: linear mixed-effects models using S4 classes. http://cran.r-project.org/web/packages/lme4/index.html

Benton, T. G., S. J. Plaistow, A. P. Beckerman, C. T. Lapsley, and S. Littlejohns. 2005. Changes in maternal investment in eggs can affect population dynamics. Proceedings of the Royal Society B 272:1351–1356.

Bilde, T., A. Foged, N. Schilling, and G. Arnqvist. 2009. Postmating sexual selection favors males that sire offspring with low fitness. Science 324:1705–1706.

Birkeland, C., and P. K. Dayton. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology and Evolution 20:356–358.

Bonduriansky, R., and T. Day. 2009. Nongenetic inheritance and its evolutionary implications. Annual Review of Ecology Evolution and Systematics 40:103–125.

Burgess, S. C., and D. J. Marshall. 2011*a*. Are numbers enough? Colonizer phenotype and abundance interact to affect population dynamics. Journal of Animal Ecology 80:681–687.

Burgess, S. C., and D. J. Marshall. 2011*b*. Temperature-induced maternal effects and environmental predictability. Journal of Experimental Biology 214:2329–2336.

Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics 27:477–500.

Cornwallis, C. K., and T. R. Birkhead. 2007. Changes in sperm quality and numbers in response to experimental manipula-

tion of male social status and female attractiveness. American Naturalist 170:758–770.
Crean, A. J., and D. J. Marshall. 2008. Gamete plasticity in a broadcast spawning marine invertebrate. Proceedings of the National Academy of Sciences USA 105:13508–13513.
Cunningham, E. J. A., and A. F. Russell. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404:74–77.
Curley, J. P., R. Mashoodh, and F. A. Champagne. 2011. Epigenetics and the origins of paternal effects. Hormones and Behavior 59:306–314.
David, G. K., D. J. Marshall, and C. Riginos. 2010. Latitudinal variability in spatial genetic structure in the invasive ascidian, *Styela plicata*. Marine Biology 157:1955–1965.
Eisenberg, D. T. A. 2011. An evolutionary review of human telomere biology: the thrifty telomere hypothesis and notes on potential adaptive paternal effects. American Journal of Human Biology 23:149–167.
Falconer, D. S., and T. F. C. Mackey. 1996. Introduction to quantitative genetics. Fourth edition. Longman, New York, New York, USA.
Fox, C. W., K. J. Waddell, and T. A. Mousseau. 1995. Parental host plant affects offspring life-histories in a seed beetle. Ecology 76:402–411.
Fullston, T., N. O. Palmer, J. A. Owens, M. Mitchell, H. W. Bakos, and M. Lane. 2012. Diet-induced paternal obesity in the absence of diabetes diminishes the reproductive health of two subsequent generations of mice. Human Reproduction 27:1391–1400.
Galloway, L. F., and J. R. Etterson. 2007. Transgenerational plasticity is adaptive in the wild. Science 318:1134–1136.
Immler, S., S. R. Pryke, T. R. Birkhead, and S. C. Griffith. 2010. Pronounced within-individual plasticity in sperm morphometry across social environments. Evolution 64: 1634–1643.
Jablonka, E., and G. Raz. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Quarterly Review of Biology 84:131–176.
Jenkins, T. G., and D. T. Carrell. 2012. The sperm epigenome and potential implications for the developing embryo. Reproduction 143:727–734.
Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.
Kott, P. 1972. Some sublittoral ascidians in Moreton Bay, and their seasonal occurrence. Memoirs of the Queensland Museum 16:233–260.
Levitis, D. A. 2011. Before senescence: the evolutionary demography of ontogenesis. Proceedings of the Royal Society B 278:801–809.
Marshall, D. J., R. M. Allen, and A. J. Crean. 2008. The ecological and evolutionary importance of maternal effects in the sea. Oceanography and Marine Biology: An Annual Review 46:203–250.
Marshall, D. J., and S. G. Morgan. 2011. Ecological and evolutionary consequences of linked life-history stages in the sea. Current Biology 21:R718–R725.
Marshall, D. J., and T. Uller. 2007. When is a maternal effect adaptive? Oikos 116:1957–1963.
Mazer, S. J., and D. L. Gorchov. 1996. Parental effects on progeny phenotype in plants: Distinguishing genetic and environmental causes. Evolution 50:44–53.
Mitchell, M., H. W. Bakos, and M. Lane. 2011. Paternal diet-induced obesity impairs embryo development and implantation in the mouse. Fertility and Sterility 95:1349–1353.
Mousseau, T. A., and C. W. Fox. 1998. Maternal effects as adaptations. Oxford University Press, Oxford, UK.
Parker, G. A., N. J. Royle, and I. R. Hartley. 2002. Intrafamilial conflict and parental investment: a synthesis. Philosophical Transactions of the Royal Society of London Series B 357:295–307.
Pembrey, M. E., L. O. Bygren, G. Kaati, S. Edvinsson, K. Northstone, M. Sjostrom, J. Golding, and A. S. Team. 2006. Sex-specific, male-line transgenerational responses in humans. European Journal of Human Genetics 14:159–166.
Pizzari, T., and G. A. Parker. 2009. Sperm competition and sperm phenotype. Pages 207–245 *in* T. R. Birkhead, D. J. Hosken, and S. Pitnick, editors. Sperm biology: an evolutionary perspective. Academic Press, Oxford, UK.
Quinn, G. P., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.
R Development Core Team. 2013. R: a language and environment for statistical computing. Version 3.0.0. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Rius, M., X. Turon, G. M. Dias, and D. J. Marshall. 2010. Propagule size effects across multiple life-history stages in a marine invertebrate. Functional Ecology 24:685–693.
Rius, M., X. Turon, and D. J. Marshall. 2009. Non-lethal effects of an invasive species in the marine environment: the importance of early life-history stages. Oecologia 159:873–882.
Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology and Evolution 15:397–402.
Simmons, L. W., A. Denholm, C. Jackson, E. Levy, and E. Madon. 2007. Male crickets adjust ejaculate quality with both risk and intensity of sperm competition. Biology Letters 3:520–522.
Stanton, M. L., and D. A. Thiede. 2005. Statistical convenience vs biological insight: consequences of data transformation for the analysis of fitness variation in heterogeneous environments. New Phytologist 166:319–337.
Uller, T. 2008. Developmental plasticity and the evolution of parental effects. Trends in Ecology and Evolution 23:432–438.
Wade, M. J. 1998. The evolutionary genetics of maternal effects. Pages 5–21 *in* T. A. Mousseau and C. W. Fox, editors. Maternal effects as adaptations. Oxford University Press, Oxford, UK.
Zeh, J. A., and D. W. Zeh. 2008. Maternal inheritance, epigenetics and the evolution of polyandry. Genetica 134:45–54.

Supplemental Material

Appendix

A table showing tests of run effects in the analysis of post-metamorphic survival and a figure showing the effect of manipulating paternal density on gamete traits (*Ecological Archives* E094-236-A1).

Ecological Archives E094-236-A1

Angela J. Crean, John M. Dwyer, and Dustin J. Marshall. 2013. Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology* 94:2575–2582.
http://dx.doi.org/10.1890/13-0184.1

APPENDIX A. A table showing tests of run effects in the analysis of post-metamorphic survival and a figure showing the effect of manipulating paternal density on gamete traits.

TABLE A1. Results of LRTs (nested model comparisons) for various random effects of run in the analysis of post-metamorphic survival. All models included the same fixed effects: paternal density, offspring density and their interaction. The first column shows the different random effects structures compared in each LRT. These are presented using the `lmer` syntax (in R). Also shown are the particular terms being tested in each comparison, the chi² statistic, degrees of freedom and *P* value.

Random effect structures compared in LRT	**Term being tested**	**chi²**	**df**	***P***
Paternal density * Offspring density | Run *vs.* Paternal density + Offspring density | Run	Paternal density:offspring density	0.134	4	0.998
Paternal density + Offspring density | Run *vs.* Offspring density | Run	Paternal density	0.603	3	0.896
Paternal density + Offspring density | Run *vs.* Paternal density | Run	Offspring density	1.749	3	0.626
1 | Run *vs.* no run random effect	Separate run intercepts	0	1	1.000

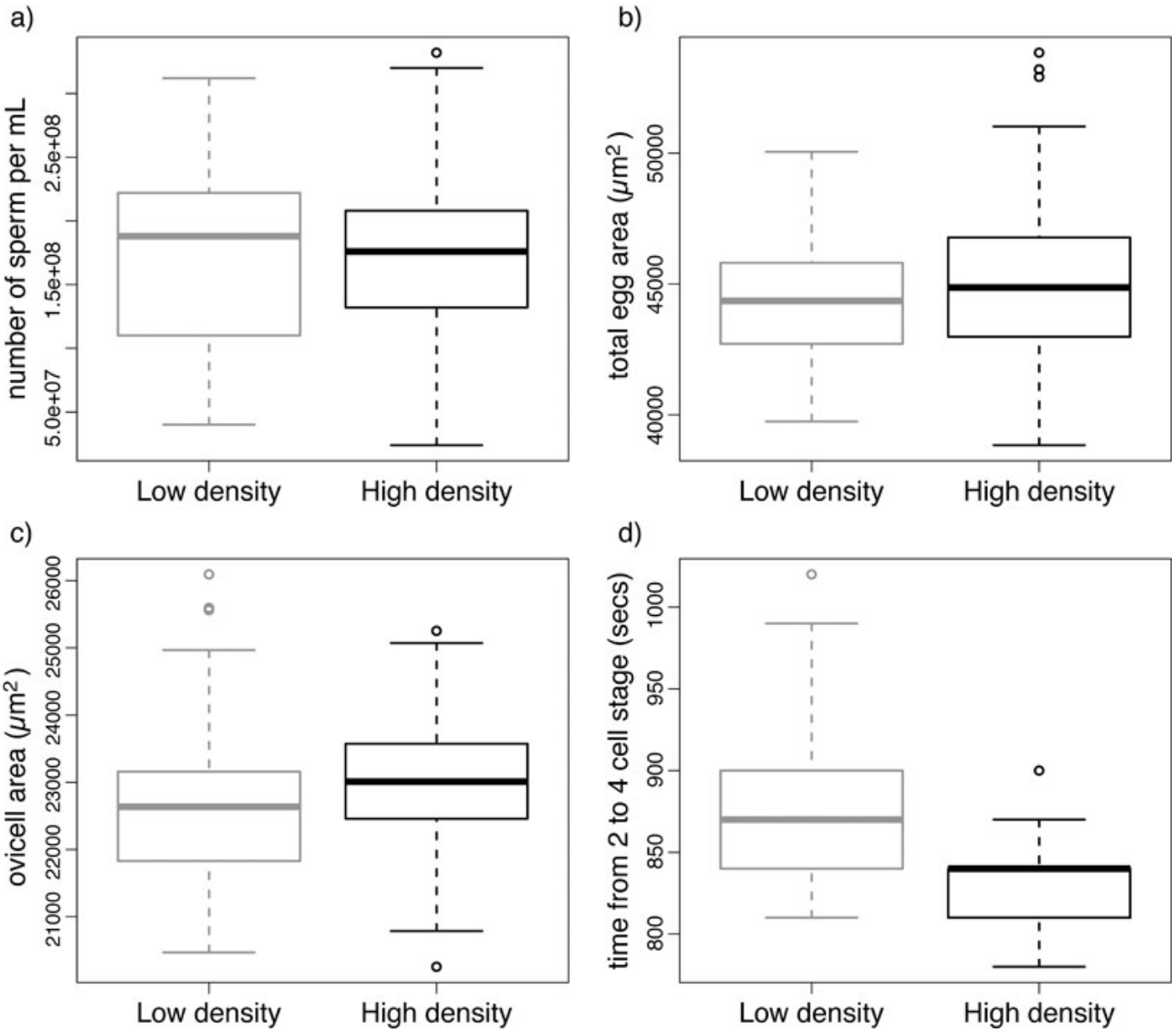

FIG. A1. Effect of manipulating paternal density on gamete traits. (a) sperm concentration, (b) total egg area, (c) ovicell area, (d) cell cleavage rate.

[Back to E094-236]