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NOTE

Within-family variation in larval viability and growth is controlled by different genes: a case study with *Crassostrea gigas*

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ABSTRACT: Variation in growth and survival of planktonic larvae has profound effects on marine population abundance. Environmental causes of differential larval growth and mortality are well studied, but genetic variation in these traits is less well understood. Here, we reveal genetic variation in survival and growth of full-sib larvae of the Pacific oyster *Crassostrea gigas* reared under identical conditions. We size-selected 1.42 million, 24 d old larvae to produce small, medium, and large size classes, constituting 93% of the population. Parental tissues and a total of 144 larvae sampled from each size class were individually genotyped with a panel of 45 mapped DNA markers. Consistent with previous work, 11 markers had genotypic frequencies that differed significantly from their Mendelian expectations and, upon mapping, are explained by 6 viability quantitative trait loci (vQTL). Notably, shell-length variance among size classes maps to 6 major growth QTL (gQTL), with just 2 collectively explaining 18% of the variance. We found little overlap between vQTL and gQTL, suggesting that variation in these larval traits is controlled by different genes. This study demonstrates the feasibility of genotyping single larvae for the identification of multiple QTL. Understanding genetic variation in larval biology is important for a comprehensive understanding of marine recruitment.

KEY WORDS: Growth · Viability · Genetics · Larvae · Bivalve · Pacific oyster · Single nucleotide polymorphism

1. INTRODUCTION

Differences in the mortality of early life stages has long been implicated as a cause of interannual variability in recruitment to fisheries and has been attributed to biological factors (starvation, predation) and physical processes (advection to unfavorable areas) (Korringa 1946, Houde 2008). Behavior and growth were subsequently recognized as important modulators of early mortality, the former providing control over dispersal and the latter reducing risk of predation. A positive association between early growth and survival, at the phenotypic level, should result in higher recruitment and fitness in the marine environment. The present study addresses whether such an association also exists at the genotypic level, leading to correlated evolution of these traits.

Mortality during planktonic larval development (type-III survivorship; Deevey 1947) is typically very high (Thorson 1950) and, though commonly attributed solely to environmental causes, is recognized, in bivalve molluscs at least, as having a substantial

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genetic basis (Plough 2016). In the Pacific oyster *Crassostrea gigas*, a large load of deleterious mutations causes the loss of 90% or more of fertilized eggs by the juvenile stage, distorting Mendelian inheritance of genetic markers in the process (Plough 2016, Yin & Hedgecock 2021). A likely by-product of high fecundity, large mutational load may characterize other marine animals besides the oyster (Plough 2016). The extent to which mutational load contributes to recruitment variability in natural populations is unknown, but unlike environmental sources of larval mortality, genetic inviability cannot be ameliorated by larval behavior or growth.

Genetic variance in the growth of juvenile and adult molluscs is well studied (reviewed by Hollenbeck & Johnston 2018), but evidence for genetic variance in larval growth rates is more limited (Ernande et al. 2003, Barros et al. 2018, Chi et al. 2020), likely owing to the resource-intensive nature of quantitative genetic studies and larval rearing. Here, we take a different approach. We use a panel of composite single nucleotide polymorphism (SNP) markers (Sun et al. 2015) to determine genomic differences among same-aged sibling *C. gigas* larvae separated by size-fractionation, while simultaneously evaluating genotype-dependent differences in viability. Mapping of quantitative trait loci (QTL) for growth (gQTL), while accounting for genotype-dependent mortality (Xu & Hu 2009), provides new insights into the genetic control of larval survival and growth.

2. MATERIALS AND METHODS

Full-sib larval *Crassostrea gigas* were produced from gametes obtained from an unrelated pair of parents derived from a natural population. To generate sufficient larvae for size-fractionation, 10 million fertilized eggs were reared in filtered seawater at 25°C in duplicate 200 l tanks (Pan et al. 2018). Survivorship was calculated at 8 time-course points by enumerating larvae in aliquots to a coefficient of variation ≤10%. Survival to Day 24 in the 2 rearing tanks did not differ and averaged 14% (86% mortality; Fig. 1a). At 8 time-points, shell length (anterior to posterior) was measured on 50 randomly selected larvae. Growth rate, calculated from the slope of the linear regression of shell length on age, did not differ between tanks (Fig. 1b): in Tank A, shell lengths at Day 24 ranged from 168 to 280 µm (mean ± SD, 222.0 ± 28.61 µm) and in Tank B, from 167 to 277 µm (221.0 ± 21.66 µm).

After determining that survival and growth did not differ between the 2 larval rearing tanks (Fig. 1), 24 d old larvae from the 2 tanks were pooled and passed through a series of sieves (nominal mesh sizes of 110, 130, 153, 180, 200, 224, and 253 µm). Of 1.42 ± 0.1 million (M) larvae, 0.35 ± 0.018 M (24%) were retained on the 200 µm screen (large), 0.6 ± 0.03 M (42%) were retained on the 180 µm screen (medium), and 0.38 ± 0.038 M (27%) were retained on the 153 µm screen (small). Larvae passing through the 153 µm mesh (7%) were not included in the genetic study. Larvae

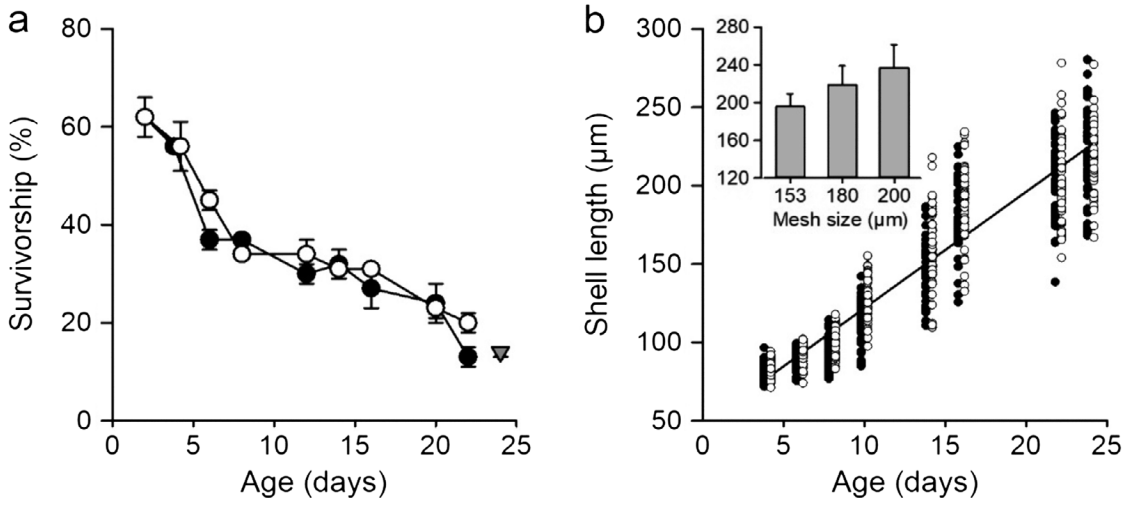

Fig. 1. Survivorship and growth of sibling larvae of *Crassostrea gigas*. (a) Percentage larval survival in duplicate 200 l tanks (open or filled symbols), calculated from the number of fertilized eggs (error bars, CV). Triangle symbol on Day 24 shows combined percentage survival, 14%, after larvae were pooled for size fractionation. (b) Shell lengths (n = 50 larvae per tank [100 total] per day; data offset on *x*-axis for clarity), as a function of age (days post-fertilization), for larvae in duplicate 200 l tanks. Growth rates did not differ between tanks (slope comparison: $F_{1,796} = 1.70$, p = 0.193). Inset: mean shell lengths of 24 d old larvae retained on 153, 180, and 200 µm mesh sieves, 195.9 ± 13.62 (±SD) µm, 218.6 ± 20.24 µm, and 236.9 ± 24.3 µm, respectively (n = 100 per size class; ANOVA, $F_{2,299} = 106.67$, p < 0.001; Tukey's test, p < 0.001 for each pair-wise comparison)

in large, medium and small size classes were enumerated and sized, with subsets preserved in 70% ethanol for DNA extraction.

DNA was extracted from parental muscle tissues and from 144 single larvae (Plough & Hedgecock 2011). Individuals were genotyped by high-resolution melting (HRM) of 45 SNP-containing amplicons (Sun et al. 2015). Thirty-two markers showed segregation in gametes from one or both parents and used for subsequent genetic analyses. The genomic locations of these markers were remapped to a chromosome-level assembly (Qi et al. 2021); to simplify presentation, we use abbreviated marker names composed of chromosome number and marker-order number (Table S1 in the Supplement at www.int-res.com/articles/suppl/m704p149_supp.xlsx). For all markers, 48 larvae (n = 16 per size class) were initially analyzed. We genotyped at 8 markers an additional 96 larvae (n = 32 per size class), which appeared, in the initial sample, to show an association of genotype frequencies with size class (i.e. total of 144 larvae).

Interval mapping of QTL was conducted for chromosomes with at least 2 markers. Parental linkage phases were determined, using JoinMap 4.1 (Van Ooijen 2011); marker *7-5* could not be placed on the linkage map and was excluded. Input to the QTL model (Luo & Xu 2003), as implemented in PROC QTL (Hu & Xu 2009; using SAS version 9.4, SAS Institute), comprised (1) parent and progeny genotypes, (2) parental linkage phases, (3) a linkage map based on the chromosome assembly, and (4) daily growth rates. Daily growth rate per size class was calculated as the gain in mean shell length from Day 2 (75 µm) to Day 24 divided by 22 d. The cross was modeled as a 4-way type, *AB* × *CD* (sire × dam), yielding progeny genotypes *AC*, *AD*, *BC*, and *BD*. Chromosomes were scanned for QTL in 1 cM (centi-Morgan) increments, using the maximum-likelihood QTL framework (convergence criterion, MAXERR, e^{-3}), with 3 genetic effects (paternal allelic substitution, maternal allelic substitution, and interaction) and the test for segregation distortion.

Chromosome-level significance thresholds (α = 0.05) for the log-likelihood ratio test statistic (LRT) were calculated for the 1 cM interval maps, following Piepho (2001). Significance thresholds determined from 1000 permutations (Churchill & Doerge 1994) identified the same gQTL as significant. Cumulative genetic mortality caused by independently assorting viability QTL (vQTL) is calculated as the multiplication product of the 6 average survival values, adjusted for sampling error (Plough & Hedgecock 2011).

3. RESULTS AND DISCUSSION

Twenty markers segregated from one or the other parent (symbolically, an *ab* × *aa* cross or vice versa, with an expected 1:1 segregation ratio), and 12 from both parents (10 *ab* × *ab* crosses, with an expected 1:2:1 segregation ratio; 2 *ab* × *ac* crosses, with an expected 1:1:1:1 segregation ratio; Table S2). Eleven markers had distorted segregation ratios (Table S2), implying differential survival of genotypes between fertilization and Day 24. These markers mapped to 6 vQTL affecting larval survival to Day 24, with a 7th peak near significantly distorted marker *7-7* falling just short of the significance threshold. These results are entirely consistent with previous work showing that a high genetic load causes substantial mortality of oyster larvae (Plough 2016, Yin & Hedgecock 2021). A large burden of deleterious mutations is likely maintained in natural populations, despite very strong selection against each individual mutation, by a high rate of mutation — a by-product of high fecundity — and by limits on the effectiveness of normalizing selection, namely, random genetic drift consistent with sweepstakes reproductive success (Hedgecock & Pudovkin 2011) and close linkage in repulsion phase (i.e. pseudo-overdominance; Yin & Hedgecock 2021).

Over the 6 independently assorting vQTL in this study, mean relative survival calculated from deviations from Mendelian proportions ranges from 0.4 to 0.75 (Table S3). The cumulative genetic mortality, 97%, obtained as the product of relative survival at independent vQTL (Table S3), is higher than the observed mortality of 86%, likely owing, in part, to non-random, size-selected sampling from the total population. Under laboratory conditions, larval mortality is almost wholly determined by genotype-dependent viability (Plough & Hedgecock 2011). Under natural conditions, genetic mortality is likely to be more than, not less than, that under laboratory conditions (Plough 2012). Environmental sources of larval mortality in nature would add to background genetic mortality caused by mutational load.

Size-fractionation of 1.42 M, 24 d old larvae produced 3 size classes (small, medium, and large), which comprised 93% of the population. Mean shell lengths differ significantly among the 3 size classes of larvae (Fig. 1b inset). Contingency chi-square tests show that genotype frequencies at 5 of 8 markers with n = 144 larvae depend on size class (Fig. 2, Table S4). Three of these markers, all on chromosome 9, also show distorted segregation ratios (Fig. 2, Table S2).

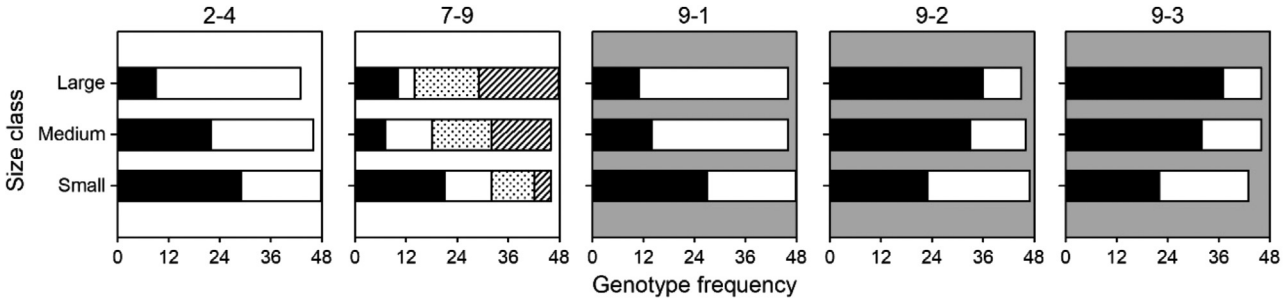

Fig. 2. Association between genotype frequency and size class at 5 markers (marker *2-4*, $\chi^2 = 14.82$, $p < 0.001$; *7-9*, $\chi^2 = 22.86$, $p < 0.001$; *9-1*, $\chi^2 = 11.84$, $p = 0.003$; *9-2*, $\chi^2 = 10.78$, $p = 0.005$; *9-3*, $\chi^2 = 8.82$, $p = 0.012$). Plots with gray shading indicate markers that are also distorted (Table S2). Black bars are homozygotes; white or patterned bars are heterozygotes (see Table S4)

Joint mapping analyses of larval size class and viability (segregation distortion), show that vQTL are more widespread than gQTL and that, in almost all instances, peaks in the viability likelihood ratio test profiles (LRT) are distinct from those associated with growth (Fig. 3; genotype data in Table S5). On chromosomes 2 and 7, where associations between growth and markers are identified (Fig. 2), vQTL and gQTL peaks are at opposite ends of the chromosome, showing little overlap of LRT profiles above significance thresholds (Fig. 3). For chromosome 9, joint test statistics (black line in Fig. 3) are highly significant, with contributions from both gQTL (red line in Fig. 3) and vQTL (blue line in Fig. 3). Of the 2 peaks for growth (at 21 and 39 cM), only the more distal overlaps with the peak for viability (39 cM). This overlap at 39 cM may be caused by 1 mutation with effects on both growth and survival or by 2 closely linked mutations with separate effects.

The gQTL have large phenotypic effects, consistent with detection of statistical significance in a study of this size (Wang & Xu 2019). Shell lengths of larvae are predicted from allelic effects for gQTL on chromosomes 2 and 9, where we could deduce the QTL genotypes based on 4 markers in 115 individuals of the 144 larvae analyzed (Table S6: markers *2-3*, *2-4*, *9-2*, and

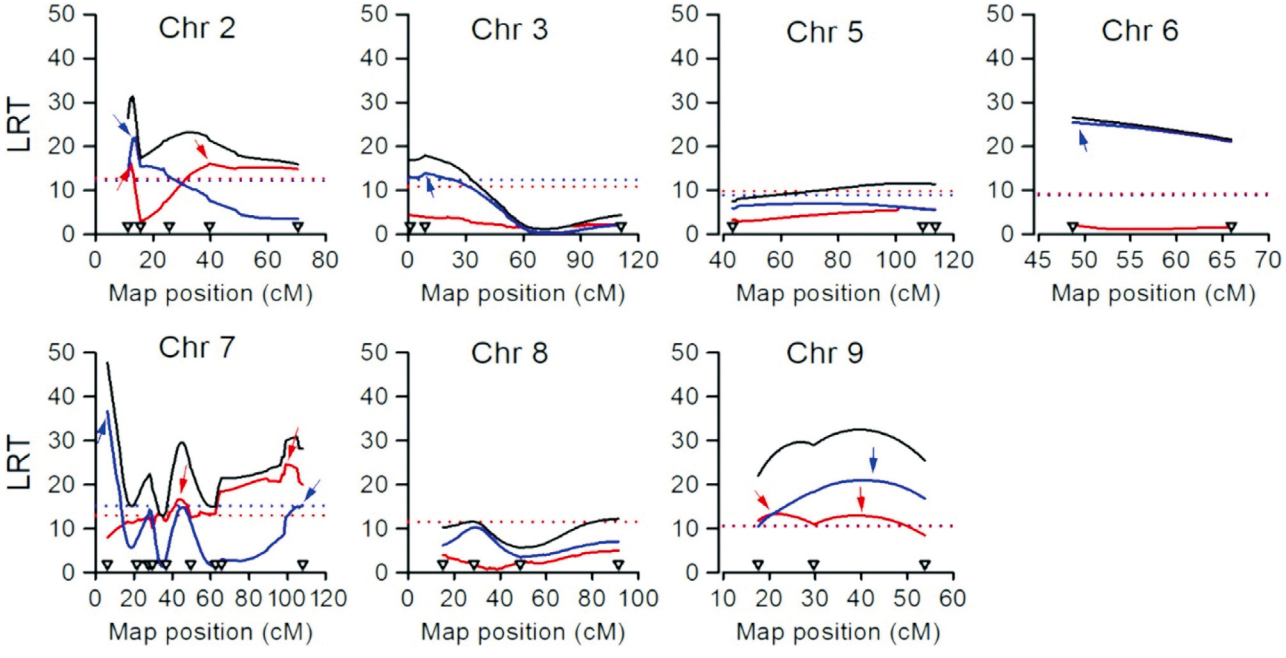

Fig. 3. Joint analysis of growth and viability quantitative trait loci (gQTL and vQTL, respectively). Red lines: profiles of likelihood ratio test statistics (LRT) for larval gQTL; blue lines: LRT profiles for larval vQTL; black lines: LRT profiles for the joint analysis of growth and segregation distortion. Dotted horizontal lines (red for gQTL, blue for vQTL): chromosome-level thresholds for statistical significance (Piepho 2001). Inverted triangles indicate the genomic positions of 29 markers across 7 chromosomes (Chr). Red and blue arrows indicate statistically significant gQTL and vQTL, respectively

9-3). The combined effects of these 2 independent gQTL, when regressed against measured shell length for each size class, explain a minimum of 18% of variance in shell length among size classes (Fig. S1).

The present study shows that, under routine culturing conditions, both growth and mortality of larval Pacific oysters depend substantially on genotype. Loci associated with viability are widespread in the genome, a conclusion consistent with a body of previous work (see references reviewed in Yin & Hedgecock 2021). The novel finding in the present study is that vQTL are largely distinct from gQTL (Fig. 3). The uncoupling of larval growth and mortality at the genotypic level provides a novel perspective on the relationship between growth and survival in natural populations with type-III survivorship and large inter-annual variability in recruitment.

If fast larval growth results in better survival in the marine environment ('bigger-is-better,' Houde 2008), natural selection should improve both traits. Our study demonstrates the feasibility of single larva QTL analyses and illustrates a possible genetic explanation as to why such a response might not occur, given that the complex sets of QTL determining survival and growth are largely non-overlapping. This finding is consistent with the low genetic correlation of growth and survival observed in quantitative genetic studies of molluscan larvae (Ernande et al. 2003, Barros et al. 2018, Chi et al. 2020). 'Bigger-is-better' may not be genetically determined. Clearly more research is needed both to quantify genetic components of variation and covariation in growth and survival of larval populations and to uncover the biochemical and physiological processes involved, since a mechanistic understanding could lead to the development of biomarkers predictive of recruitment success.

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