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Genetic inviability is a major driver of type III survivorship in experimental families of a highly fecund marine bivalve

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Abstract

The offspring of most highly fecund marine fish and shellfish suffer substantial mortality early in the life cycle, complicating prediction of recruitment and fisheries management. Early mortality has long been attributed to environmental factors and almost never to genetic sources. Previous work on a variety of marine bivalve species uncovered substantial genetic inviability among the offspring of inbred crosses, suggesting a large load of early-acting deleterious recessive mutations. However, genetic inviability of randomly bred offspring has not been addressed. Here, genome-wide surveys reveal widespread, genotype-dependent mortality in randomly bred, full-sib progenies of wild-caught Pacific oysters (*Crassostrea gigas*). Using gene-mapping methods, we infer that 11–19 detrimental alleles per family render 97.9–99.8% of progeny inviable. The variable genomic positions of viability loci among families imply a surprisingly large load of partially dominant or additive detrimental mutations in wild adult oysters. Although caution is required in interpreting the relevance of experimental results for natural field environments, we argue that the observed genetic inviability corresponds with type III survivorship, which is characteristic of both hatchery and field environments and that our results, therefore, suggest the need for additional experiments under the near-natural conditions of mesocosms. We explore the population genetic implications of our results, calculating a detrimental mutation rate that is comparable to that estimated for conifers and other highly fecund perennial plants. Genetic inviability ought to be considered as a potential major source of low and variable recruitment in highly fecund marine animals.

Keywords: early life-history mortality, fisheries, genetic load, mutation, recruitment

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Introduction

World fisheries are a critical natural resource, providing more than 20% of the protein that humans consume worldwide and employment to 10–12% of the world's population (Pontecorvo *et al.* 1980; Botsford *et al.* 1997; Dyck & Sumaila 2010). However, over the past 50 years, it has become increasingly clear that many fisheries are overexploited, in serious decline, or on the verge of collapse (Botsford *et al.* 1997; Pauly *et al.* 2002; Beddington *et al.* 2007; Worm *et al.* 2009). Currently, substantial effort and debate is focused on identifying the best methods and tools to rebuild and sustainably manage global fish stocks going forward (Pauly *et al.* 2002; Beddington *et al.* 2007; Worm *et al.* 2009).

A long-standing challenge in fisheries management is natural variability in stock–recruitment relationships and the resulting uncertainty in setting sustainable catch limits (Hjort 1914; Fogarty *et al.* 1991; Cushing 1996; Myers 2001). Marine fisheries primarily target species with a 'periodic' life history, characterized by long life, delayed reproduction, high fecundity and high early mortality (type III survivorship; Rickman *et al.*

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2000; Llodra 2002; Winemiller 2005). This periodic life history is adapted to interannual environmental variability, which causes recruitment numbers to vary commonly by several orders of magnitude (Roughgarden *et al.* 1988; Llodra 2002). Oceanographic and ecological factors underlying recruitment variability, such as lack of food availability, transport by currents to unsuitable habitat and predation, have been well understood for a century (Hjort 1914; Cushing 1990) and, together with biological factors, such as adult spawning strategies, larval duration and larval behaviour (Cowen *et al.* 2006), explain significant amounts of recruitment variability (Houde 2008). Still, predictions of future recruitment based on current stock abundance are often inaccurate (Myers 2001; Houde 2008), which suggests, perhaps, that not all factors causing early mortality have been identified. One possibility that has rarely, if ever been considered is that endogenous, genetic factors might play a role in early mortality.

The earliest evidence for genotype-dependent mortality in marine species with periodic life histories came from experimental studies of bivalve molluscs, which revealed that segregation ratios for molecular markers in progeny produced by controlled pair-crosses commonly deviated from Mendelian expectations (e.g. Wada 1975; Wilkins 1976). That such distortions are caused by selection against closely linked, recessive, deleterious mutations was subsequently implicated by numerous reports showing that both expressed (Foltz 1986) and unexpressed markers (Hu & Foltz 1996) were affected, that distorted transmission occurred in crosses of wild-caught parents but was exacerbated in families known to be inbred (McGoldrick & Hedgecock 1997; Bierne *et al.* 1998) or produced from small hatchery stocks (Wilkins 1976), and that distortions occurred unpredictably among markers and families (Mallet *et al.* 1985; McGoldrick & Hedgecock 1997). Viability selection against loci linked to markers was later definitively shown in inbred oyster families, by comparing segregation ratios at different life stages, leading to the inference that natural populations carry a large load of early-acting, lethal or nearly lethal, mostly recessive mutations (Bierne *et al.* 1998; Launey & Hedgecock 2001; Bucklin 2002). Subsequent application of quantitative trait locus (QTL) mapping methods to inbred F_2 families showed, further, that highly deleterious, mostly recessive alleles were scattered throughout the genome, caused mortality, typically, of more than 90% of fertilized eggs, and were expressed mostly in larval stages or during metamorphosis (i.e. corresponding to the type III survivorship curve; Plough 2011; Plough & Hedgecock 2011). Selection against these alleles and their dominance can be increased by a nutritionally poor diet, but overall genetic mortality remains high even in a high-quality, nutrient-replete diet, indicating that much of the load may not be conditional on environment (Plough 2012).

Although recent studies have provided definitive evidence for a high load of recessive mutations in oysters, they have not examined genetic inviability of randomly bred individuals, which could offer insight into what role, if any, mutational load might have in the natural early mortality of marine animals with periodic life histories. The null hypothesis is that early viability of randomly bred individuals should not be affected by recessive mutational load, because these mutations should largely be suppressed in heterozygous condition by fully functional, wild-type alleles.

Here, we examine segregation ratios of microsatellite DNA markers and single nucleotide polymorphisms (SNPs) in adult full-sib progeny from four pair-crosses of wild-caught Pacific oysters reared in Puget Sound and Willapa Bay, WA. Again, our null hypothesis is that we should see much less genotype-dependent mortality in these randomly bred families than we detected in previous studies of inbred families. Applying the same QTL methods previously employed for inbred crosses (Plough & Hedgecock 2011; Plough 2012), we investigate for the first time, the degree to which mortality of randomly bred oysters depends on endogenous differences in individual viability. We take a laboratory-based, as opposed to a field-based approach to estimate genetic mortality because full-sib families cannot be readily identified and followed through their life cycle in the field and significant sources of natural early mortality can be eliminated in the hatchery (e.g. predation, food limitation, migration, etc.; Hjort 1914; Korringa 1946; Rumrill 1990). Although caution is required in interpreting the relevance of experimental results for natural field environments, we argue that, because type III survivorship is such a strong characteristic of both hatchery and field environments, our results have implications for understanding low and variable recruitment in highly fecund marine animals, while suggesting the need for additional experiments under near-natural conditions (Benincà *et al.* 2008, Sommer *et al.* 2015).

Methods

Crosses, culturing methods and sampling

In July 2006, four full-sib families (F12, F20, F24 and F45) were created by pair-crossing wild Pacific oysters collected in Dabob Bay, WA, a randomly mating population founded by massive seed importation over decades (Mann 1979; Quayle 1988; X. Sun & D. Hedgecock, unpublished data). Crosses were performed in the

research facility of the Taylor Shellfish Farms hatchery in Quilcene, WA (see timeline in Table S1, Supporting Information), and larvae from each family were cultured, following published experimental procedures (Hedgecock & Davis 2007), in separate 100-l vessels of filtered sea water, at an initial stocking density of 10 larvae/ml, with a mixed-algal diet of *Isochrysis galbana*, *Chaetoceros calcitrans* and *Tetraselmis suecica*. Survival from fertilization to the D-hinge larval stage (day 2) across the four families ranged from 40% to 60%, which is typical (e.g. Ernande *et al.* 2003; Plough & Hedgecock 2011). No other estimates of larval or early juvenile (spat) survival were made during the hatchery phases of this experiment, but hatchery logs record nothing out of the ordinary with regard to larval mortality (typically high; e.g. Lannan 1980a; Mallet & Haley 1983, 1984) or to early seed mortality (typically low; Hedgecock & Davis 2007). Larval periods ranged from 18 to 24 days, and after metamorphosis and setting, a minimum of ~4000 juvenile oysters for each family were selected randomly (without regard to size or shape) for rearing in separate, downwelling, and indoor nursery silos.

By October 2006, juvenile oysters were large enough to be placed into pearl nets in Dabob Bay for further growth. In June 2007, oysters were retrieved from Dabob Bay, stocked into replicate bags at 100 oysters per bag, and deployed to Willapa Bay, WA, and Totten Inlet, WA (Table S1, Supporting information). Excess oysters were put into bags in Thorndyke Bay; samples for genetic analysis were subsequently taken from these bags, in December 2007. Although survival was not recorded in these early field stages of culture, obvious mortality would have been noted as a departure from the typically quite high survival at these stages (Hedgecock & Davis 2007). Survival of families in the Willapa Bay and Totten Inlet localities was recorded in April 2008 (survival from stocking at 1 year to day 644 post-fertilization) and averaged 93.8%. All bags in Willapa Bay were lost to winter storms, so a new set of six replicate bags of 40 oysters each per family was made for this locality from the Totten planting. By October 2008 (day 837 post-fertilization, ~2.3 years), cumulative survival in Willapa Bay averaged 74.3%, although survival in F20 and F45, 92% and 86%, respectively, was significantly higher than that in F12, 56% (contingency chi-square = 77.7, 2 d.f., $P < 0.0001$). Additional individuals were taken from F12, F20 and F45, to increase sample size; adductor muscle tissues were preserved in 70% ethanol, at 4 °C. Following Plough & Hedgecock (2011), samples for genotyping were taken at 1.4 and 2.3 year so that a calculation of genetic mortality over the life cycle could be made.

DNA extraction and genotyping methods

DNA was extracted from 10 to 25 mg of preserved adductor muscle tissue using either the Qiagen DNeasy Animal Tissue or the Gentra Pure-gene tissue kit (Qiagen, Valencia, CA, USA), following manufacturers' protocols. Microsatellite markers cloned from the Pacific oyster were PCR amplified in individual reactions and sequenced on the ABI Prism 377 DNA sequencer (Perkin Elmer, Waltham, MA, USA) as described previously (Launey & Hedgecock 2001; Plough & Hedgecock 2011; Plough 2012). SNP markers were isolated from expressed sequence tags (EST) for the Pacific oyster (Hedgecock *et al.* 2015) and genotyped via one of three methods. Ten SNPs in F45 were genotyped at the Roswell Park Cancer Institute, Buffalo, NY, USA, using the iPLEX® Gold mass array platform (Sequenom, San Diego, CA, USA; Gabriel *et al.* 2009). The rest of the SNPs in F45 and the other three families were among 1536 SNPs genotyped with an Illumina GoldenGate bead array (Shen *et al.* 2005) at the USC Keck Epigenome Center) for individuals from five mapping families, including the parents and first 46 progeny of F20, F24 and F45; nearly 1100 of these are placed on the Pacific oyster linkage map (Hedgecock *et al.* 2015). Next, high-resolution melt (HRM) assays were developed, using a Roche LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA), for genotyping 53 of the SNPs mapped with the Golden Gate data (see Sun *et al.* 2015). HRM analyses were carried out for segregating markers in the remaining individuals of F20, F24 and F45 and in F12, once HRM assays on the F12 parents revealed which markers were segregating. See Tables S2–S6 (Supporting information) for complete details on marker mating types, genotyping platforms and the specific markers interrogated in each family.

Analysis of segregation data

As a first examination of segregation at markers across the genome, genotype data for each marker were tested individually for deviations from expected Mendelian ratios, using goodness-of-fit chi-square tests, with significance reported at both the $\alpha = 0.05$ level and the Bonferroni-adjusted level. Marker segregation data can be categorized by mating type, which can, in turn, be categorized by the number of alleles segregating in the genotypes of the two parents. For the microsatellites, mating types were observed with as few as two alleles segregating (sire × dam; AA × AB, AB × AA, AB × AB; 11 of 92 cases), but more commonly three or four alleles were segregating (e.g. AB × AC, AC × AD; 81 of 92 cases; see Table 1). SNPs were almost exclusively biallelic in these families and exhibited mating

Table 1 Numbers of markers yielding segregation data, by cross-type and family, followed, in parentheses, by numbers of markers with significant deviations of genotypic proportions from expected Mendelian ratios at the nominal $\alpha = 0.05$ level

	Family				
Cross type	12	20	24	45	Totals
AA × *AB*	14 (5)	15 (8)	2 (0)	42 (20)	73 (33)
AB × *AB*	5 (3)	4 (3)	6 (1)	8 (3)	23 (10)
AB × *AC*	2 (2)	4 (3)	3 (2)	1 (1)	10 (8)
AB × *CD*	20 (13)	15 (12)	15 (9)	22 (20)	72 (54)
Totals	41 (23)	38 (26)	26 (12)	73 (44)	178 (105)

types AB × AA (or AA × AB) most commonly (63 of 86 cases) and AB × AB less frequently (22 of 86, with one AB × AC cross; see Results, Tables S2–S6, Supporting information). Null alleles were detected at a small fraction of microsatellite markers (<10%), as has been observed previously in experimental crosses of oysters (e.g. Launey & Hedgecock 2001; Plough & Hedgecock 2011). Although problematic in wild population samples, null alleles are easily detected by observations of unexpected genotypes in experimental progenies (e.g., seeing BB in offspring from an AB × AA cross; Plough & Hedgecock 2011). In many cases, the genotypes can be recoded with no ambiguity (e.g. cross type AB × CØ can be recoded as AB × CD because the null allele represents a fourth parental allele detected in AØ and BØ rather than AD and BD offspring). Markers with null alleles that produced ambiguous genotypes in the offspring (e.g. cross type AB × AØ produces AA and AØ, which cannot be distinguished) were removed from our analyses.

Microsatellite genotypes were scored in duplicate reactions for at least 5% of individuals and an average of 17 markers, in each family. Genotyping error averaged 2.51% across all families. Genotyping error rates for SNPs on the Sequenom, Illumina GoldenGate and HRM platforms were likewise determined from replicate reactions to be 0.9% (1/107; unpublished), 0% (0/12 937; Hedgecock *et al.* 2015), and 0.2% (1/449; Sun *et al.* 2015), respectively.

Contingency chi-square tests for homogeneity of genotypic proportions

To test for the possibility that genotype-dependent mortality could have occurred long after settlement, we performed contingency chi-square tests of genotypic proportions between the 1.4- and 2.3-year-old samples, calculating *P*-values via Monte Carlo simulation in R v3.0.2 (R development Core Team 2013). One-hundred forty of the 179 markers were available for this analysis: family 24 was only sampled at one time point, and therefore could not be included, and 13 markers across the three other families lacked genotype data at the 2.3-year time point. *P*-values across the 140 markers were adjusted for multiple comparisons with a sequential Bonferroni correction.

Linkage map construction and QTL mapping

Linkage maps were constructed separately for the four experimental families (F12, $n = 167$; F20, $n = 127$; F24, $n = 94$; F45, $n = 169$) in JoinMap 4.1, using the Kosambi mapping function with a minimum likelihood of the odds (LOD) score of 3.0 for linkage group (LG) assignments (Van Ooijen 2006). Separate maps were created for each family, because marker order has been shown to differ across families (Hubert & Hedgecock 2004) and not all markers were segregating or genotyped in all four families. For LG 2 in Family 24, sparse marker coverage produced a longer than expected interval between Cg145 and Cg157, compared with previous maps, so we used the interval length from F45 and previous maps.

Assignment of markers to LGs was largely in accord with previously published maps, with an average of 5 markers per LG (Hubert & Hedgecock 2004; Plough & Hedgecock 2011). Forty-one (24 microsatellites, 17 SNPs), 38 (24 microsatellites, 14 SNPs), 26 (18 microsatellites, eight SNPs) and 73 (26 microsatellites, 47 SNPs) markers were genotyped in families 12, 20, 24 and 45, respectively (Table 1, Tables S3–S6, Supporting information).

Phase information, marker locations, and parent and progeny genotypes are inputs for the viability model of Luo & Xu (2003) implemented in PROC QTL, a user-defined procedure for SAS (SAS Institute Inc., 2009). The viability model incorporates a QTL mapping procedure that scans the genome in specified increments (1 cM in this study) for the presence of loci associated with viability selection (genotype deficiencies), under a maximum-likelihood framework. Segregation and phase data for each marker are converted to a 4-allele cross type, *ab* × *cd*, with four genotypes. In the absence of viability selection, the genotypic frequencies at any point in the genome are expected to be Mendelian, that is 0.25: 0.25: 0.25: 0.25; sampling error and viability selection alter these expected proportions. The viability model was run on the adult genotype data from each of the four families separately (seven of 10 LGs for F12, eight of 10 LGs for F20 and F24, and 10 of 10 LGs for F45). Significance thresholds were set using a permutation approximation method based on the likelihood ratio test (LRT) profile from each family establishing

genome-wide thresholds at the $\alpha = 0.01$ level (Piepho 2001). Multiple QTL on a single LG were identified when the LRT statistic fell by at least 4.60 (~2 LOD) between two QTL peaks (Lander & Botstein 1989).

Genetic effects from viability QTL-associated markers

Once significant QTL were found, a series of nested chi-square tests (Foltz 1986) was employed to distinguish statistically the fitness effects of individual paternal alleles (the sire effect), maternal alleles (the dam effect) and interaction between parental alleles. These tests were performed at the position of each QTL; genotype arrays (counts) were generated by multiplying the estimated genotype frequencies by the average sample sizes across all markers within a family, taking into account missing genotype data. Each test had one degree of freedom and was independent of the other tests. Tests were considered significant if their *P*-values fell below a Bonferroni-corrected threshold level of $\alpha = 0.05$.

Calculating the load of deleterious mutations in parents

In each family, the number of deleterious loci per parent was calculated by summing the occurrences of significant sire (S) and dam (D) effects across viability QTL (vQTL). In two cases (of 37 total), only the interaction effect (I) was significant, so one was added to the total for each parent, because both AC and BD genotypes were deficient, relative to AD and BC genotypes, requiring two independent dominance interactions. Counts for all significant effects were then summed across the four families and divided by eight to produce an average for each parent.

Testing for epistasis

Digenic interactions between vQTL were assessed with contingency chi-square tests of genotypic associations between markers most closely linked to QTL, and for all markers within a family (Plough & Hedgecock 2011; Plough 2012). Tests were adjusted for multiple comparisons to control the false discovery rate (Benjamini & Hochberg 1995).

Estimating mortality at viability QTL

Estimated genotypic frequencies at vQTL peaks were used to calculate survival and mortality attributable to each vQTL, following methods in Plough & Hedgecock (2011). Briefly, mortality was calculated from the relative survival of the four genotypes at each QTL peak, where the relative survival of each genotype, w_{ij} at a QTL is w_{ij}/w_{max}, and w_{max} is the proportion of the highest frequency genotype (Luo & Xu 2003). Average relative survival, S, at a QTL is as follows:

$$\bar{S} = \frac{(w_{11}/w_{max} + w_{12}/w_{max} + w_{21}/w_{max} + w_{22}/w_{max})}{4} = \frac{1}{4w_{max}}$$

Average relative mortality at this QTL, M, is $1 - \bar{S}$ or $1 - \frac{1}{4w_{max}}$. In finite samples, chance fluctuations in genotype proportions will produce a nonzero estimate of mortality by this equation, even in the absence of selection, so we correct for this sampling effect by means of simulation. Relative survival in simulations with no selection averaged 0.835, 0.825, 0.79 and 0.84 for families 12, 20, 24 and 45, respectively (cf. the theoretical expectation of 1.0), leading to upward adjustments of average relative survival at vQTL (Table 2). Because genotype frequency data come from adult samples, the inferred genetic mortality at each marker applies to the entire the life cycle, from egg to adult (e.g. Plough & Hedgecock 2011).

Results

Patterns of segregation distortion at single markers

Genotypic proportions deviate significantly from expected Mendelian segregation ratios in 56%, 68%, 42% and 59% of markers in the four families, respectively (Table 1). All four parental alleles can be distinguished at most microsatellite markers in these random pair-crosses (i.e. mating type *ab* × *cd* or *ab* × *ac*), confirming that parents are unrelated and most progeny are heterozygous, as expected from a randomly mating population (see Methods). Of the 82 fully informative cross types (last two rows, Table 1), 62 are significantly distorted (numbers in parentheses, Table 1). Almost all distorted markers (60 of 62) display deficiencies of heterozygous genotypes (and thus excesses of other heterozygous genotypes). In most cases (57 of 62), two heterozygous genotypes sharing a parental allele are deficient, suggesting linkage of that parental marker allele with a deleterious allele, having additive or partially dominant effects on viability (Tables S3–S6, Supporting information). Commonly, however, three of four genotypes are deficient, suggesting linkage of two parental marker alleles with independent deleterious mutations (41 of 62 cases).

A greater proportion of microsatellite loci exhibit segregation distortion compared with SNP markers (67 of 92 vs. 34 of 86, respectively; exact test for independence of counts for distorted and nondistorted, across marker

Table 2 Viability quantitative trait locus (vQTL), survival and genetic effects results for the four wild families

Family	vQTL	Linkage group	Pos.	LRT	*AC*	*AD*	*BC*	*BD*	Genetic effects	w_{max}	Survival	Adj. surv.
12	1	1	48.2	21.71	0.13	0.3	0.44	0.13	I	0.438	0.571	**0.587**
12	2	2	11.2	155.98	0.03	0.75	0.16	0.06	S, D, I	0.753	0.332	**0.337**
12	3	3	26.6	22.42	0.16	0.14	0.33	0.37	S	0.374	0.668	0.69
12	4	3	44.9	26.86	0.15	0.13	0.32	0.4	S	0.398	0.629	**0.648**
12	5	4	39.0	60.30	0.22	0.21	0.06	0.51	D, I	0.505	0.495	**0.507**
12	6	4	49.7	50.54	0.18	0.08	0.48	0.25	S, D	0.485	0.516	0.529
12	7	6	56.3	73.67	0.26	0.41	0.32	0.01	S, I	0.411	0.608	**0.626**
12	8	6	95.8	26.26	0.33	0.09	0.32	0.26	D	0.328	0.763	0.792
12	9	10	4.0	52.69	0.19	0.07	0.48	0.26	S, D	0.482	0.519	0.532
12	10	10	42.0	50.63	0.05	0.2	0.25	0.5	S, D	0.499	0.501	**0.514**
20	1	3	9.7	73.67	0.04	0.22	0.11	0.64	S, D, I	0.635	0.394	**0.402**
20	2	4	28.8	66.54	0.42	0.02	0.42	0.13	D	0.422	0.592	0.612
20	3	4	40.8	63.23	0.53	0.09	0.29	0.09	D	0.532	0.47	**0.482**
20	4	5	23.8	30.23	0.05	0.4	0.24	0.31	D, I	0.396	0.631	**0.653**
20	5	7	1.0	59.95	0.6	0.22	0.06	0.12	S, D, I	0.6	0.416	0.426
20	6	7	13.0	98.45	0.61	0.28	0.07	0.03	S, D, I	0.614	0.407	**0.416**
20	7	8	1.0	57.33	0.13	0.04	0.42	0.4	S	0.423	0.591	**0.61**
20	8	10	4.8	25.80	0.14	0.15	0.23	0.48	S	0.481	0.52	**0.535**
24	1	4	30.4	86.39	0.68	0.01	0.13	0.18	S, D, I	0.681	0.367	**0.376**
24	2	5	16.0	23.54	0.17	0.11	0.16	0.56	S, D, I	0.559	0.448	**0.46**
24	3	6	55.9	51.74	0.24	0.01	0.52	0.23	S, D	0.524	0.477	**0.492**
24	4	7	24.9	74.92	0.64	0.2	0.01	0.16	S, D, I	0.639	0.391	**0.401**
24	5	8	0.0	80.65	0	0.19	0.15	0.66	S, D, I	0.656	0.381	**0.39**
24	6	10	41.0	31.85	0.06	0.36	0.49	0.08	I	0.49	0.51	**0.527**
24	7	10	56.0	36.60	0.25	0.32	0.41	0.01	D, I	0.414	0.604	0.628
45	1	2	56.3	179.07	0.12	0.77	0.07	0.04	S, D, I	0.774	0.323	**0.328**
45	2	3	34.0	81.55	0.18	0.1	0.62	0.1	S, D, I	0.62	0.403	**0.411**
45	3	4	1.0	27.11	0.1	0.23	0.28	0.39	S, D	0.393	0.636	0.656
45	4	4	35.9	33.27	0.11	0.23	0.22	0.45	S, D	0.448	0.558	**0.574**
45	5	5	50.7	113.31	0.43	0.04	0.46	0.07	D	0.462	0.542	**0.556**
45	6	6	11.8	29.45	0.18	0.44	0.12	0.26	S, D	0.44	0.569	**0.584**
45	7	7	0.9	31.91	0.27	0.12	0.43	0.18	D	0.43	0.581	0.598
45	8	7	28.6	37.30	0.27	0.11	0.45	0.17	S, D	0.454	0.551	**0.565**
45	9	8	38.4	30.21	0.35	0.16	0.38	0.12	D	0.376	0.665	**0.686**
45	10	9	14.8	58.50	0.56	0.16	0.19	0.09	S, D, I	0.563	0.444	**0.454**
45	11	10	1.0	59.80	0.5	0.15	0.08	0.27	S, I	0.5	0.5	**0.513**
45	12	10	27.2	26.93	0.38	0.13	0.35	0.14	D	0.381	0.657	0.678

QTL, survival and genetic effects results for the four wild families. Genotype frequencies estimated by the model at QTL are displayed in columns *AC, AD, BC* and *BD* (cross type for all positions is *AB* × *CD*). Genetic effects are estimated from the nested chi-square tests of genotype counts at each QTL peak position, which are generated by multiplying the average sample size from a family by the estimated genotype frequencies. Significant sire effects (S), dam effects (D) and interaction effects (I) are listed for each QTL (Bonferroni-corrected $\alpha = 0.05$ level). Average survival and adjusted survival (corrected for sample size through simulation: see Methods) at each QTL are reported, along with w_{max}, the maximum genotype frequency at a given QTL. Estimates of cumulative survival use adjusted survival estimates and only QTL that are unlinked (>50 cM apart) or the more deleterious of QTL that are linked (QTL used to calculate cumulative survival given in bold).

type, $P < 0.0001$). However, the proportion of mating types also differs significantly between the two marker types: mating types AA × AB and AB × AA are the most common for SNPs (63 of 86), while they constitute only a small fraction of mating types for microsatellites (10 of 92; exact test for independence of marker types vs. mating types, AA × AB vs. all others, $P < 0.0001$). There are two genetic reasons why a SNP is less likely to show distortion than a microsatellite in our study. First, markers homozygous in one parent lack unambiguous haplotype associations with linked deleterious mutations in that parent (Fig. S1, Supporting information). Second, chance clustering of SNPs in regions of the genome lacking deleterious mutations may bias

against observing segregation distortion for this marker type. While microsatellite markers were chosen to be well distributed across the genome (Plough & Hedgecock 2011), SNP markers were selected and genotyped for this study before linkage information was available (Hedgecock *et al.* 2015). By chance, over 23 of 86 SNPs cluster on LG 1 (LG 1) (Table S2, Supporting information), which exhibits no distortions in three families and a minor distortion in the fourth, and no SNP marker occurs on LG 2, which shows the most extreme distortions of any LG (LRT values >180; Fig. 1).

Genome scans for viability QTL

Information from distorted segregation ratios is integrated through QTL mapping, to determine the number, location and effect of causal deleterious mutations throughout the genome (Plough & Hedgecock 2011). We detect 10, 8, 7 and 12 vQTL in families 12, 20, 24 and 45, respectively, all of which are above genome-wide significance thresholds ($\alpha = 0.01$; Fig. 1, Table 2). QTL are broadly distributed across the genome in each of the families, although all families lack vQTL on at least one LG that has marker coverage. Across families, vQTL appear to be in different genomic locations (e.g. only families 12 and 45 have significant QTL on LG two), which suggests that mutations leading to inviability are found at a large number of loci in the oyster genome (Fig. 1).

Parsing the gene-action at vQTL (maternal, paternal, or interaction effects, Fig. 2) provides further insight into patterns of viability selection and allows calculation of the total number of alleles causing mortality. Nested chi-square tests of genotype arrays at vQTL reveal that allele substitution effects (either paternal or maternal) are significant for 54 (73%) of the 74 possible effects at 37 identified vQTL (Fig. 2; Table 2). Significant interaction (dominance) effects are found across all four

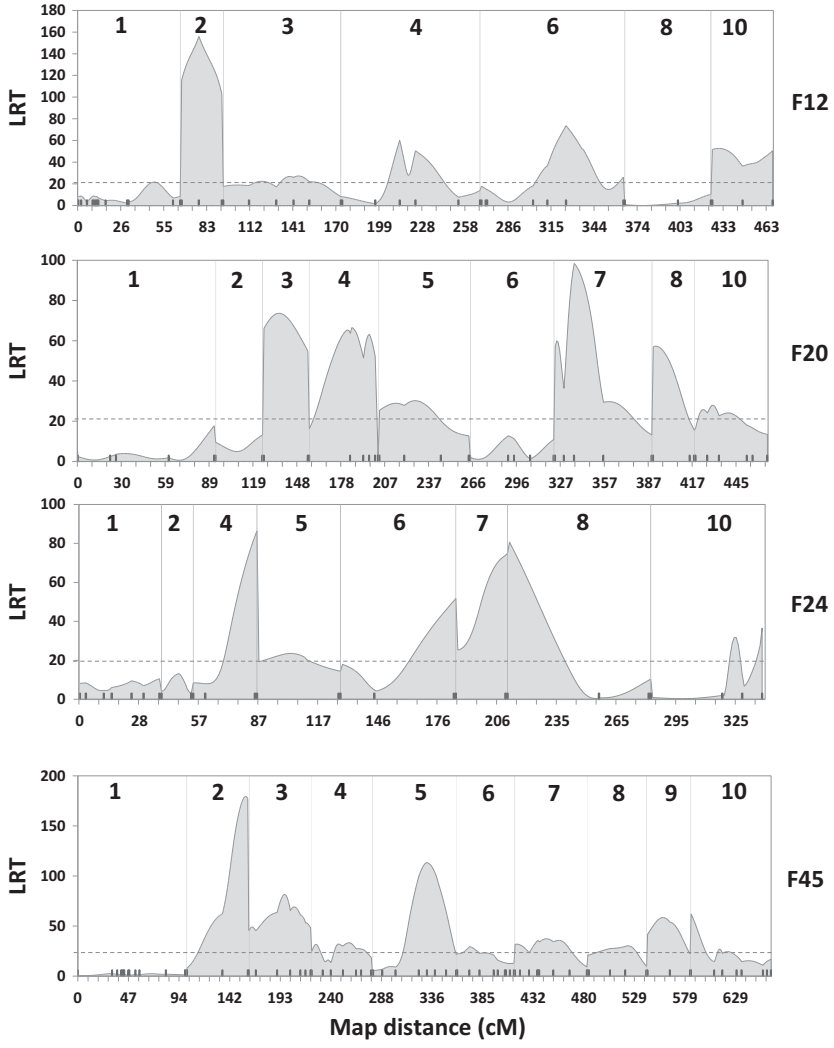
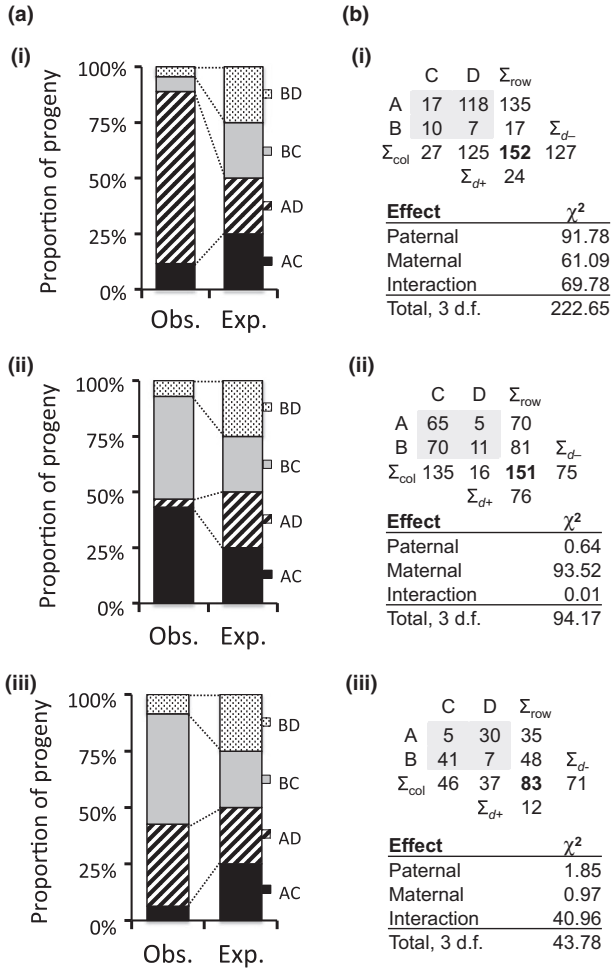


Fig. 1 Genome-wide scans for viability quantitative trait locus in the four random-bred families. The *x*-axis displays the centimorgan (cM) position for the linkage map in each family, and the *y*-axis is the likelihood ratio test statistic. Long vertical lines mark the ends of linkage groups, which are numbered 1–10. Small grey bars above the *x*-axis indicate the position of markers used in the mapping procedure. The dotted gray line indicates the genomewise threshold value for significance ($\alpha = 0.01$), 21.7, 21.7, 21.5 and 22.8 for families 12, 20, 24 and 45, respectively.



(i)

	C	D	Σ_{row}	
A	17	118	135	
B	10	7	17	Σ_{d-}
Σ_{col}	27	125	**152**	127
		Σ_{d+}	24	

Effect	χ^2
Paternal	91.78
Maternal	61.09
Interaction	69.78
Total, 3 d.f.	222.65

(ii)

	C	D	Σ_{row}	
A	65	5	70	
B	70	11	81	Σ_{d-}
Σ_{col}	135	16	**151**	75
		Σ_{d+}	76	

Effect	χ^2
Paternal	0.64
Maternal	93.52
Interaction	0.01
Total, 3 d.f.	94.17

(iii)

	C	D	Σ_{row}	
A	5	30	35	
B	41	7	48	Σ_{d-}
Σ_{col}	46	37	**83**	71
		Σ_{d+}	12	

Effect	χ^2
Paternal	1.85
Maternal	0.97
Interaction	40.96
Total, 3 d.f.	43.78

Fig. 2 Nested chi-square partitioning results for three example viability quantitative trait locus (vQTL) that exhibit (i) significance of all three allelic effects (vQTL 1, linkage group (LG) 2, Family 45), (ii) maternal effects only (vQTL 5 LG 5, Family 45) and (iii) interaction effects only (vQTL 6 LG 10, family 24). A: Graphical representation of observed and expected genotype proportions for three vQTL. B: Tables of raw genotype counts and chi-square partitions. Σ_{row}, Σ_{col}, $\Sigma_{d+/-}$ represent the row sum (Sire allelic effect), column sum (Dam allelic effect) and interaction sum, respectively for the three vQTL. See Table 2 for all vQTL chi-square effect results.

families, at 18 of 37 vQTL; they are the sole significant effect for only two vQTL (Table 2, Fig. S2, Supporting information). Significant paternal and maternal allelic substitution effects are detected in almost equal frequency, 25 paternal vs. 29 maternal, indicating no parent-of-origin effects. Co-occurrence of paternal and maternal effects (at 19 of 37 vQTL) suggests segregation of two deleterious alleles, one from each parent, a result consistent with patterns of genotype deficiencies observed at the majority of markers (Tables S3–S6, Supporting information). Counting co-occurrences of paternal and maternal allele substitution effects and interaction effects (see Methods), we have evidence for 15, 11, 13 and 19 deleterious mutations in families 12, 20, 24 and 45, respectively, yielding an average, across the eight parents, of 7.25 deleterious loci per diploid genome.

Mortality caused by each vQTL is generally quite high, ranging from 0.237 to 0.663 (Fig. 4, Table 2) and averaging 0.46. If vQTL are unlinked and act independently, they should have multiplicative effects on offspring survival in each family; thus, mortality, based on survival adjusted for sampling, at i, independent vQTL, can be calculated as $1 - \Pi\bar{S}_{i_{adj}}$. If, however, vQTL act in concert (epistatically) to produce mortality, then multiplying survival at individual vQTL would overestimate cumulative mortality. Pairwise tests of epistasis (contingency chi-square tests) between markers that are closely linked to each other reveal highly significant dependence, as expected (Fig. 3; Fig. S3, Supporting information); among unlinked markers, however, <1% of interactions (21 of 3945 combinations) are nominally significant ($P > 0.01$ for all), none after correction for the false discovery rate. As no evidence of epistasis is found in any family, we take unlinked vQTL as independent sources of inviability, yielding cumulative, egg-to-adult genetic mortality estimates of 97.9%, 98.3%, 99.3% and 99.8% in families 12, 20, 24 and 45, respectively (Fig. 4; Table 2, Fig S3, Supporting information).

Timing of viability selection and recorded adult mortality

Genotype-dependent mortality is determined in samples taken at ~530 and 837 days post-fertilization (Table S1, Supporting information). Recorded mortality for families 12, 20 and 45 between ~1 year and 1.4 year of age averages only 6%. By 2.3 year of age, cumulative mortality in these three families is 44%, 8%, and 14%, respectively (Table S1, Supporting information), but this late mortality is independent of prior genetic mortality, as contingency chi-square tests of genotypic proportions between the 530- and 837-day sampling points are significant for only eight of 140 loci, seven of which are already distorted at 1.4 year of age and simply undergo further distortion in the later sample.

Discussion

Distortion of Mendelian segregation ratios in randomly bred families

The majority of microsatellite DNA and SNP markers typed in four full-sib families, which were produced by crosses of wild-caught, adult Pacific oysters, show seg-

regation ratios that are significantly distorted from Mendelian expectations. This is, surprisingly, an overwhelming rejection of our null hypothesis that genotype-dependent mortality ought to be greatly reduced in randomly bred compared to inbred oysters, owing to suppression of recessive deleterious mutations. The extent of segregation distortion cannot be attributed to genotyping error or to other artefacts associated with microsatellite markers, such as null alleles. Compared to microsatellites, SNPs show lower proportions of segregation distortion, but this is attributable to the predominance among SNPs of AA × AB (or AB × AA) mating types, which lack power to detect deleterious mutations segregating in the marker-homozygous parent, and to the uneven genomic distribution of SNPs used in this study. This latter explanation is supported by more extensive SNP-mapping data for families F12, F20 and F45 (Hedgecock *et al.* 2015); for SNPs mapped to all LGs with an average spacing of 1 cM, the proportion of distorted segregation ratios is significantly higher than it is for the small, unevenly distributed subset of SNPs in this study (902 of 1846 vs. 30 of 86, respectively, $P = 0.02$).

Distortions of segregation ratios have been widely reported in experimental crosses of the Pacific oyster and other bivalve mollusc species over the past 40 years (e.g. Wada 1975; Wilkins 1976; Beaumont *et al.* 1983, 1988, 1990; Gaffney & Scott 1984; Foltz 1986; Thiriot-Quievreux *et al.* 1992; Hu *et al.* 1993; Hu & Foltz 1996; McGoldrick & Hedgecock 1997; Bierne *et al.* 1998; Launey & Hedgecock 2001; Bucklin 2002; Plough & Hedgecock 2011). The last three studies took samples at different time points, which showed that distortions are not present immediately after fertilization but accumulate afterwards. This rules out meiotic drive in parents as a cause of segregation distortion and leaves zygotic mortality, specifically viability selection against partially dominant mutations, as the only possible explanation. A similar conclusion was reached by several earlier studies (e.g. Beaumont *et al.* 1988, 1990; Thiriot-Quievreux *et al.* 1992). Distortions of Mendelian segregation ratios have, so far, been reported in only a few other highly fecund aquatic species, which are also expected to have high mutational loads (e.g. Liu *et al.* 2006; Zhu *et al.* 2013). Such species have not traditionally been as amenable as bivalve molluscs to breeding experiments, and removal of distorted markers for linkage mapping suggests that a reporting bias may also be at play (e.g. Hubert *et al.* 2010).

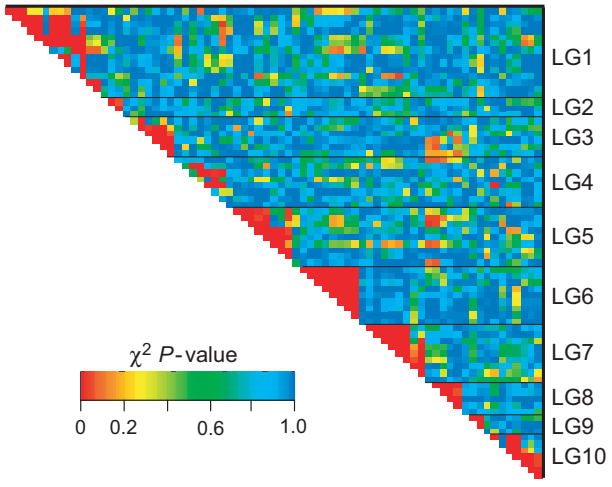


Fig. 3 Pairwise chi-square tests of the bilocus association of genotypes in family 45. Each block represents the chi-square test *P*-value (uncorrected) with warm colours (red) indicating significance and cold colours (blue) indicating nonsignificance. Markers are arranged in a pairwise fashion by ascending LG, which are marked with black lines; the diagonal of the triangular matrix has markers from the same LG side by side, and significant tests represent expected linkage associations. All red blocks (low *P*-values) off the diagonal were either above the $\alpha = 0.05$ level or were not significant after adjustment for the false discovery rate.

The architecture of genetic inviability

We apply QTL-mapping methods to examine the genetic architecture underlying these widespread distortions of segregation ratios. The mapping analysis reveals strong viability selection at 11–19 vQTL per family, owing to partially dominant deleterious paternal and maternal mutations, many of which also show significant dominance interactions. Summing up allelic effects for each parent, we calculate that wild adults each carry, on average, seven or eight partially dominant mutations. Obviously, parent oysters carrying these mutations did survive. In their progeny, slightly more than half of the heterozygous carriers of these mutations survive, based on average survival at vQTL (Table 2). Pairwise contingency chi-square tests across the genome reveal significant interactions only among linked vQTL, so unlinked vQTL are independent sources of mortality. Overall survivorship, calculated as the product of survival across unlinked vQTL per family, is remarkably low, ranging from 0.2% to 2.1%. In other words, ~99% of experimental progeny had to have died before we took samples for genotyping, to produce the widespread distortions of Mendelian segregation ratios observed.

This 99% genetic inviability in random-bred families is higher but probably not significantly higher than what we previously reported for inbred crosses, 90–96% (Plough & Hedgecock 2011; Plough 2012). Intuitively, one expects inbred crosses to suffer greater mortality from inbreeding depression, but evidence is mixed in the literature. Higher mortality is reported for random-

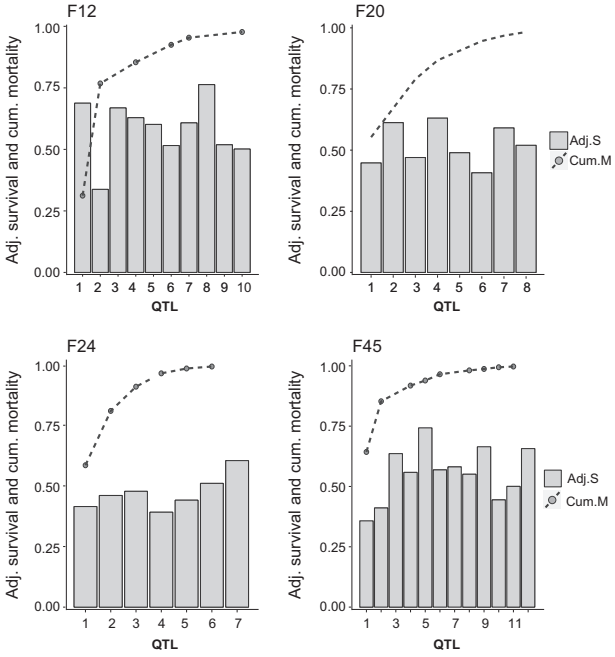


Fig. 4 Survival/cumulative mortality results for each quantitative trait locus (QTL) across the four random-bred families. Bars represent adjusted survival for each QTL, and filled circles represent cumulative mortality calculated for independent (unlinked) QTL.

bred relative to inbred crosses in some studies (e.g. Lannan 1980b; Mallet & Haley 1983, 1984), but the opposite is reported in many other studies (eastern oyster, Longwell & Stiles 1973; catarina scallop, Ibarra *et al.* 1995; Pacific oyster, Hedgecock *et al.* 1995, McGoldrick & Hedgecock 1997; Evans *et al.* 2004). Inbreeding depression might vary among studies, because inbreeding levels vary (e.g. Zheng *et al.* 2012), because inbreeding depression varies among families with the same degree of inbreeding (e.g. Liu *et al.* 2011), or because selection (purging) removes deleterious alleles in the generations leading up to any given experiment with inbred families. Purging has been demonstrated for a variety of species (Crnokrak & Barret 2002), including bivalves (e.g. McGoldrick & Hedgecock 1997; Zheng *et al.* 2012).

The important point is that, while QTL-mapping methods reveal high genetic inviability in both inbred and random-bred families, the genetic architectures of the inviability are strikingly different. In inbred families, it is largely identical-by-descent homozygotes that are inviable, reflecting a recessive mutational load uncovered by experimental crosses; in random-bred families, it is heterozygotes that are inviable, reflecting a surprisingly large load of partially dominant deleterious mutations in wild-caught parents.

Although our previous inbreeding experiments were designed to measure the viability of identical-by-descent homozygotes, we did, in fact, detect a number of partially dominant deleterious mutations in inbred families (Launey & Hedgecock 2001; Plough & Hedgecock 2011). In these studies, we used a two-locus model to jointly estimate dominance, selection and map distance from segregation data (genotype counts); this model was limited to mating types producing a marker homozygote hypothetically linked to a deleterious recessive mutation (e.g. deficiencies of AA from AB × AB or AB × AC crosses). Three of 20 cases in Launey & Hedgecock (2001, Table 3) and two of 11 tested cases in Plough & Hedgecock (2011, Table 2) showed partial dominance in the two-locus model. Moreover, in Plough & Hedgecock (2011), five AB × AC and one AB × CD mating types could not be treated with the two-locus model but showed heterozygote deficiencies similar to those reported here. In retrospect, we now better appreciate that experimental inbred families were carrying partially dominant mutations, which evidently had escaped selection in prior generations.

Genetic inviability likely corresponds with type III survivorship

The estimate of 99% genetic inviability is based on distortions of Mendelian segregation ratios in adults that were sampled at ~530 days post-fertilization or later (Table S1, Supporting information), to capture the full developmental spectrum of deleterious load (Plough & Hedgecock 2011). Actual, cumulative, egg-to-adult mortality in these families has to have been at least as high as genetic inviability but could have been higher, owing to environmental causes of mortality. Survival of young juveniles and adults in previous field growth trials is typically >90% (Hedgecock & Davis 2007) and ranged, here, from 90% to 96% for three families, from day 351 to 644 post-fertilization (Table S1, Supporting information). Mortality great enough to accommodate 99% genetic inviability must, therefore, have occurred during early life stages in the hatchery, where high mortality is typical (Helm & Bourne 2004). This timing would be consistent with the developmental patterns of selective and actual mortality previously observed in hatchery-reared inbred families (Launey & Hedgecock 2001; Plough & Hedgecock 2011; Plough 2012) and with type III survivorship in general.

How well do hatchery experiments represent nature?

Is genotype-dependent mortality an artefact of the hatchery environment and therefore unrepresentative of

genetic inviability in the wild? Based on our experience and the literature, actual hatchery mortality, from fertilized egg to spat (the early, post-metamorphic juvenile stage), is typically on the order of 99%, consistent with type III survivorship. The percentage of fertilized eggs that reach the D-hinge larval stage at day 2 in a hatchery typically ranges from 30% to 85% (Helm & Bourne 2004); in our study, it ranged from 40% to 60%. Survival from day 2 to metamorphosis is typically low, 30–75% (e.g. Lannan 1980a,b; Mallet & Haley 1983, 1984; Gallager & Mann 1986; Hedgecock *et al.* 1995, Ernande *et al.* 2003; Helm & Bourne 2004; Taris *et al.* 2006; Plough & Hedgecock 2011; Plough 2012; Joaquim *et al.* 2014). Metamorphosis success is rarely quantitatively measured but is only about 10% of apparently competent pediveliger larvae (Ernande *et al.* 2003; Plough 2011; Plough & Hedgecock 2011). Survival as high as 93%, from day 2 to 16, and metamorphosis success as high as 86% (both values not counting return to D-hinge; Rico Villa *et al.* 2008) are outliers, possibly owing to differences in methodology. In their well-replicated study, Ernande *et al.* (2003) found 51% of fertilized eggs at the D-hinge stage, 6.1% at day 24, and 0.9% at spat. Thus, the level of actual mortality necessary to accommodate 99% genetic inviability is consistent with egg-to-spat survival rates typically seen in controlled rearing environments.

Early mortality in nature, where factors, such as predation and mismatch with food availability, contribute to mortality, could easily absorb the genetic inviability observed in the hatchery. Natural mortality of bivalve molluscs and other marine species with type III survivorship is probably two or more orders of magnitude greater than hatchery mortality, largely because of predation (Korringa 1941, 1946; Thorson 1950; Rumrill 1990; Houde 2008). Coastal environments, where wild oyster larvae would naturally develop, may also be food-limited (Fenaux *et al.* 1994; Fotel *et al.* 1999; Bos *et al.* 2006) and more stressful than hatchery environments, in which larvae are supplied with food ad libitum. That hatchery mortality is largely explained by genetic inviability, leaving little room for environmental causes of mortality, is consistent with the absence, in this artificial environment, of the major sources of natural mortality.

Genetic inviability could be conditional on environment, a genotype × environment interaction, in which mutations that are deleterious in the hatchery are near-normal in the natural environment. This issue has been approached by manipulating environmental factors in hatchery experiments. For example, Beaumont *et al.* (1988, 1990) showed for juvenile mussels that distorted segregation ratios, which were large enough to imply 75% mortality, were largely unaffected by different salinities or temperatures. Plough (2012) recorded a fourfold difference in survival to the spat stage for two F_2 oyster families, each reared on single-algal and mixed-algal diets (n = 3086, 60-day-old spat for the 1-algal diet vs. n = 12 183 60-day-old spat for the 3-algal diet). Genetic inviability was greater on the single-algal compared to the mixed-algal diet (98.8% vs. 92.8% in one family; 98.3% vs. 84.1% in the other), because of an increase in selection but also a shift towards partial dominance of deleterious mutations (Plough 2012). The mixed-algal diet promoted better growth and survival but did not eliminate genetic inviability altogether, suggesting that the genetic load identified in the hatchery is only weakly conditional on environment. In light of these findings, the mixed-algal diet used to rear families for this study should have suppressed the deleterious effects of vQTL and biased against observing mortality of heterozygous, randomly bred individuals. The heavy mortality observed in these experiments is unlikely, therefore, to be attributable solely to the hatchery environment in which the oysters were reared.

The question of how well hatchery or laboratory experiments represent nature arises legitimately in many areas of science. Experiments will clearly be necessary to falsify the hypothesis that the genetic inviability observed here is an artefact of hatchery rearing. Fortunately, mesocosms offer one means of rearing experimental progenies under near-natural conditions (Benincà *et al.* 2008; Sommer *et al.* 2015) and the possibility of further testing the implication of our findings for natural populations.

Population genetics of deleterious mutations in the Pacific oyster

If these mutations reduce heterozygote viability in nature, how can they be so abundant in the natural population from which we obtained the wild, parent oysters? Such deleterious mutations should only exist at very low frequencies in natural populations, unless opposed by mutation and, possibly, random genetic drift.

First, let us consider how high the mutation rate would have to be to account for the transmission of so many viability alleles from the parents. From classical, mutation–selection, equilibrium theory for partially dominant lethal alleles, assuming selection coefficients, $s \approx 1.0$, as previously estimated for recessive viability-reducing alleles in the oyster (Launey & Hedgecock 2001; Plough & Hedgecock 2011), the number of lethal mutations carried by an individual in a diploid population is $n_{le} \approx 2\bar{q}n$, where $\bar{q}$ is the equilibrium frequency of lethal mutations and n is the number of loci at which lethal mutations can arise (Hedrick *et al.* 1999). From vQTL-mapping data, we estimate $n_{le} \approx 7.25$ per individual wild parent; if we assume that these mutations

can occur in $n = 5000$ genes in Pacific oysters (Harrang *et al.* 2013) then the average frequency of these partially dominant lethal mutations is 7.25×10^{-4}, that is each one is, indeed, expected to be quite rare.

Next, substituting this equilibrium frequency into the equation, $\bar{q} \approx u/h$ (Nei 1968), and solving for u with dominance $h = 0.5$, as implied by the relative survival of affected heterozygotes at vQTL, yields a per-locus mutation rate of 3.6×10^{-4}, only slightly higher than the estimated rate of 10^{-4} to 10^{-5} for lethal mutations in conifers (Lande *et al.* 1994). This, in turn, yields a genomic lethal mutation rate of $U = 1.81$, which is 90 times higher than the estimate for Drosophila (Simmons & Crow 1977) but not even two times higher than the upper end of the genome-wide range of lethal mutations in conifers, 0.1–1.0 (Lande *et al.* 1994; Karkkainen *et al.* 1996). Assuming that our calculation is approximately correct, the 90-fold greater genomic mutational load in the oyster compared to the fruit fly matches the contrast between the probability of 0.95 that a fertilized Drosophila egg develops to adulthood (Simmons & Crow 1977) and the probability of 0.01, determined here, that a fertilized oyster egg does so. Thus, the cumulative effect of numerous deleterious mutations, each one being rare, could account for our observations of genetic inviability at the family level. Such a large mutational load in the Pacific oyster is consistent with large mutational loads for deleterious recessive alleles documented for conifers and some other long-lived perennial plants, such as mangrove and eucalyptus, especially when map-based or genomic approaches are employed (e.g. Remington & O'Malley 2000; Myburg *et al.* 2014). For the oyster and other highly fecund marine animals, a high load of deleterious mutations affecting early viability may be an acceptable trade-off for the advantages of the periodic life history (Williams 1975).

Classical, mutation–drift, equilibrium theory, however, may overestimate the lethal mutation rate necessary to support the observed mutational load, because marine populations may be far from demographic and genetic equilibria, owing to variability in recruitment (Hjort 1914; Fogarty *et al.* 1991; Cushing 1996; Myers 2001; Houde 2008) and sweepstakes reproductive processes (Hedgecock & Pudovkin 2011), in which a relatively small fraction of the spawning population may be responsible for the bulk of recruitment. Some features of oyster population biology that could increase the load but not necessarily the rate of lethal mutations or that could increase the variance in or initial frequency of lethal mutations include sweepstakes reproductive success, low effective population size ($N_e \sim 150$; Hedgecock 1994, Li & Hedgecock 1998, X. Sun & D. Hedgecock, unpublished data), active transposable elements (see below), and the large number and potential clustering of mutations arising during premeiotic cell divisions (Klekowski & Godfrey 1989; Gao *et al.* 2011, 2014). The importance of premeiotic cell divisions for mutation accumulation is illustrated by molecular evidence for male-driven evolution in mammals and birds, in which males have three- to six-fold higher mutation rates than females (Ellegren & Fridolfsson 1997; Nachman & Crowell 2000; Makova & Li 2002). A better estimate of the lethal mutation rate in marine species with type III survivorship may ultimately be provided by coalescent population genetic theory allowing for multiple-mergers in genealogies (Steinrücken *et al.* 2013).

A high mutational load in the Pacific oyster is consistent with the very high levels of nucleotide and gap polymorphisms revealed by DNA sequences (Sauvage *et al.* 2007; Harrang *et al.* 2013). SNP frequency across four Pacific oyster genomes is 2.3% (Zhang *et al.* 2012), which is >40 times that observed in humans (Sachidanandam *et al.* 2001 but comparable to that in another highly polymorphic marine species, the purple sea urchin (Sodergren *et al.* 2006). Also, genome sequences from both inbred and wild oysters uncovered 3094 short indels, causing frameshifts in the coding regions of 2665 genes; thus, gap polymorphisms may be an important source of deleterious mutations. Transposable elements may also contribute to a high mutation rate in this species. Alignment of DNA sequences from a resequenced wild oyster to the reference genome assembly, which was made from an inbred oyster, identified 20 605 deletions of 100 bp or greater; more than 80% of these indels overlapped with known transposable elements (Zhang *et al.* 2012). In summary, structural and sequence polymorphisms in the Pacific oyster genome are consistent with a high rate of mutation, thus providing additional basis for the abundance of highly deleterious variants causing selection in the progenies of wild oyster crosses.

Implications for fisheries recruitment

Over the century since Hjort's influential paper (Hjort 1914), considerable evidence has been amassed on the environmental factors that cause high mortality in the early life stages of reproductively prolific marine organisms (Houde 2008). Although fisheries oceanographers have recently shifted focus to the influence of the abundance and quality of spawning adults on recruitment variability (Houde 2008), the possibility that any part of early mortality has endogenous causes has rarely, if ever, been considered. The current study demonstrates that a substantial fraction, up to 99% of the offspring produced by wild oysters, are genetically inviable and

that their deaths explain much of the observed early mortality in culture (Plough & Hedgecock 2011) and, perhaps, in the wild (Korringa 1946; Rumrill 1990). As mortality in the wild may well reach 99.99% or higher (Houde 2008), there is scope for both exogenous and endogenous causes of early mortality. Two additional, environmentally dependent factors could contribute to interannual variability in genetic inviability and ultimately in recruitment, one of the most vexing problems in fisheries management (Fogarty *et al.* 1991; Cushing 1996; Rickman *et al.* 2000). Sweepstakes reproductive processes (Hedgecock & Pudovkin 2011), which amplify variance in reproductive contributions among spawning adults, could cause genetic inviability to vary from cohort to cohort. Likewise, genotype-by-environment interaction could change the strength of selection or the degree of dominance of viability mutations, as shown by studies of inbred F_2 families of the oyster (Plough 2012), leading to different degrees of genetic inviability across patches of a heterogeneous ocean environment or across years that differ in major oceanic conditions relevant to recruitment success (e.g. temperature, food availability or quality). Whether genetic inviability is a major source of early mortality and recruitment variability in other marine animals with periodic life histories is an open question.

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L.P. and D.H. designed and conceived of the study; L.P. and G.S. performed molecular work and collected genotype data; L.P. and D.H. performed all of the data analysis and statistical analysis; L.P. and D.H. drafted the manuscript. All authors gave final approval for publication.

Data accessibility

Genotype data from the parents and offspring used in the mapping analysis deposited in Dryad (doi: 10.5061/dryad.7b6t9).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Illustration of mating type influence on detection of segregation distortion in SNPs.

Fig. S2 Chi-square pie charts showing the partition of significant allelic effects for all 38 QTL across the four families.

Fig. S3 Pairwise chi-square tests of the di-genic interaction of genotypes in families 12, 20, and 24.

Table S1 History of Dabob Bay wild families and timeline of genetic sampling.

Table S2 Complete marker list, including details on marker type, linkage group, and genotyping platform.

Table S3 Marker segregation data for family 12.

Table S4 Marker segregation data for family 20.

Table S5 Marker segregation data for family 24.

Table S6 Marker segregation data for family 45.