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Heterosis for yield and crossbreeding of the Pacific oyster *Crassostrea gigas*

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Abstract

Hybrid vigor or *heterosis* for growth and survival was demonstrated in experimental crosses of inbred Pacific oysters by Hedgecock et al. [Hedgecock, D., McGoldrick, D.J., Bayne, B.L., 1995. Hybrid vigor in Pacific oysters: an experimental approach using crosses among inbred lines. Aquaculture 137, 285–298.]. Substantial evidence for the pervasiveness of heterosis accumulated since then suggests a role for crossbreeding in commercial improvement. Here, we summarize evidence for yield heterosis in juvenile (seed) and adult oysters resulting from four diallel mating experiments. In pair crosses of parental inbred lines, we quantify heterosis by potence, $h_p = Q/L > 1.0$, where Q is twice the deviation of a hybrid from the mid-parent value and L is the absolute difference between the mean trait-values of the two parental inbred lines, these contrasts being estimated from ANOVA. For larger incomplete diallel crosses, in which partially inbred parental lines were not reared, we present estimates of Griffing's [Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9, 463–493.] general and specific combining abilities (GCA, SCA) and, where possible, reciprocal hybrid effects (R). GCA is significant in two of four crosses analyzed, while SCA is significant in all four crosses, particularly at the seed stage, and R is significant in all three crosses, in which reciprocal comparisons were possible. The reciprocal effect is partitioned into maternal (extra-nuclear) and non-maternal (extra-nuclear × nuclear interaction) effects; the latter are significant in 4 of 5 cases, while maternal effects are significant in only 2 of 5 cases. Improvement of commercial oyster seed can be achieved by a combination of selection among inbred lines and crossbreeding of elite inbred lines; pervasive differences between reciprocal hybrids may constrain the direction of interline crosses. Because correlation of seed and adult yield is positive but weak, we propose to retain top inbred parent lines based on seed yield and to re-test the most promising crosses on a much larger production scale.

Keywords: Heterosis; Pacific oyster; *Crassostrea gigas*; General and specific combining abilities; Reciprocal effects; Crossbreeding

1. Introduction

The Pacific oyster *Crassostrea gigas*, which has been exported to all continents but Antarctica (Mann, 1979), has had the highest production of any aquatic species for the past several years, 4.4 Mt, nearly one-tenth of global aquaculture production, in 2004 (most recent statistics at FAO, ftp://ftp.fao.org/fi/stat/summary/default.htm). On the U.S. west coast, the Pacific oyster industry is based largely on hatchery seed and is thus amenable to genetic improvement programs. Two joint university–industry research programs funded by the U.S. Department of Agriculture (USDA) have worked for a decade on genetic improvement of Pacific oysters in the western region. The Molluscan Broodstock Program (MBP), using family selection, has obtained 10–20% gains per generation for general yield across different rearing environments

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(Langdon et al., 2003). At the same time, a USDA Western Regional Aquaculture Center (WRAC) project examined the potential for exploiting hybrid vigor or *heterosis* for yield by crossbreeding of inbred lines.

Heterosis for growth and survival in bivalve molluscs was inferred from positive correlation of these fitness-related traits with allozyme heterozygosity (see reviews by Zouros and Pogson, 1994; Britten, 1996) and subsequently demonstrated experimentally in crosses of inbred Pacific oysters (*C. gigas*) by Hedgecock et al. (1995). The experimental approach enabled breeding of F_2 populations, with which definitive evidence for a large load of deleterious recessive mutations was obtained (Launey and Hedgecock, 2001). A large genetic load simultaneously explains oft-observed distortions of Mendelian ratios in studies of inheritance and supports the dominance theory of heterosis (Crow, 1998), at least for larval and early juvenile survival. These advances in our understanding of the causes of heterosis, however, were based on a handful of crosses and could not address broader questions pertinent to the utilization of heterosis for improvement of commercial stocks, i.e. how widespread is the phenomenon of heterosis in crosses of inbred lines, what are the relative contributions of additive and non-additive components of genetic variance to yield generally, and can the combining ability of parental lines be tested in an efficient and cost-effective manner. Here, we summarize evidence for yield heterosis in juvenile (seed) and harvest-size adult oysters, resulting from four incomplete diallel crosses, factorial matings using a set of six to nine inbred lines as both male and female parents. We partition variance in yield in these experiments into additive and non-additive genetic components, and present conclusions about the potential for crossbreeding to improve commercial oyster yields.

2. Materials and methods

2.1. Inbred lines and diallel crosses

All inbred stocks were derived by brother-sister mating within pedigreed families produced by the MBP from self-recruiting naturalized populations of *C. gigas* in Dabob and Willapa Bays, WA, USA, and Pendrell Sound, B.C., Canada (Langdon et al., 2003). Most inbred families produced in our diallel crosses were, thus, second-generation full-sib mated lines with inbreeding coefficients of $f=0.375$. Some lines, notably *1.035* and *1.051* in crosses made in 2003 were G_3 ($f=0.5$). We named crosses by year and cross number (e.g. 03x6). Inbred families were given a four-element name, giving birth-year and cross, hatchery of origin, generation of inbreeding (G_t), and the numerical name for the random-bred G_0 family, from which the line was initially derived (e.g. 02x2-T-2-c*1.035* denotes the G_2 of *1.035*, an MBP cohort-1 family propagated in the second experiment of 2002 at the Taylor Resources hatchery). A shortened identification for this family, 02x2 *35*, fits on a tag and provides essential cross-referencing to more complete pedigree records. Hybrids from factorial crosses are similarly identified, e.g. 02x2 *35×51* is the F_1 hybrid between a *1.035* ♂ and a *1.051* ♀ from cross 02x2).

Four partial diallel crosses, 01x1, 01x4, 03x6, and 03x8, furnished the material for this study. 01x1, a cross of lines *1.002*, *1.010*, *1.035*, *1.038*, *1.046*, and *1.051*, produced 23 of the 36 possible families (missing all but the *35* and *51* parental inbred families, and all members of the half-sib families from male *10* and female *2*. 01x4, a cross of lines *1.009*, *1.028*, *1.033*, *1.035*, *1.041*, *1.046*, and *1.053*, produced only 19 of the 49 possible families (missing all parental inbred families, the maternal half-sib families from *46* and *33*, which was omitted from subsequent analyses, and crosses *28×9* and *35×9*. 03x6, a cross of lines *1.020*, *1.026*, *1.035*, *1.036*, *1.045*, *1.047*, *1.051*, *1.052*, and *2.092*, produced 49 of the 81 possible families (missing parental inbred families *20*, *35*, *51*, and *52*, the maternal half-sib families for *20* and *51*, the paternal half-sib family for *52*). 03x8, a cross of lines *1.003*, *1.009*, *1.019*, *1.021*, *1.040*, *2.061*, and *2.094*, produced only 26 of the 36 possible families (missing parental inbred families *3* and *40*, the maternal half-sib families for *40* and *21*, which was omitted from subsequent analyses, and individual crosses *3×94*, *40×3* and *94×61*. In most cases, missing families were lost shortly after fertilization or during early larval stages, a likely consequence of inbreeding depression in the case of inbred lines (Hedgecock et al., 1995) or poor gamete quality in the case of maternal or paternal half-sibs (Lannan, 1980; Gaffney et al., 1993).

2.2. Biopsy, sexing, and genotyping of potential parents

We routinely took biopsy samples from 8–10 prospective parents per inbred family to check sex and pedigree (oysters are sequential hermaphrodites and have no external secondary sexual traits); either mantle (from oysters relaxed in Mg_2SO_4) or adductor muscle (from oysters completely removed from the shell and stored refrigerated in a zip-lock plastic bag) was sampled and preserved in ethanol for subsequent DNA extraction. A smear of gonad tissue was inspected under a light microscope to confirm presence of eggs or sperm.

We typed microsatellite markers in prospective parents and their parents, using methods described by Li et al. (2003) and Hubert and Hedgecock (2004). For 01x1, we used microsatellite markers *ucdCgi-001*, *ucdCgi-002*, *ucdCgi-006*, *ucdCgi-028*, *imbCG-49*, *imbCG-108*, *um2L-10* to analyze parents and putative progeny of MBP crosses producing the inbred stocks. In 2003, we used a set of 11 microsatellite DNA markers (*ucdCgi-002*, *ucdCgi-028*, *um2L-10*, *ucdCgi-184*, *ucdCgi-196*, *ucdCgi-117*, *ucdCgi-160*, *ucdCgi-141*, *ucdCg-157*, *ucdCgi-200*, and *ucdCgi-162*) distributed across the 10 linkage groups of the Pacific oyster (Hubert and Hedgecock, 2004) to confirm parentage and pedigree of all broodstock.

2.3. Culture methods and phases

We used a four-phase culture system for all crosses. We harvested eggs and sperm by strip spawning, made factorial

crosses nearly simultaneously in an array of 1-l plastic beakers, and transferred fertilized eggs to 100-l tanks for larval rearing (Phase I), using standard hatchery methods (Breese and Malouf, 1975). Families were not replicated in Phase I. Following a two- to three-week larval period, we used epinephrine to induce metamorphosis of larvae without attachment to cultch (Coon et al., 1986). Preliminary experiments showed that competent larvae from a given larval culture could be accumulated over several days in a refrigerator, with no measurable effect on subsequent metamorphosis and growth in the indoor upwelling system. Thus, by simultaneously treating the accumulated competent larvae with epinephrine, we eliminated differences in time of metamorphosis, which might have contributed to within-family variance in subsequent early growth.

Metamorphosed larvae were transferred to small down-welling tubes in an indoor replicated nursery culture (Phase II) at a density of 10,000 pediveligers per tube. A packed-volume method was used to determine and control the number of newly metamorphosed seed put into each nursery tube. Juvenile oysters were first size-fractionated on a series of Nytex screens to produce more uniform seed sizes and increase the accuracy of counts from a packed volume. When seed could be retained on a 7-mm^2 mesh (~75 days post-fertilization), we transferred them to an outdoor replicated nursery culture system consisting of suspended rotating seed cages (Phase III), at densities of 400 seed per cage. In these rotating seed cages, seed are tumbled and redistributed with each tidal cycle, encouraging more uniform growth and hardening of the shell. When field-reared seed could be retained on a 19-mm^2 mesh (115–145 days post-fertilization), we transferred them to on-bottom cages at densities of ~100 oysters per cage; cages were clipped to a nylon/dacron rope, which was staked to the intertidal substrate (Phase IV). The initial count and weight of the seed stocked into each cage were recorded. Each family was put into 6 cages, which were then deployed in a randomized block design in Thorndyke Bay, WA, for periods of 11 to 23 months, depending on experiment. Routine flipping of cages inhibited the growth of barnacles, which settle densely at this site and can greatly increase the cost of measuring yield. At the transition from Phase III to Phase-IV culture, 03x6 and 03x8 were moved from Thorndyke Bay to Totten Inlet in South Puget Sound, WA, to protect these crosses from winter storm damage.

2.4. Measuring yield

As oysters are held communally in cages and are thus in competition, we followed the MBP practice of ignoring individual measurements and focusing on cage-yield as the unit of interest (Hedgecock et al., 1997; Langdon et al., 2003). Yield data were collected mostly at the conclusion of Phases III (seed) and IV (harvest), although for 03x6 we weighed 100 seed from Phase II, as they were stocked into the Phase III cages. Yield is a function of both growth and survival, but mortality was negligible in our experiments (e.g. 3.4% and 8.0% in Phase IV of 01x1 and 01x4, respectively), enabling yield to be quantified by average or total weight of oysters in a cage. Replicate cages were randomized along lines at *iso*-tidal heights in the field; variation among replicate cages per family provided an estimate of within-family error variance. We obtained Phase-IV harvest yield data from all four crosses. Sufficiently replicated data on seed yield were obtained in experiments 01x1, 01x4, and 03x6; for the latter cross, we collected data on yield at the end of Phases II and III (called Seed-2 and Seed-3, respectively).

2.5. Statistical analyses, estimates and contrasts

For pair crosses of parental inbred lines, we quantified heterosis by potence, $h_p = Q/L > 1.0$, where Q is twice the deviation of a hybrid from the parental mean (the mid-parent value) and L is the absolute difference between the mean trait-values of the two parental inbred lines, these contrasts being estimated from ANOVA followed by appropriate tests (Griffing, 1990; Hedgecock et al., 1995). For larger, incomplete diallel crosses, in which most inbred parental lines were not reared, we estimated Griffing's (1956) general and specific combining abilities (GCA, SCA) and, where possible, reciprocal hybrid effects (R), using a random effects model.

We used Griffing's (1956) Method 4 to analyze diallel crosses without reciprocal hybrids (i.e. all ij crosses, for which $i>j$, making $p(p-1)/2$ F_1 hybrids from p parent lines). This method allowed more parents to be included in analyses with some loss of information from reciprocal crosses. For crosses 01x1, 01x4, 03x6, we substituted data for certain maternal half-sib families for missing paternal half-sib data or vice-versa (e.g. in 01x1, maternal half-sib line *10* data substituted for the missing paternal line *10* half-sib data). We substituted the occasional *ji*th hybrid for the *ij*th hybrid in 01x4 and 03x8 (e.g. data from 35×28 substituted for 28×35 in 01x1). Finally, we substituted the grand mean for missing cells in two crosses, 20×51 and 35×52 in analyses of 03x6 data for 9 parent lines and 3×40 in the analysis of 03x8 data for 6 parent lines. The linear model for Method 4 was

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk},$$

where μ was the grand mean, g_i and g_j were the additive effects of the two inbred parent lines, i.e. the general combining abilities (GCA) of the ith sire and jth dam line, s_{ij} was the non-additive interaction or special combining ability of cross ij (SCA, $i<j$), and e_{ijk} was error among replicates. We used Method 3 to analyze cases for which data on all $p(p-1)$ F_1 hybrids from p parent lines were available; this enabled estimation of an additional term in the linear model, r_{ij}, the estimate of extra-nuclear effects (R) causing differences between reciprocal hybrids, which should have identical nuclear-gene effects (i.e. AB=BA). R was further partitioned, $r_{ij}=m_i+m_j+n_{ij}$, where m_i is the maternal effect of parent line i, m_j is the maternal effect of parent line j, and n_{ij} is the non-maternal extra-nuclear×nuclear interaction effect of the ijth or jith hybrid. We used the DIALLEL-SAS05 program of Zhang et al. (2005; Zhang and Kang, 1997), with SAS 9.1, to carry out these analyses. Components of variance, $V(g)$, $V(s)$, and $V(r)$ are estimated by this program and tested under the random effects model (model 2) of Griffing (1956). Proportional contributions of the causal components of variance in yield are calculated from their expected contributions to yield variance: $V(Y)=2V(g)+V(s)+$

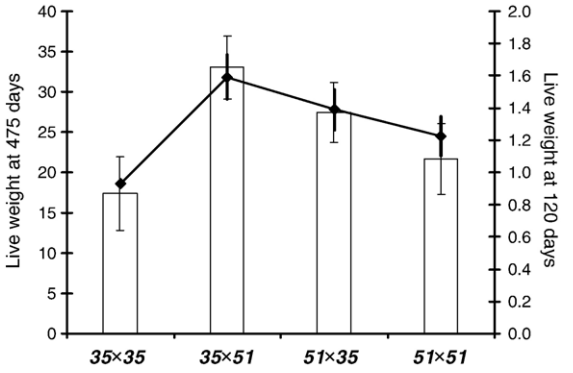

Fig. 1. Heterosis for growth is illustrated by the mean sizes of inbred and hybrid seed oysters at 120 days of age (line with diamonds and heavy, uncapped error bars, right *Y*-axis, in grams) and adult oysters at 475 days of age (bars with lighter, capped error bars, left *Y*-axis, in grams). The four families were produced by a cross of inbred parent lines *35* and *51*, as part of cross 01x1.

$V(e)$ for Method 4 and $V(Y)=2V(g)+V(s)+V(r)+V(e)$ for Method 3.

3. Results

3.1. Genotyping of prospective parents

We typed microsatellite DNA markers to confirm parentage and pedigree for 370 prospective parents of the four experimental crosses; of these, 39 (10.5%) had genotypes incompatible with their parents and were rejected as contaminants. Another 51 individuals (13.8%) were rejected for lack of information or uncertainty in genotyping. Contamination was especially heavy in stocks examined in 2003; for 18 inbred families (275 individuals), we found 35 contaminants altogether. Contamination was unevenly distributed across lines: five lines had no contaminants, five lines had one, three lines had two contaminants, and five lines had from three to six contaminants.

3.2. Potence in the 51 × 35 cross

A cross of inbred lines *35* and *51* made within 01x1 illustrated well the phenomenon of heterosis (Fig. 1). At both seed and harvest stages, both reciprocal hybrids were heavier than the better parent line *51*, a result consistent with Griffing's (1990) statistical definition of hybrid vigor ($h_p>1.0$). Since stocking density in cages was uniform (101.1 ± 1.7) and overall mortality was low (5.3%), mean live weight is an adequate measure of yield. Potence for 115-day-old seed yield was well above 1.0 and highly significant for the *35 × 51* hybrid ($h_{35}=3.51$, $P=0.0004$) and above 1.0 but not significant for the *51 × 35* hybrid ($h_{53}=2.12$, $P=0.064$). The average weight of the *35 × 51* seed was 150% of the mid-parent value. Hybrid *35 × 51* was 0.2 g heavier than the reciprocal *51 × 35* hybrid ($P=0.01$); mean weights for the two inbred oysters were not significantly different from each other. Potence at final harvest was well above 1.0 for both reciprocal hybrids ($h_{35}=7.12$, $P<0.0001$; $h_{53}=3.94$, $P=0.017$). At 33.1 g, the average *35 × 51*

Table 1
General and specific combining abilities of inbred lines of Pacific oysters *Crassostrea gigas* in diallel crosses without reciprocal hybrids (Method 4)

Cross	Stage	*p*	Model *F*	r^2	*V*(*g*)	*V*(*s*)	*V*(*e*)
01x1	Seed-3	6	$12.50_{14,\ 46}^{***}$	0.79	0.084**	0.034**	0.014
					0.78	**0.16**	**0.06**
01x1	Adult	6	$2.72_{16,\ 63}^{**}$	0.41	110,718**	−31,517	280,548
					0.44	**0.0**	**0.56**
01x4	Seed-3	6	$7.07_{14,\ 15}^{***}$	0.87	0.0292*	0.0157*	0.0094
					0.70	**0.19**	**0.11**
01x4	Adult	6	$3.11_{14,\ 64}^{***}$	0.40	317,923**	−48,520	201,892
					0.76	**0.0**	**0.24**
03x6	Seed-2	9	$25.38_{35,\ 140}^{***}$	0.86	0.561	12.816***	0.565
					0.08	**0.88**	**0.04**
03x6	Seed-2	7	$23.53_{20,\ 80}^{***}$	0.85	−0.995	12.133***	0.464
					0.0	**0.96**	**0.04**
03x6	Seed-3	9	$4.32_{35,\ 136}^{***}$	0.53	0.580*	2.104***	0.903
					0.28	**0.51**	**0.22**
03x6	Seed-3	7	$3.67_{20,\ 78}^{***}$	0.48	0.125	2.635***	1.030
					0.06	**0.67**	**0.26**
03x6	Adult	9	$1.67_{35,\ 117}^{*}$	0.33	80.96*	56.51	284.74
					0.32	**0.11**	**0.57**
03x8	Adult	6	$15.60_{19,\ 62}^{***}$	0.76	12.585	246.96***	17.54
					0.09	**0.85**	**0.06**

Cross label is year and experiment number; Seed-2, Seed-3, and adult yield are from Phase II, Phase III, and Phase IV, respectively; *p*, number of parent lines, model *F* from general linear model, with degrees of freedom and significance (*$P<0.05$, **$P<0.01$, ***$P<0.001$); r^2, proportion of variance explained; *V*(*g*) and *V*(*s*) are variance components for general and specific combining abilities, respectively, *V*(*e*) is mean square error; bold numbers below variances are proportional contribution to yield variance $V(Y)=2V(g)+V(s)+V(e)$ (negative components taken as zero; significance levels as above).

hybrid oyster was 13.7 g heavier than the mid-parent mean weight of 19.4 g, while the hybrid *51×35* oyster averaged 8.1 g more than the mid-parent. Hybrid *35×51* was 5.6 g heavier than the reciprocal *51×35* hybrid ($P=0.02$); mean weights for the two inbred oysters were not significantly different. Note the similarity of results for the seed and adult stages (Fig. 1).

3.3. General and specific combining abilities

Inbred parent lines were often missing from diallel crosses, owing to their decreased survival and growth (inbreeding depression), and entire half-sib families were also occasionally missing, owing evidently to poor gamete quality (Lannan, 1980).

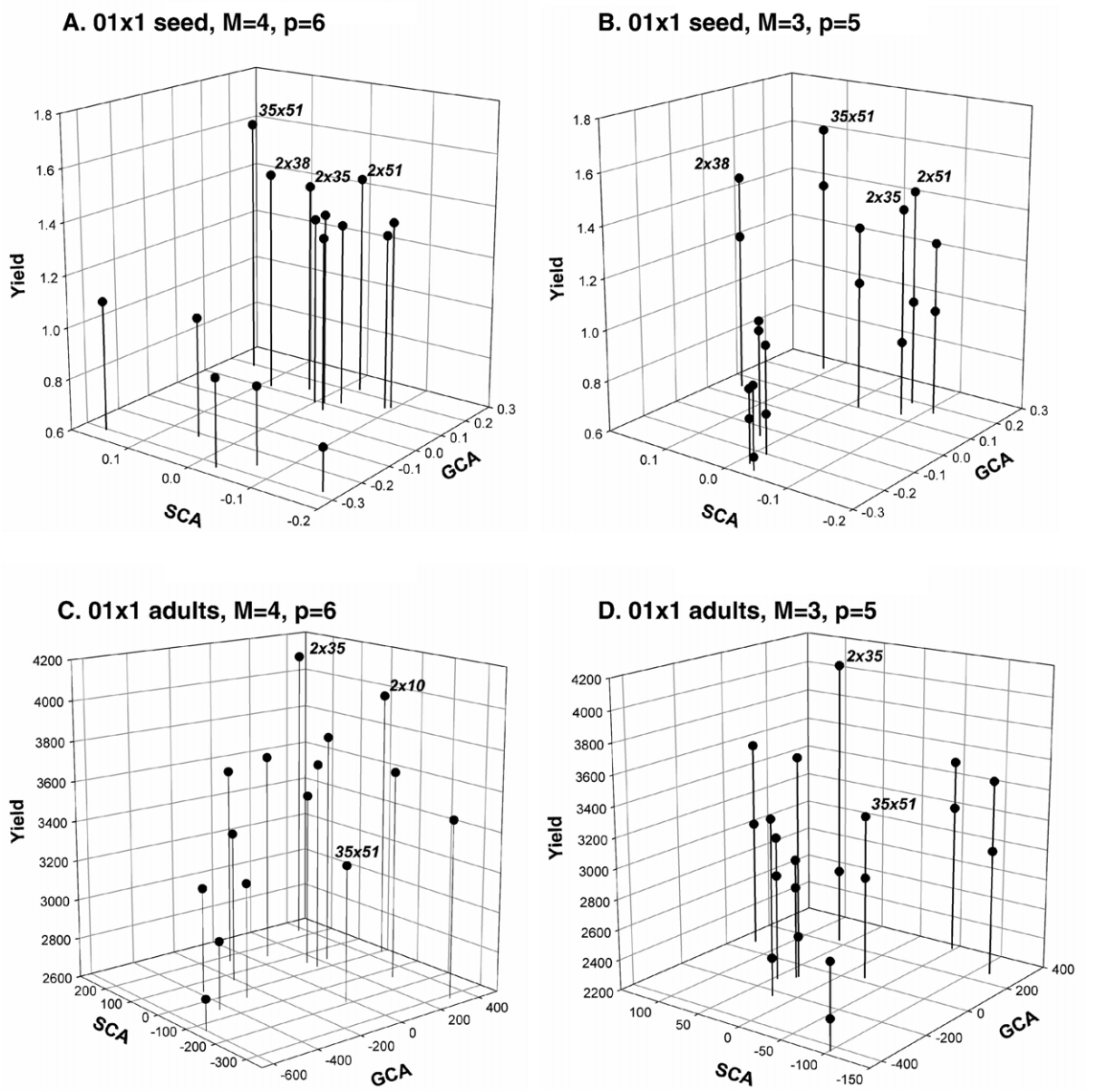

Fig. 2. Yield of hybrid families produced by the 01x1 partial factorial cross, as a function of the general combining abilities of inbred parent lines (GCA) and specific combining abilities of individual crosses (SCA). Average weight of individual seed-size oysters per cage (g) at 120 days (panels A and B) and yield per cage (g) at harvest (475 days, panels C and D) are analyzed, using two different methods and numbers of parent lines (panels A and C, Method 4, no inbreds and no reciprocal hybrids, with 6 parent lines; panels B and D, Method 3, no inbreds but reciprocal hybrids included, with 5 parent lines). GCA and SCA are deviation in grams from the grand mean yield in each experiment. The parameter, R_{ij}, representing the difference in yield between reciprocal hybrids, is calculated in Method 3 and displayed as the distance between the two balls on each stick (i.e. reciprocal hybrids have the same GCA and SCA). Significance of variance components associated with GCA and SCA are given in Tables 1 and 2. Higher-yielding or reference crosses are labeled in each panel.

Data from partial diallel crosses were used to estimate general (additive) and specific (non-additive) combining abilities and reciprocal effects, using the methods of Griffing (1956). We first used Method 4 to maximize the number of parents by not requiring reciprocal hybrids (Table 1). This method permits calculation of general and specific combining abilities but not reciprocal effects. Eight of 10 models investigated are highly significant ($P<0.01$, Table 1), and these explain from 40% to 80% of variance in yield. The variance component associated with GCA, $V(g)$, is significant in five cases and, when doubled to account for contributions from both parents, comprises from 28% to 78% of the sum of yield-variance components. Variance

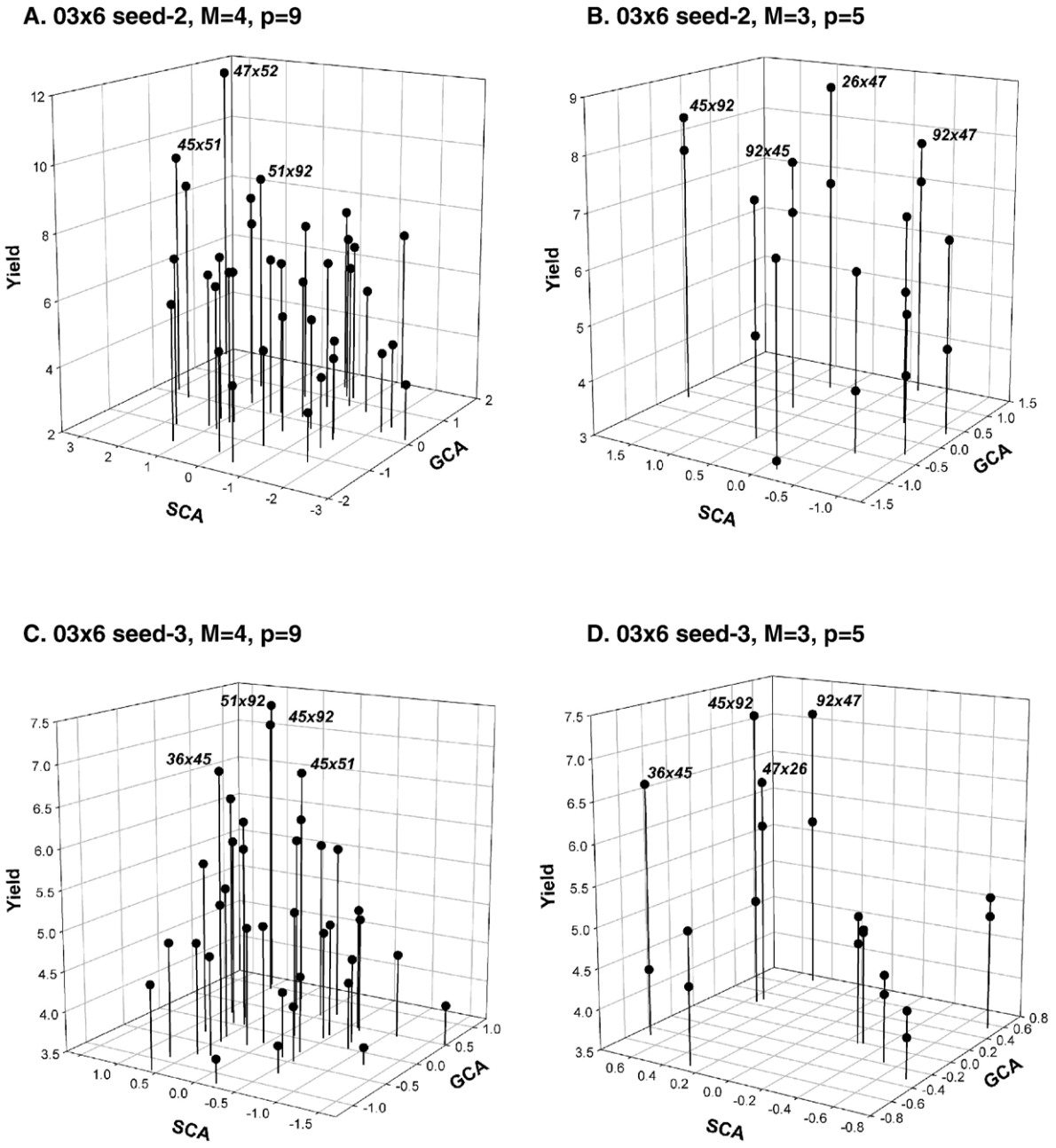

Fig. 3. Yield of hybrid families produced by the 03x6 partial factorial cross, as a function of the general combining abilities of inbred parent lines (GCA) and specific combining abilities of individual crosses (SCA), as in Fig. 2. SCA is significant in all analyses, GCA accounts for little of the variance in yield in this cross, and reciprocal effects are significant at the Seed-2 but not the Seed-3 stage (see Tables 1 and 2).

Table 2
General and specific combining abilities of inbred lines of Pacific oysters *Crassostrea gigas* in diallel crosses with reciprocal hybrids (Method 3)

Cross	Stage	*p*	Model *F*	r^2	*V*(*g*)	*V*(*s*)	*V*(*r*)	*V*(*e*)
01x1	Seed-3	5	$11.17_{19,\ 54}$***	0.80	0.0614	0.0399***	0.0482***	0.0207
					0.53	**0.17**	**0.21**	**0.09**
01x1	Adult	5	$11.09_{21,\ 85}$***	0.73	63,242*	3724	240,870***	99,955
					0.27	**0.01**	**0.51**	**0.21**
03x6	Seed-2	5	$7.56_{19,\ 75}$***	0.66	−0.408	6.431***	3.414***	1.379
					0.0	**0.57**	**0.30**	**0.12**
03x6	Seed-3	5	$2.29_{19,\ 71}$**	0.38	−0.195	1.730*	0.746	1.598
					0.0	**0.42**	**0.18**	**0.39**
03x8	Adult	5	$12.17_{19,\ 90}$***	0.72	41.06	53.26***	78.08***	15.84
					0.37	**0.24**	**0.33**	**0.07**

Cross label is year and experiment number; Seed-2, Seed-3, and adult yield are from Phase II, Phase III, and Phase IV, respectively; *p*, number of parent lines, model *F* from general linear model, with degrees of freedom and significance (*$P<0.05$, **$P<0.01$, ***$P<0.001$); r^2, proportion of variance explained; *V*(*g*), *V*(*s*), and *V*(*r*) are variance components associated with general and specific combining abilities and reciprocal differences, respectively, *V*(*e*) is mean square error; bold numbers below variances are proportional contribution to total yield variance, $V(Y)=2V(g)+V(s)+V(r)+V(e)$ (negative components taken as zero; significance levels as above).

associated with specific combining ability, *V*(*s*), on the other hand, is significant in seven cases and comprises 16% to 96% of the causal components of variance.

Comparing components of yield variance between stages within crosses, SCA or *V*(*s*) decreases (in significance, percent contribution, or both) from seed to adult stages in 01x1, 01x4, and 03x6 (Table 1). On the other hand, *V*(*e*) increases with stage in all three experiments, suggesting that variance among replicate cages increases during the culture cycle. GCA or *V*(*g*) also increases in significance or percent contribution in 01x4 and 03x6 but not in 01x1 (Table 1). In one cross, however, 03x8, *V*(*s*) is the only significant component of variance in adult yield, accounting for 85% of the causal components of yield variance.

In 01x1, seed oysters from Phase III averaged 1.32 g. General effects (g_i) were highly significant for lines *1.046* (−0.34 g) and *1.051* (+0.13 g). Five of 15 estimates of SCA (s_{ij}) were significant and contributed to at least two of the top-yielding crosses at this stage, *35×51* (+0.15 g) and *2×38* (+0.08 g) (Fig. 2A). At harvest, mean yield in 01x1 was 3500 g per cage; only two g_i and none of the SCA were significant. The highest performing cross, *2×35*, combined positive GCA (g_2=109.5 g+g_{35}=119.8 g=229.3 g) with positive SCA ($s_{2\times35}$=235.6 g), while the top-yielding cross at the seed stage, *35×51* ranked only 11th at harvest (Fig. 2C). Likewise, in 01x4, seed oysters averaged 0.94 g. Five of six general effects were highly significant, while only 3 of 15 SCA were significant. At harvest, mean yield in 01x4 was 2237 g per cage; only three g_i and none of the SCA are significant. Again, the highest performing cross, *28×46*, combined positive GCA (g_{28}=44.5 g+g_{46}=449.7 g=493.8 g) with positive SCA ($s_{2\times35}$=79.0 g).

In contrast to results in 2001, crosses in 2003 showed evidence for stronger non-additive components of yield variance. For 03x6, the largest cross with 9 parent lines, the average weight of 100 Phase II seed was 6.37 g. Seven of nine general effects were significant, contributing from −1.07 g to +1.22 g to average yield; 25 of 36 specific effects were significant, contributing from −2.82 g to 3.41 g to average yield.

Table 3
Original and random model *F*-tests of maternal (M) and non-maternal (NM) components of variance in diallel crosses of inbred lines of Pacific oyster *Crassostrea gigas* with reciprocal hybrids (Method 3)

Cross	Stage	Source	SS	*df*	MS	*F*	df_{Adj}	F_{Adj}
01x1	Seed-3	M	0.779	4	0.19465	9.420***	6	2.954
		NM	0.395	6	0.066	3.189**	54	3.189**
01x1	Adult	M	13476019	4	3369005	33.705***	6	10.421**
		NM	1939709	6	323285	3.234**	85	3.234**
03x6	Seed-2	M	11.874	4	2.968	2.153	6	0.255
		NM	69.958	6	11.660	8.457***	75	8.457***
03x6	Seed-3	M	6.980	4	1.745	1.093	6	0.424
		NM	24.667	6	4.111	2.575*	71	2.573*
03x8	Adult	M	1450	4	362.6	22.881***	6	10.983**
		NM	198	6	33.01	2.083	90	2.083

Cross labeled by year and experiment number. Seed-2, Seed-3, and adult yields are from Phase II, Phase III, and Phase IV, respectively. Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

The top-performing cross at this early seed stage, *47×52*, combined positive GCA (+1.73 g) and SCA (3.41 g), while the second ranked cross, *45×51*, achieved high yield through high SCA (2.94 g) with only a minor contribution of GCA (+0.12 g) (Fig. 3A). At the end of Phase III culture for 03x6, the average weight of seed per cage was 5.16 g. Only 5 of 9 general effects were significant, contributing from −0.84 g to +0.57 g to average yield; only 9 of 36 specific effects were significant, contributing from −1.69 g to 1.11 g to average yield. The top-performing cross at this later seed stage, *51×92*, combined positive and similar GCA and SCA (+1.06 g), as did the second ranked cross, *45×92* (GCA, +0.95 g; SCA, +0.98 g) (Fig. 3C). At harvest, when average weight of animals per cage had reached 125 g, the linear model for 03x6 was barely significant (Table 1), and only 1 of 9 general effects and 3 of 36 SCA were significant. Still, the top-yielding cross, *45×52*, combined positive GCA and SCA (+10.7 g and +16.3 g, respectively).

In order to construct a Method 4 analysis of 03x6 data incorporating all 9 parents, it was necessary to use data from reciprocal half-sib families (*35* maternal and *51* paternal) and to substitute the mean yield for two missing cells, *20×51* and *35×52*. It was also possible, however, to analyze a reduced set of 7 parents, which required substituting only 4 crosses from the maternal *35* half-sib family. The high level of agreement between the partitioning of variance in these two sets of results suggests that substitutions in the 9-parent set had little impact on the outcome (Table 1).

3.4. Reciprocal effects

We next used Method 3, which requires a full set of reciprocal hybrids, to check the results of Method 4 analyses and to estimate reciprocal effects for reduced sets of parents within crosses 01x1, 03x6 and 03x8 (Table 2). For 01x1 and 03x6, agreement between estimates of GCA and SCA for the two methods can be visualized in Figs. 2 and 3, comparing panels A with B and C with D for labeled crosses. Tables 1 and 2 also show agreement in significance and proportional contributions of $V(g)$ and $V(s)$ components. The model F for Method 3 analysis of 03x6 adult data was not significant ($P=0.088$), so it was not included in Table 2.

Reciprocal effects were highly significant in four of five analyses, and in the fifth analysis (for 03x6 Seed-3), the probability of $V(r)$, 0.054, fell just short of significance. $V(r)$ accounted for 21% to 51% of the causal components of yield variance. Disparity in yield between reciprocal hybrids was illustrated by the large distances between the two balls on each stick in Figs. 2B,D and 3B,D. The reciprocal effect was broken down into maternal and non-maternal components for the five crosses analyzed by Method 3 (Table 3). Maternal effects were significant in two of the five crosses (01x1 and 03x8 adults), while non-maternal effects were significant in four of the five crosses, being absent only from 03x8.

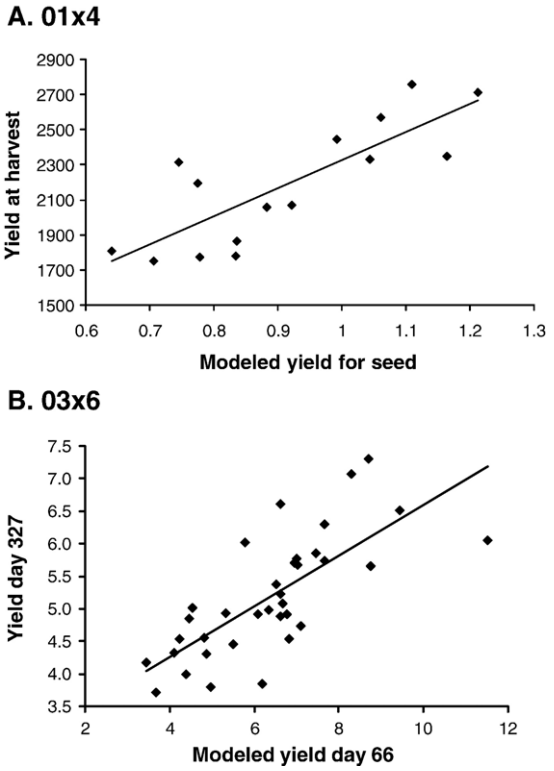

Fig. 5. Correlation of yield at a later time point with yield at an earlier time point based on a linear model with GCA and SCA components. A) cross 01x4, correlation of adult harvest yield at Phase IV with modeled seed yield at Phase III (M=4, $p=6$; $Y=1605.2X+718.39$; $r^2=0.63$; $F=25.16$, $df=1, 13$, $P<0.001$). B) cross 03x6, correlation of seed yield at Phase III (day 327) with modeled seed yield at Phase II (day 66) (M=4, $p=9$; $Y=0.390X+2.70$; $r^2=0.53$; $F=40.62$, $df=1, 34$, $P<0.001$).

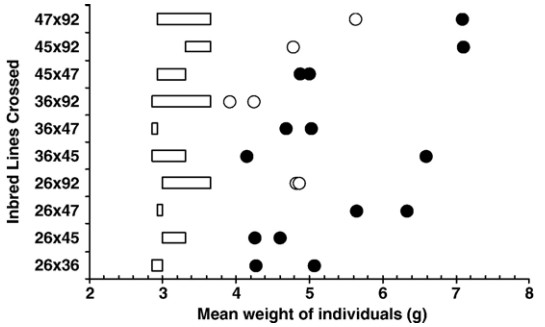

Fig. 4. Hybrid vigor in a complete 5×5 diallel within cross 03x6 (cf. Fig. 3D). For each cross on the *Y*-axis, the open rectangle shows the range in final seed yield (mean weight of individuals per bag at 327 days) between the two parental lines, whose yields are given by the projections of the rectangle's end lines onto the *X*-axis. Circles to the right of the rectangles, show the greater yield of all hybrids relative to the better of their inbred parents, i.e. $h_p>1.0$; unfilled circles are not significantly greater than the highest yielding parent line; filled circles are significantly greater, at an experiment-wide $\alpha=0.05$ level.

3.5. Potence for hybrid yield in the complete 5×5 diallel in 03x6

Within cross 03x6, a complete 5×5 diallel cross with reciprocals and inbred parent lines was available for analysis by Method 1 of Griffing (1956); analysis of the Seed-3 yield gave results comparable to those for Method 3 (Table 2), with non-significant $V(g)$, highly significant $V(s)$, and mildly significant $V(r)$. The proportional contributions of these variance components to the sum of the causal components in the Method 1 analysis, compared to those from the Method 3 analysis (Table 2), were weighted more towards $V(s)$ at 70% and less towards $V(r)$ and $V(e)$ at 11% and 20%, respectively. The reciprocal effect was solely non-maternal ($P=0.016$), as in the Method 3 analysis (Table 3).

This complete diallel cross permitted calculation of yield potence for 10, 2×2 factorial crosses embedded within it. Potence was greater than 1.0 (hybrid yield exceeded the yield of its better parent) for all 20 hybrid-crosses, ranging from $h_{36\times92}=1.9$ to $h_{47\times26}=100$, and significantly exceeded 1.0 for 11 hybrids (Fig. 4). In only two crosses, *36×45* and *45×92*, was the difference between reciprocal hybrids significant ($P<0.01$ in both cases); that line *45* was the maternal parent in the first case and the paternal parent in the second case illustrated the non-maternal nature of the reciprocal effect observed in the complete diallel. Indeed, the only significant non-maternal factors in the complete analysis were associated with these two crosses, $N_{36\times45}$ contributing 0.91 g and $N_{45\times92}$, 1.04 g to a mean individual weight of 4.80 g.

3.6. Correlation of yield at seed and adult stages

If seed yield predicted harvest yield, then parent lines with good combining ability could be identified at Phase III or possibly Phase II of culture, eliminating the cost for Phase IV. We explored the correlation of seed yield, modeled on estimates of GCA and SCA (Method 4), with final yield in experiments 01x1, 01x4, and 03x6. We also tested the correlation of modeled yield at Phase II with yield at Phase III in 03x6. Correlation of seed and adult yield in 01x1 and 03x6 was positive but not significant; correlation between seed and adult yield in 01x4 and between Seed-2 and Seed-3 yield in 03x6 was significant (Fig. 5).

4. Discussion

4.1. The pervasiveness and genetic basis of yield heterosis in the Pacific oyster

Heterosis for growth and survival in the Pacific oyster *C. gigas* was first demonstrated experimentally in two, 2×2 factorial crosses among inbred lines (Hedgecock et al., 1995, 1996). We adopted Griffing's (1990) operational definition of heterosis, potence, $h_p=Q/L>1.0$, where Q is twice the deviation of a hybrid from the parental mean (the mid-parent value) and L is the absolute difference between the mean trait-values of the two parental inbred lines, these contrasts being estimated from ANOVA followed by appropriate tests of significance. This definition of heterosis differs from that typically employed by animal geneticists, percent deviation from the mid-parent, which does not specify whether hybrid performance exceeds that of the better parent. The experimental approach confirmed the non-additive nature of genetic variance for fitness-related traits in this bivalve mollusc and led to experimental demonstration of mutational load as a cause of marker-associated inbreeding depression and a likely cause of heterosis (Bierne et al., 1998; Launey and Hedgecock, 2001; Bucklin, 2002). Much experimental evidence for heterosis in growth and survival of larval stages has accumulated in the interim since the initial reports (Hedgecock et al., 1995, 1996). Pace et al. (2006), for example, showed that heterosis for larval growth or size at age was evident in 21 or 23 comparisons obtained in four, 3×3 factorial crossing experiments.

Our purpose, here, was to summarize an accumulated body of evidence concerning heterosis for yield of juvenile and adult oysters, traits of interest in oyster farming (Hedgecock et al., 1997; Langdon et al., 2003). Because oysters on the U.S. West Coast are increasingly reared for the half-shell market as single oysters in cages, in which they compete for phytoplankton resources, oyster breeders must, like plant breeders, treat the cage (=plot) as the unit of replication and measurement (see Sheridan et al. (1996) and Sheridan (1997) for a different point of view and approach). This reduces the power of statistical analysis, since the number of measurements is reduced from the 50 individuals that are typically measured at each water change in a larval culture (e.g. Pace et al., 2006) to six or fewer replicate cages per family per site. Nevertheless, even with this level of replication, we obtained significant linear models in almost all cases (Tables 1 and 2), although error variance appeared to increase with stage of culture. The questions motivating this study were how widespread is the phenomenon of heterosis in crosses of inbred lines evaluated in aquaculture systems, what are the relative contributions of additive and non-additive components of genetic variance to yield generally, can the combining ability of parental lines be tested in an efficient and cost-effective manner, and can crossbreeding be used to improve commercial oyster yields.

Diallel crosses of inbred lines of the Pacific oyster *C. gigas* derived from naturalized populations in the Pacific Northwest USA consistently showed heterosis for yield, suggesting that this is a pervasive phenomenon.

Demonstrating heterosis by the operational definition, $h_p > 1.0$, requires yield data for F_1 hybrids and their parent lines, which were available only for the *35×51* cross within 01x1 (Fig. 1) and a complete, 5×5 diallel subset within 03x6 (Fig. 4). In all of these cases, hybrid yield exceeded the yield of the better parent line, and in 16 of 22 cases, this difference was significant, despite the limitation of an average of 4 replicates for inbreds and 4.6 replicates for hybrids. This degree of heterosis has not been observed in farmed fish, though most studies employ the difficult to interpret deviation-from-mid-parent definition of heterosis and use crosses among intraspecific populations or varieties rather than crosses among inbred lines (e.g. Gjerde and Refstie, 1984; Bentsen et al., 1998; Bryden et al., 2004; but see Wohlfarth, 1993). Cross-breeding is rare in fish breeding programs with the notably exceptions of those for common carp and channel catfish (Hulata, 2001).

In most factorial crosses, it was not possible to rear all parental lines alongside the F_1 hybrids. For these experiments, we calculated general and specific combining abilities, using methods described initially by Griffing (1956) and implemented in DIALLEL-SAS05 (Zhang et al., 2005; see also Zhang and Kang, 1997) for incomplete diallel crosses. Such diallel crosses produce a set of F_1 hybrids that are representative of individuals in the natural populations from which the inbred parents were derived. The advantage of the diallel experiment over observations of wild individuals is that it enables us to partition variance in yield (size-at-age) into causal components. Results from these four experiments showed that both additive and non-additive components of variance are important contributors to oyster yield (Figs. 2 and 3). Yield generally increased with GCA, as expected, but high-yielding hybrids with high, positive SCA and little GCA were also observed. The non-additive genetic component of yield variance is often the largest. This is most clearly seen at the seed stages; $V(s)$ is significant for all three crosses, in which seed yield was measured, 01x1, 01x4, and 03x6, accounting for a remarkable 88–96% of yield variance of Phase II seed in the last cross (Table 1). The role of non-additive genetic variance is much less evident at the adult stage, $V(s)$ being significant in only one of four crosses (03x8), though accounting for 85% of yield variance in that case. While variance components may change with age, increased error variance at the adult vs. Phase III seed stage (56% vs. 6% in 01x1, 24% vs. 11% in 01x4, 57% vs. 22% in 03x6, based on analyses in which reciprocals were not considered) suggests instead that replication of Phase-IV cages was not sufficient, except in 03x8, for which $V(e)$ accounted for only 6% of yield variance. Nevertheless, results based on analyses without reciprocals (Method 4) may have missed substantial reciprocal effects. For example, analysis of 01x1 adult yield without reciprocals indicated no significant $V(s)$, whereas variance among reciprocal hybrids, $V(r)$, was highly significant in an analysis that included reciprocal hybrids (Table 2).

4.2. Reciprocal effects

The large differences in yield between reciprocal hybrids, which were observed in all three of the crosses amenable to analysis by Method 3, were a surprising and novel result of this study. $V(r)$ accounted for a remarkable 21% to 51% of yield variance. Maternal (extra-nuclear) effects for adult yield were significant both in 01x1, in which GCA was significant, and in 03x8, in which GCA was negligible. Bentsen et al. (1998) observed small but significant reciprocal (maternal) effects at harvest of a diallel cross of tilapia strains. Reciprocal affects on body dimensions and weight, accounting for up to 26% of variance, were reported by Rantala and Roff (2006) for a diallel cross of inbred lines of the cricket *Gryllus firmus*. Persistence of maternal effects into adulthood has until recently been neglected as a substantial source of variance in quantitative or fitness-related traits (Roff and Sokolovska, 2004).

Even more surprising in our study was the finding, upon partitioning the reciprocal effect into maternal and non-maternal components, that non-maternal effects were prevalent and often highly significant. To our knowledge, non-maternal effects have not been reported previously for any animal, though they have been reported in maize (Zhang et al., 1996).

To put these reciprocal effects into perspective, the largest reciprocal effect contributed ±704 g (23%) to a mean harvest yield of 3088 g in 01x1, while the largest non-maternal effect contributed ±287 g (9.3%). Similarly, reciprocal and non-maternal reciprocal effects contributed up to 29% and 19%, respectively, of Phase II seed yield in 03x6. Significant non-maternal contributions to reciprocal variance suggest that strong interactions of extra-nuclear and nuclear factors are important in determining yield. In this regard, it is interesting that whole-transcriptome profiling of oyster larvae produced by a 2×2 full factorial cross of inbred lines *35* and *51* uncovered a maternal pattern in mitochondrial gene expression (Hedgecock et al., 2007; J. P. Curole, E. Meyer, D. T. Manahan, and D. Hedgecock, in prep.). It will be necessary to explore interactions between nuclear and mitochondrial genes in more detail, at both the quantitative and functional genomic levels.

4.3. Predicting harvest yield from seed yield

We observed positive correlation between yield of young seed (Phase II) and yield of older seed (Phase III) or adults at harvest, but only half of these correlations were significant (Fig. 5). Again, we may not have had sufficient replication during final growout to resolve genetic determinants of yield. Another potentially confounding factor with 03x6 was relocation of this cross from Thorndyke Bay, where the older seed were reared, to Totten Inlet, where the oysters were reared to final harvest. This caveat raises the topic of hybrid × environment interaction, which we have not begun to explore, in part because of the difficulty of carrying out diallel crosses in one environment. Still, the positive and sometimes significant correlations between seed and adult stages that we have observed are encouraging. If we could reasonably predict yield at harvest by measures of yield and its additive and non-additive genetic components at the seed stage, then we could not only save the enormous labor costs of growout but we could also test more lines each year in several environments. Lines with promising special combining ability could then be crossed subsequently to produce large quantities of seed for planting and testing at commercial scales.

5. Conclusion

Commercial breeding programs can improve the yield of Pacific oysters, especially in regions, such as the U.S. West Coast, where farmers depend on seed produced in hatcheries. Using diallel crosses among inbred parent lines, we found that yield increased with general combining ability, as expected (Langdon et al., 2003), but that high-yielding hybrids with high, positive special combining ability and low GCA were also common. These results suggest that improvement of commercial oyster seed could be achieved by a combination of selection among inbred lines and selection for specific combining ability. Also noteworthy in these experiments were the large differences between reciprocal hybrids, which constrain the direction of line crosses in the production of high-yielding hybrid seed. We conclude with a few thoughts on how these results might be implemented in a commercial crossbreeding program.

5.1. A two-tiered scheme for identifying elite lines for commercial seed production

We believe a two-tiered system of crosses will be needed to identify elite inbred lines for commercial hybrid seed production. With maize, elite lines are identified annually by crossing thousands of inbred lines, producing millions of hybrid combinations, which are then simultaneously planted and evaluated for yield. In this way, the commercial corn breeder exerts tremendous selection for combining ability, taking the best pair of inbred lines out of the thousands tested. With oysters, however, rearing even a 7 × 7 diallel cross of inbred lines to harvest size is difficult and impractically labor intensive; indeed, we succeeded only four times in three years (in experiments 01x1, 01x4, 03x6, and 03x8). We now believe that testing of inbred lines at a young seed stage may suffice to identify sets of top inbred parents for further testing. We can increase replication at these early stages and begin to test in different environments. Following this first tier of crosses, we recommend re-testing top parent lines on a much larger production scale, against current outbred industry stock, to identify elite lines for further amplification and commercial seed production. Only one commercial-scale trial has been conducted to date, which suggested that *51 × 35* hybrids were better performing than current industry stock (J. P. Davis and S. Matson, unpublished), even though this cross was clearly inferior to several other hybrid combinations in crosses 01x1 and 03x6, including the reciprocal *35 × 51*.

5.2. Maintenance of inbred lines

A disadvantage of crossbreeding compared to selection as a strategy for improving yield of oysters is the extra effort and expense of maintaining inbred lines, particularly since growth and survival are reduced by inbreeding depression. We manage this cost by planting three cages of inbred seed to evaluate the performance of inbred lines; we keep the top 50% of families in each group planted simultaneously. This practice amounts to selection among lines, largely on the basis of additive genetic variance. As these selected lines begin to be used in crosses, models with fixed effects may be more appropriate than models with random effects (Zhang and Kang, 1997). Since the non-additive components of genetic variance (dominance, epistasis), which are the basis of crossbreeding, are uncorrelated with the additive component, lines with good specific combining ability could be lost by this practice. Nevertheless, culling of inbred lines is a practical necessity faced by commercial breeders.

Two other requirements for a successful line- and crossbreeding program are genotyping to confirm pedigree and good record keeping. We found that an average of 10% of prospective parents for the crosses reported here were contaminants, confirming the necessity of validating parent pedigrees for each diallel cross; contamination of experimental bivalve populations has

been reported previously (e.g. Mallet et al., 1985; Foltz, 1986; Zouros et al., 1992; Li and Guo, 2004) and must be confronted in all such studies. Having confirmed pedigree, tracking of hybrid performance is the next requirement. A uniform convention for naming lines and crosses, which is essential for good record keeping, proved surprisingly difficult to establish. A name must be simple enough for labeling and visual identification in the field but complex enough to identify and track pedigree unambiguously. The nomenclature developed in the course of this study (see Section 2.1) for inbred families accomplishes these objectives.

5.3. Crossbreeding and polyploids

The oyster industry has experienced a marked shift in demand for triploid hatchery seed (Nell, 2002). Today, on the U.S. West Coast, 40–50% of hatchery seed are triploid, and for certain companies the percentage of triploid seed is as high as 100% for single oysters and 50% for shucked meat product. Thus, a commercial crossbreeding program may need to focus on production of triploid as well as diploid hybrid seed. Since triploid seed is currently produced by fertilizing diploid eggs with sperm from tetraploid males (Eudeline et al., 2000), we can only utilize general rather than specific combining ability to improve triploids, owing to meiotic segregation in the hybrid female parent. To take full advantage of the non-additive components of genetic variation for yield, industry will need to incorporate specific combining ability into the tetraploid lines. This can be done by chemically inducing triploidy in the fertilized eggs of diploid hybrids and using the resulting triploids to found tetraploid stocks. The suggestion from the maize literature is that heterosis compounds with higher ploidy levels: just as AB is better than AA at the diploid level, ABC > AAB at the triploid level, and ABCD > AABC at the tetraploid level (Birchler et al., 2003).

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References

Bentsen, H.B., Eknath, A.E., Palada-de Vera, M.S., Danting, J.C., Bolivar, H.L., Reyes, R.A., Dionisio, E.E., Longalong, F.M., Circa, A.V., Tayamen, M.M., Gjerde, B., 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. Aquaculture 160, 145–173.

Bierne, N., Launey, S., Naciri-Graven, Y., Bonhomme, F., 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. Genetics 148, 1893–1906.

Birchler, J.A., Auger, D.L., Riddle, N.C., 2003. In search of the molecular basis of heterosis. Plant Cell 15, 2236–2239.

Breese, W.P., Malouf, R.E., 1975. Hatchery Manual for the Pacific Oyster *Crassostrea gigas* Gould. Sea Grant Program Publ. No. ORESU-H-75-002. Oregon State University, Corvalis, Oregon, USA. 22 pp.

Britten, H., 1996. Meta-analysis of the association between multilocus heterozygosity and fitness. Evolution 50, 2158–2164.

Bryden, C.A., Heath, J.W., Heath, D.D., 2004. Performance and heterosis in farmed and wild Chinook salmon (*Oncorhynchus tshawytcha*) hybrid and purebred crosses. Aquaculture 235, 249–261.

Bucklin, K.A., 2002. Analysis of the genetic basis of inbreeding depression in the Pacific oyster *Crassostrea gigas*. Ph.D. dissertation in Genetics, University of California Davis, Davis.

Coon, S.L., Bonar, D.B., Weiner, R.M., 1986. Chemical production of cultchless oyster spat using epinephrine and norepinephrine. Aquaculture 58, 255–262.

Crow, J.F., 1998. 90 years ago: the beginning of hybrid maize. Genetics 148, 923–928.

Eudeline, B., Allen, S.K., Guo, X.M., 2000. Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. Aquaculture 187, 73–84.

Foltz, D.W., 1986. Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. Evolution 40, 869–870.

Gaffney, P.M., Bernat, C.M., Allen, S.K., 1993. Gametic incompatibility in wild and cultured populations of the eastern oyster, *Crassostrea virginica* (Gmelin). Aquaculture 115, 273–284.

Gjerde, B., Refstie, T., 1984. Complete diallel cross between 5 strains of Atlantic salmon. Livest. Prod. Sci. 11, 207–226.

Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9, 463–493.

Griffing, B., 1990. Use of a controlled-nutrient experiment to test heterosis hypotheses. Genetics 126, 753–767.

Hedgecock, D., McGoldrick, D.J., Bayne, B.L., 1995. Hybrid vigor in Pacific oysters: an experimental approach using crosses among inbred lines. Aquaculture 137, 285–298.

Hedgecock, D., Langdon, C., Blouin, M., Allen, S.K., 1997. Genetic Improvement of Cultured Pacific Oysters by Selection. Special Report, vol. 968. Agricultural Experiment Station, Oregon State University, Corvallis, OR, USA. 40 pp.

Hedgecock, D., McGoldrick, D.J., Manahan, D.T., Vavra, J., Appelmans, N., 1996. Quantitative and molecular genetic analysis of heterosis in bivalve molluscs. J. Exp. Mar. Biol. Ecol. 203, 49–59.

Hedgecock, D., Lin, J.-Z., DeCola, S., Haudenschild, C., Meyer, E., Manahan, D.T., Bowen, B., 2007. Transcriptomic analysis of growth heterosis in larval pacific oysters (*Crassostrea gigas*). Proc. Natl. Acad. Sci., U.S.A. 104, 2313–2318.

Hubert, S., Hedgecock, D., 2004. A linkage map of microsatellite DNA markers for the Pacific oyster *Crassostrea gigas*. Genetics 168, 351–362.

Hulata, G., 2001. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111, 155–173.

Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Improved family yields of Pacific oysters *Crassostrea gigas* Thunberg derived from selected parents. Aquaculture 220, 227–244.

Lannan, J.E., 1980. Broodstock management of *Crassostrea gigas*: IV. Inbreeding and larval survival. Aquaculture 21, 352–356.

Launey, S., Hedgecock, D., 2001. High genetic load in the Pacific oyster. Genetics 159, 255–265.

Li, L., Guo, X., 2004. AFLP-based genetic linkage maps of the Pacific oyster *Crassostrea gigas* Thunberg. Mar. Biotechnol. 6, 26–36.

Li, G., Hubert, S., Bucklin, K., Ribes, V., Hedgecock, D., 2003. Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. Mol. Ecol. Notes 3, 228–232.

Mallet, A.L., Zouros, E., Gartner-Kepkay, K.E., Freeman, K.R., Dickie, L., 1985. Larval viability and heterozygote deficiency in populations of marine bivalves: evidence from pair-matings. Mar. Biol. 87, 165–172.

Mann, R. (Ed.), 1979. Exotic Species in Mariculture. MIT Press, Cambridge.

Nell, J.A., 2002. Farming triploid oysters. Aquaculture 210, 69–88.

Pace, D., Marsh, A.G., Green, A., Leong, P., Hedgecock, D., Manahan, D.T., 2006. Physiological bases of genetically determined variations in growth of marine invertebrate larvae (*Crassostrea gigas*). J. Exp. Mar. Biol. Ecol. 335, 188–209.

Rantala, M.J., Roff, D.A., 2006. Analysis of the importance of genotypic variation, metabolic rate, morphology, sex and development time on immune function in the cricket, *Gryllus firmus*. J. Evol. Biol. 19, 834–843.

Roff, D.A., Sokolovska, N., 2004. Extra-nuclear effects on growth and development in the sand cricket *Gryllus firmus*. J. Evol. Biol. 17, 663–671.

Sheridan, A.K., 1997. Genetic improvement of oyster production — a critique. Aquaculture 153, 165–179.

Sheridan, A.K., Smith, I.R., Nell, J.A., 1996. Reducing the impact of environmental variation in a growth rate improvement program for the Sydney rock oyster *Saccostrea commercialis*. Aquaculture 143, 145–154.

Wohlfarth, G.W., 1993. Heterosis for growth-rate in common carp. Aquaculture 113, 31–46.

Zhang, Y., Kang, M.S., 1997. DIALLEL-SAS: a SAS program for Griffing's diallel analyses. Agron. J. 89, 176–182.

Zhang, Y.D., Kang, M.S., Magari, R., 1996. A diallel analysis of ear moisture loss rate in maize. Crop Sci. 36, 1140–1144.

Zhang, Y., Kang, M.S., Lamkey, K.R., 2005. DIALLEL-SAS05: a comprehensive program for Griffing's and Garder–Eberhart Analyses. Agron. J. 97, 1097–1106.

Zouros, E., Pogson, G.H., 1994. Heterozygosity, heterosis and adaptation. In: Beaumont, A.R. (Ed.), Genetics and Evolutionof Aquatic Organisms. Chapman & Hall, London, pp. 135–146.

Zouros, E., Freeman, K.R., Ball, A.O., Pogson, G.H., 1992. Direct evidence for extensive paternal mitochondrial-DNA inheritance in the marine mussel *Mytilus*. Nature 359, 412–414.