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Transcriptome Analysis of Reciprocal Hybrids Between *Crassostrea gigas* and *C. angulata* Reveals the Potential Mechanisms Underlying Thermo-Resistant Heterosis

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Abstract

Heterosis, also known as hybrid vigor, is widely used in aquaculture, but the molecular causes for this phenomenon remain obscure. Here, we conducted a transcriptome analysis to unveil the gene expression patterns and molecular bases underlying thermo-resistant heterosis in *Crassostrea gigas* ♀ × *Crassostrea angulata* ♂ (GA) and *C. angulata* ♀ × *C. gigas* ♂ (AG). About 505 million clean reads were obtained, and 38,210 genes were identified, of which 3779 genes were differentially expressed between the reciprocal hybrids and purebreds. The global gene expression levels were toward the *C. gigas* genome in the reciprocal hybrids. In GA and AG, 95.69% and 92.00% of the differentially expressed genes (DEGs) exhibited a non-additive expression pattern, respectively. We observed all gene expression modes, including additive, partial dominance, high and low dominance, and under- and over-dominance. Of these, 77.52% and 50.00% of the DEGs exhibited under- or over-dominance in GA and AG, respectively. The over-dominance DEGs common to reciprocal hybrids were significantly enriched in protein folding, protein refolding, and intrinsic apoptotic signaling pathway, while the under-dominance DEGs were significantly enriched in cell cycle. As possible candidate genes for thermo-resistant heterosis, GRP78, major egg antigen, BAG, Hsp70, and Hsp27 were over-dominantly expressed, while MCM6 and ANAPC4 were under-dominantly expressed. This study extends our understanding of the thermo-resistant heterosis in oysters.

Keywords Thermo-resistant heterosis · Transcriptome · Hybrid oysters · Non-additive expression · Over-dominance

Introduction

Heterosis or hybrid vigor refers to the biological phenomenon in which hybrids exhibit superior performance, such as improved biomass production, developmental rates, yields, and stress tolerance, compared to its parents (Springer and Stupar 2007; Chen 2013). Crossbreeding is widely applied to crops and livestock like maize, rice, cattle, and poultry (Birchler et al. 2003; Green 2009), as well as to aquatic animals, including fish, abalone, scallop, and oyster (Rahman et al. 2013; de la Cruz and Gallardo-Escárate 2011; Wang et al. 2011; Hedgecock et al. 2007). Given its significance in agriculture, considerable interest has been focused on how heterosis contributes to increased trait in hybrids. Early studies proposed dominance, over-dominance, and epistasis genetic hypotheses to explain heterosis (Birchler et al. 2003). Moreover, complementation of genes from the parents may contribute to heterosis in maize (Lai et al. 2010) and *Arabidopsis* (Childs et al. 2010). Heretofore, the consensus on heterosis is that no single hypothesis applies to every species or every phenomenon.

Phenotypic evolution usually proceeds by variations in the spatial and temporal modes of gene expression (Doebley and Lukens 1998). Thus, it is reasonable to assume that differential expression gene between hybrids and parents may be responsible for the heterosis (Swanson-Wagner et al. 2006; Zhai et al. 2013). Transcriptomic studies have yielded comprehensive insights into heterosis in some species. For example, Hedgecock et al. (2007) speculated that non-additive gene

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expression levels form the base of growth heterosis. Fujimoto et al. (2012) suggested that the early increase in activity of genes involved in photosynthesis resulted in a larger leaf area in hybrid *Arabidopsis*. Li et al. (2016) observed that several non-additive genes related to circadian rhythm regulation and flowering time were upregulated in hybrid rice. Understanding the mechanisms of heterosis can help in the exploitation of heterosis (Somerville and Somerville 1999). However, only a few studies revealed differentially expressed genes, potentially associated with heterosis.

The Pacific oyster (*Crassostrea gigas*) is one of the most cosmopolitan bivalves in aquaculture due to its high meat quality, rapid growth, and excellent environmental tolerance (Shatkin et al. 1997; Langdon et al. 2003). Since 2008, *C. gigas* have suffered severe mass mortalities associated with complex interactions between the host, the high temperatures, and pathogen (de Lorgeril et al. 2018). Fujian oyster (*Crassostrea angulata*) is another important shellfish species advantageous for its strong adaptability to warm seawater (Li et al. 2017; Ghaffari et al. 2019). To obtain new varieties combining the growth advantage of *C. gigas* and heat resistance of *C. angulata*, hybrid oysters were artificially bred by crossing *C. gigas* and *C. angulata*. In the field, the hybrid oysters exhibited rapid growth and high-temperature tolerance traits and were thus suitable for large-scale aquaculture (Tan et al. 2020; Jiang et al. 2021, 2022a). Although several physiological and immune analyses have been conducted (Meng et al. 2021; Jiang et al. 2022b), the genetic and molecular mechanisms associated with thermo-resistant heterosis are still poorly understood in hybrid oysters.

In this study, we used RNA sequencing (RNA-seq) technology to analyze two reciprocal hybrids and its parental species, *C. gigas* and *C. angulata*. The objectives of this study were (1) to assess the deviation of hybrid gene expression from the mid-parent value, (2) to determine whether certain gene expression patterns in hybrids correlate with thermo-resistant heterosis, and (3) to identify potential candidate genes or pathways contributing to thermo-resistant heterosis.

Materials and Methods

Biological Material

The *C. gigas*, *C. angulata*, and their reciprocal hybrids are neither an endangered nor protected species. All experiments in this study were conducted according to national and institutional guidelines.

In 2019, *C. gigas* and *C. angulata* broodstocks were obtained from Rongcheng, Shandong Province, and Zhangzhou, Fujian Province, respectively. The population construction was performed according to Jiang et al. (2021). Briefly, the parents of *C. gigas* and *C. angulata* were used to construct the hybrids (*C. gigas* ♀ × *C. angulata* ♂: GA; *C. angulata* ♀ × *C. gigas* ♂: AG) using a reciprocal cross. Meanwhile, purebreds (*C. gigas* ♀ × *C. gigas* ♂: GG; *C. angulata* ♀ × *C. angulata* ♂: AA) were also constructed. After settlement, spats were transported to Rongcheng for field grow-out rearing. After 1 year, the pure (GG and AA) and hybrid (GA and AG) oysters were collected and transported to an aquafarm in Laizhou, Shandong Province. One hundred oysters with the similar sizes (6.0–7.5 cm) from each cross were selected, placed in rectangular cement ponds, and maintained for a 15-day acclimation period at ambient seawater temperature (22 ± 0.5 °C) and salinity (30 ± 0.5 psu). During the feeding trial, oysters were fed with fresh *Phaeodactylum tricornutum* Bohlin three times a day. One third of the seawater was replaced daily to ensure fresh water quality.

Thermal Experiment

The thermal experiment was performed in twelve 100-L polyethylene buckets with fresh seawater as previously described with slight modifications (Jiang et al. 2022b). In detail, the seawater temperature was gradually elevated from 22 to 35 °C at a rate of 1 °C h^{-1}. Subsequently, 20 healthy oysters in each cross were directly transferred to the buckets for 96 h. The water parameters were kept at nominal values during the whole experiment (temperature 35 ± 0.5 °C, salinity 30 ± 0.5 psu). Triplicates for each cross were set in thermal stress experiment. Only highly active oysters were used for tissue extraction. After 96 h, the survival rates of hybrid and purebred crosses were calculated. Simultaneously, three oysters per bucket were randomly chosen ($n = 3$ replicate buckets for each cross), and the gills were sampled, treated with liquid nitrogen, and stored at -80 °C.

RNA Extraction, Library Construction, and Sequencing

In each cross, the gills of three oysters were equally mixed as a biological sample, and each cross included three biological replicates. Total RNA samples were extracted based on the instructions of TRIzol reagent (Invitrogen, USA), quantified by a NanoPhotometer® spectrophotometer (Implen, CA, USA) and assessed for the integrity with the RNA 6000 Nano Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). RNA samples with an RNA integrity number (RIN) value approximately greater than 7 were used in downstream applications. A total amount of 1 µg RNA per sample was used as input material for the RNA sample preparations. The library was constructed using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations. The messenger RNA (mRNA) was enriched

from total RNA using the poly-T oligo-attached magnetic beads. mRNA was fragmented using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5×). After that, using the cleaved RNA fragments as the template, random hexamer primer and M-MuLV Reverse Transcriptase were used for first-strand complementary DNA (cDNA) synthesis. This was followed by the synthesis of second-strand cDNA, end repair, and adaptor ligation; 250–300-bp fragments were selected using AMPure XP system (Beckman Coulter, Beverly, USA) for PCR amplification. High-throughput sequencing of RNA library was performed using the Illumina NovaSeq platform to generate 150-bp paired-end reads.

Identification of Differential Expression Genes

Clean reads were acquired after removing low-quality and primer/adapter-contaminated reads. The Q20, Q30, and GC contents of the clean reads were checked in the Fastp software v0.20.0. The clean data were aligned to the reference genome (GenBank assembly accession: GCF_902806645.1) of *C. gigas* using Hisat2 v2.0.5. The featureCounts v1.5.0-p3 was used to count the number of reads mapped to the reference genome. The fragments per kilobase per million fragments (FPKM) method (Trapnell et al. 2010) was used to estimate the expression levels of all genes. R package DESeq2 was used to identify the differentially expressed genes (DEGs) between hybrid and purebred crosses. Differential expression quantification was based on a logarithmic scale (log2 fold change), the adjusted P value (P_{adj}) (Benjamin–Hochberg approach) indicating the statistical significance of the observed changes. $P_{adj} < 0.05$ and |log2 (fold change)|> 1 were set as the thresholds for significantly differential expression.

Additive and Dominance Effect Analysis

The d/a ratio, also referred to as potence (Griffing 1990), provides a standardized measure of the F1 hybrid expression level relative to the average of the parental levels (Guo et al. 2006), as well as an indicator of genetic non-additivity

(Vuylsteke et al. 2005). To assess the expression level of each gene with FPKM ≥ 1 in the hybrid crosses relative to the purebred crosses, we used the d/a ratio from quantitative genetics as a measure (Guo et al. 2006; Bi et al. 2014).

$$\frac{d}{a} = \frac{F_1 - \mu}{P_1 - \mu}$$

Specifically, d represents the dominant effect, a represents the additive effect, F_1 is the gene expression level in GA or AG, P_1 is the gene expression level in GG, and μ is the average gene expression level in the purebred crosses. If $d/a = 0$, then the expression level of GA or AG is equal to the average expression level of purebred crosses. If $0 < d/a < 1$, then the GA or AG expression is biased towards the GG levels. If $d/a = 1$, then the gene shows complete dominant effect from the *C. gigas* allele. If $d/a > 1$, then the hybrid expression level of this gene is beyond the range of purebred crosses. Likewise, in case of $d/a < 0$, genes show hybrid expression levels skewed towards AA levels (Bi et al. 2014).

To further break down the number of genes that expressed at the additive and non-additive levels, the degree of dominance was measured according to the following formula (Xu 2013; Liang 2017):

$$\frac{d}{|a|} = \frac{F_1 - \mu}{|P_1 - \mu|}$$

The genes in reciprocal hybrids were binned into seven expression patterns (Table 1).

Gene Ontology Annotation and Pathway Enrichment

Gene Ontology functional enrichment analysis of non-additive DEGs with different genetic patterns was carried out using the clusterProfiler v4.0.2 R package. Gene Ontology (GO) terms with $P_{adj} < 0.05$ were significantly enriched by DEGs. Meanwhile, the non-additive DEGs with different genetic patterns were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Significantly enriched pathways were obtained using a P_{adj} value < 0.05.

Table 1 Classification of expressed genes in reciprocal hybrids between *C. gigas* and *C. angulata*

Category	Under-dominance (UD)	Dominance (D−)	Partial dominance (PD−)	Additivity (ADD)	Partial dominance (PD+)	Dominance (D+)	Over-dominance (OD+)
$d/|a|$	$(-\infty, -1.2)$	$[-1.2, -0.8)$	$[-0.8, -0.2)$	$[-0.2, 0.2]$	$(0.2, 0.8]$	$(0.8, 1.2]$	$(1.2, +\infty)$
GA (all genes)	7752 (52.49%)	1202 (8.14%)	1450 (9.82%)	716 (4.85%)	784 (5.31%)	383 (2.59%)	2481 (16.80%)
AG (all genes)	5082 (34.41%)	1496 (10.13%)	2291 (15.51%)	1179 (7.98%)	1324 (8.97%)	606 (4.1%)	2790 (18.89%)
GA (DEGs)	369 (31.78%)	44 (3.79%)	24 (2.07%)	50 (4.31%)	83 (7.15%)	60 (5.17%)	531 (45.74%)
AG (DEGs)	55 (22.00%)	29 (11.60%)	16 (6.40%)	20 (8.00%)	38 (15.20%)	22 (8.80%)	70 (28.00%)

Quantitative Real-Time PCR Validation

To validate the RNA-seq, we selected 7 heat response–related genes for quantitative real-time polymerase chain reaction (qRT-PCR) on Roche LightCycler 480 (Roche, Switzerland) using Green® Premix Ex Taq™ II (Takara, China). The remaining RNA samples after RNA-seq analysis were used for qRT-PCR. Primers were designed using the NCBI online Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are listed in Table S1. As a control for the input RNA, the level of a housekeeping gene, elongation factor 1-α (EF1-α), was also measured. The qRT-PCRs were carried out in 20 µL volumes containing 10 µL of TB Green PCR Master Mix, 1 µL each of forward and reverse primers, 1 µL of diluted cDNA, and 7 µL of H_2O. The conditions were 95 ℃ for 3 min, 40 cycles of 95 ℃ for 5 s, 60 ℃ for 30 s, and 40 ℃ for 1 min. Amplification efficiency of PCR was assessed by drawing standard curves from a serial dilution analysis of cDNA. All data was determined using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Statistical Analysis

Survival rates were analyzed using the SPSS 26.0 software. One-way analysis of variance (ANOVA) and LSD test were used to analyze the difference of survival rate among hybrids and purebreds. Differences were considered statistically significant if P is < 0.05. Heterosis for survival rate is defined by the hybrid potence (h_p). The formula to calculate h_p was taken from Hedgecock et al. (2007) as

$$h_p = \frac{2[F_1 - (P_1 + P_2)/2]}{|P_1 - P_2|}$$

where F_1 is the mean survival rate of GA or AG, and P_1 and P_2 are the mean survival rate of purebred crosses GG and AA, respectively.

Results

Hybrid Survival and Heterosis

After 96 h of heat stress, reciprocal hybrids exhibited a higher survival rate compared with GG ($P < 0.05$) and AA ($P > 0.05$) (Table 2). The survival rate of AA was higher, although not significantly so, than GG ($P = 0.087$). Hybrid potence for the two reciprocal hybrids were greater than 1.0 (GA: $h_p = 2.54$; AG: $h_p = 2.38$), and GA was larger than AG. The reciprocal hybrids between *C. gigas* and *C. angulata* displayed classical hybrid vigor or survival heterosis.

Table 2 The survival rate and heterosis of four crosses

Crosses	Survival rate (%)	h_p
GA	60.00 ± 7.20^a	2.54
AG	58.89 ± 10.10^a	2.38
GG	34.44 ± 8.31^b	–
AA	48.89 ± 6.85^{ab}	–

Different superscript letters indicate a significant difference ($P < 0.05$)

Transcriptome Assemblies

All 12 RNA libraries were of high quality with RIN values ranging from 7.4 to 8.5 after an RNA quality evaluation. More than 5.88 Gb of raw bases for each library and 530 million 150-bp paired-end reads with an average of 44 million raw reads for each of the 12 libraries were acquired (Table 3). After filtering low-quality data, 505 million clean reads were acquired with an average Q30 value of 94.17% and an average GC content of 40.69%. The average mapping rate of clean reads was 79.07% in GG, 79.76% in GA, 78.10% in AG, and 74.98% in AA, respectively. These outcomes indicated that the sequencing data were of adequate quality for downstream analysis. Transcriptome data are available at SRA archive from the GenBank database under the accession number PRJNA900034.

Identification of DEGs

To explore further the possible mechanisms and genes involved in the thermo-resistant heterosis of hybrid oyster, we compared the transcriptome profiles of hybrids (GA and AG) versus those of purebreds (GG and AA) (Fig. 1). The highest number of DEGs was observed in the "GA vs AA" group whereas the lowest was seen in the "AG vs GG" group after 96 h of heat stress (Fig. 1a). Relative to GG, 510 and 282 DEGs were upregulated and downregulated in GA (Fig. 1c) while 233 and 144 DEGs were upregulated and downregulated in AG (Fig. 1e). Also, relative to AA, 1212 and 822 DEGs were upregulated and downregulated in GA (Fig. 1d) while 396 and 180 DEGs were upregulated and downregulated in AG (Fig. 1e). Among these DEGs, 404, 143, 1397, and 143 genes were specifically expressed in "GA vs GG," "AG vs GG," "GA vs AA," and "AG vs AA" groups, respectively; 12 DEGs were co-expressed between the four comparison groups (Fig. 1b). The numbers of DEGs showed an expression bias in the direction between hybrids and purebreds, while hybrids' gene expression patterns were more like GG other than AA.

Table 3 Sequencing and assembly statistics of the transcriptome data

Sample	Raw reads	Clean reads	Q30 (%)	GC (%)	Total reads	Mapped reads	Mapped ratio
GG_1	41,084,196	39,191,574	94.33	41.00	18,281,208	14,322,587	78.35
GG_2	41,769,614	39,937,676	93.66	40.49	18,519,694	14,587,246	78.77
GG_3	43,214,076	41,528,114	93.9	40.77	19,375,547	15,520,060	80.1
AA_1	44,451,662	41,936,676	94.55	40.31	19,664,341	14,862,396	75.58
AA_2	43,888,728	40,942,748	94.25	40.05	19,559,915	14,687,291	75.09
AA_3	45,868,718	42,522,228	94.53	40.05	20,445,875	15,182,212	74.26
AG_1	46,397,934	44,110,888	93.9	39.20	20,249,849	15,271,632	75.42
AG_2	47,492,132	46,050,856	94.33	43.38	21,473,842	17,247,668	80.32
AG_3	44,515,244	42,968,684	94.33	40.95	19,596,675	15,395,478	78.56
GA_1	42,878,894	41,191,818	94.04	40.75	18,990,748	15,187,645	79.97
GA_2	44,359,822	42,388,478	94.34	40.65	19,533,783	15,446,565	79.08
GA_3	44,138,910	42,403,638	93.87	40.66	19,342,742	15,520,659	80.24

Pattern of Hybrid Gene Expression

We used a *d/a* rate to compare the transcript levels of crossbreds relative to *C. gigas* and *C. angulata* for detecting whether global gene expression levels in the hybrids resemble one parent over the other. The results showed that the distribution of the *d/a* ratios was clearly biased towards *C. gigas* (Fig. 2a). To examine this further, we assessed the *d/a* ratios of DEGs of reciprocal hybrids. Compared to all genes, DEGs exhibited a higher degree of bias toward the *C. gigas* genome parent (Fig. 2b). Furthermore, obvious differences in *d/a* rate distribution were found between reciprocal hybrids. The proportion of genes that showed *C. gigas* genome bias expression was larger in GA. The *d/a* ratio distributions for DEGs in GA peak around 2.
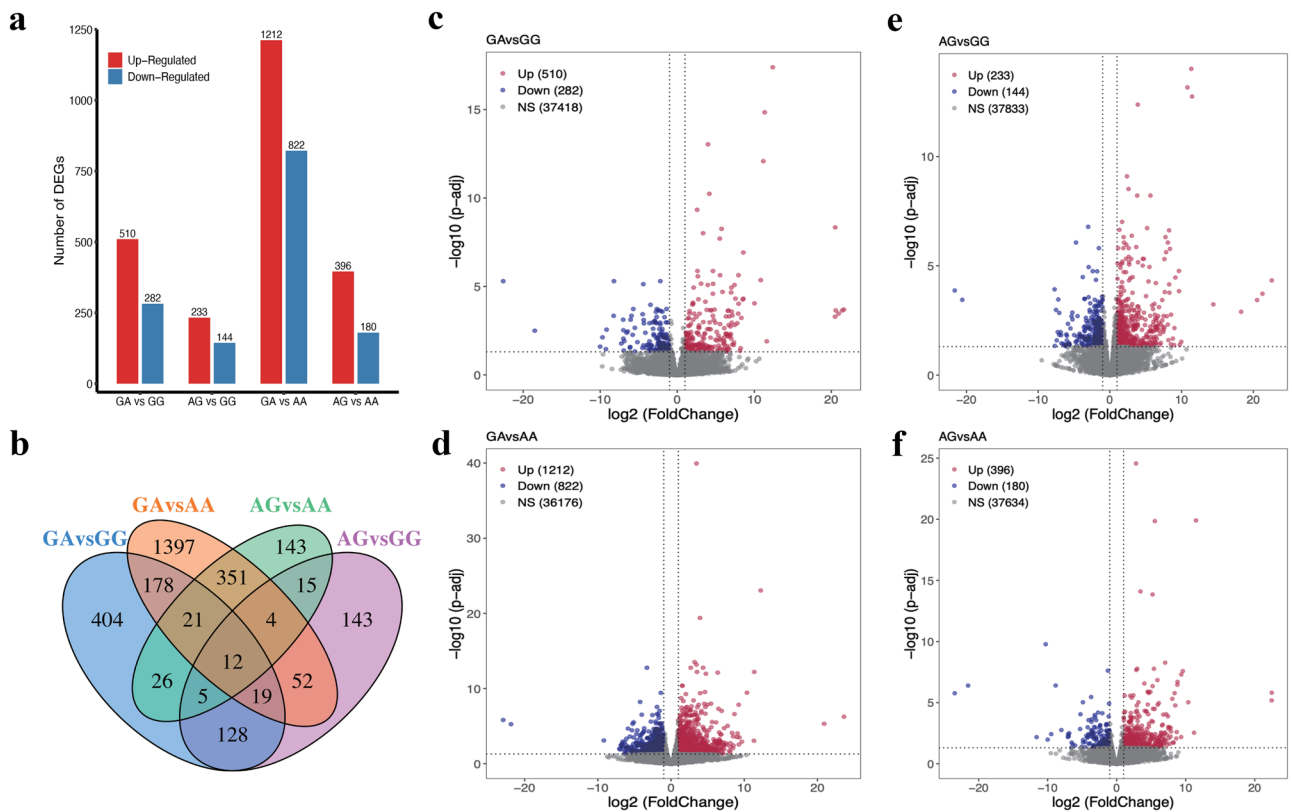

Fig. 1 Comparison of differentially expressed genes (DEGs). The number of upregulated and downregulated DEGs in each group (**a**) and Venn diagram of overlapping DEGs among these four groups (**b**); volcano plot of DEGs in GA vs GG (**c**), GA vs AA (**d**), AG vs GG (**e**), and AG vs AA (**f**)
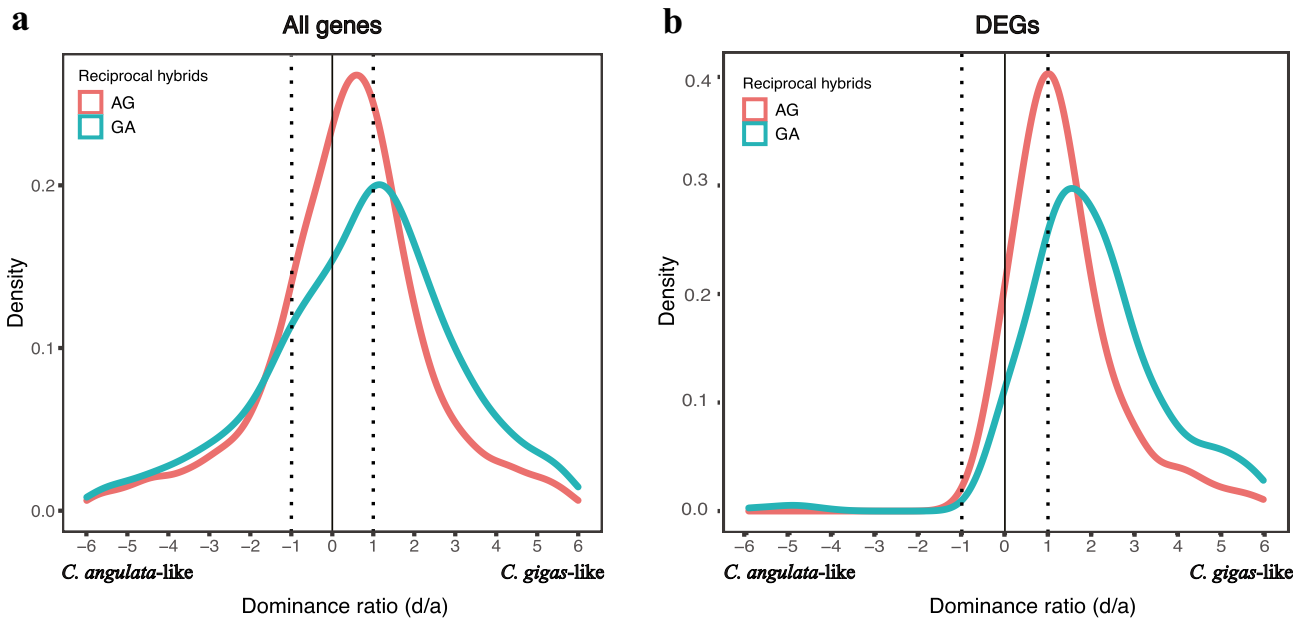
a All genes

b DEGs

Fig. 2 Results of using the *d/a* ratio to quantify the level of deviation in transcript expression of the reciprocal hybrids relative to the mid-parent value of *C. gigas* and *C. angulata*. **a** Reciprocal hybrids' *d/a* ratio distribution of all genes; **b** reciprocal hybrids' *d/a* ratio distribution of DEGs

To evaluate additive, dominant, and over-dominant expression, we divided genes in the reciprocal hybrids into 7 categories (Table 1). As for all genes, the percentage of non-additivity in GA and AG was 95.15% and 92.02%, respectively; the percentage of non-additivity in GA and AG was 95.69% and 92.00% in terms of DEGs, respectively. Over-dominance (OD) and under-dominance (UD) accounted for more than half of genes, indicating that, for GA and AG, most of the genes were expressed at a level beyond the range of the purebreds. Notably, UD accounted for the highest proportion of the 7 categories for all genes, while OD accounted for the highest proportion for DEGs. GA (45.74%) showed more OD expression than AG (28.00%) in terms of DEGs.

Functional Analysis of Non-additive DEGs

To further explore the potential mechanism of heterosis, we identified the molecular function of non-additive DEGs, taking into account their genetic model (Fig. 3). In GA, the non-additive DEGs were mostly enriched ($P < 0.05$) in deoxyribose phosphate biosynthesis and metabolic processes, nucleobase-containing small molecule interconversion and 9 + 2 motile cilium (UD DEGs); in oxidoreductase activity (D − DEGs); in amino acid transport (PD − DEGs); in oxidoreductase activity (PD + DEGs); and in protein folding and refolding, unfolded protein binding, and protein folding chaperone and chaperone complex (OD DEGs) (Fig. 3a; Table S2). In AG, the non-additive DEGs were mainly involved ($P < 0.05$) in extracellular transport, cilium-dependent cell motility, 9 + 2 motile cilium, and MCM complex (UD DEGs); in MutSalpha

complex binding (D − DEGs); and in the regulation of response to butyrate, intrinsic apoptotic signaling pathway, positive regulation of cell migration by vascular endothelial growth factor signaling pathway, and protein refolding (OD DEGs) (Fig. 3b; Table S3).

A total of 7 and 14 significant ($P < 0.05$) KEGG pathways were identified by the UD and OD genes in GA, respectively (Fig. 4a; Table S4), while only 4 significant ($P < 0.05$) KEGG pathways were identified by the OD genes in AG (Fig. 4; Table S5).

In GA, significant pathways enriched from the UD genes were mainly related to pyrimidine metabolism, pentose phosphate pathway, cell cycle, drug metabolism, and DNA replication; those from the OD genes were mainly related to chaperones and folding catalysts, antigen processing and presentation, protein processing in endoplasmic reticulum, longevity-regulating pathway, spliceosome, and proteasome. In AG, significant pathways enriched from the OD genes were mainly related to chaperones and folding catalysts, longevity-regulating pathway, pyrimidine metabolism, and protein processing in endoplasmic reticulum.

Quantitative Real-Time PCR

We selected seven genes (GRP78, major egg antigen, BAG3, HSP27, HSP70, dnaJ, and Birc3) to verify the differential expression of genes identified by RNA-seq. The qRT-PCR results indicated that the relative expression of each gene was consistent with the trend of RNA-seq (Table 4), suggesting the accuracy of RNA-seq analysis.
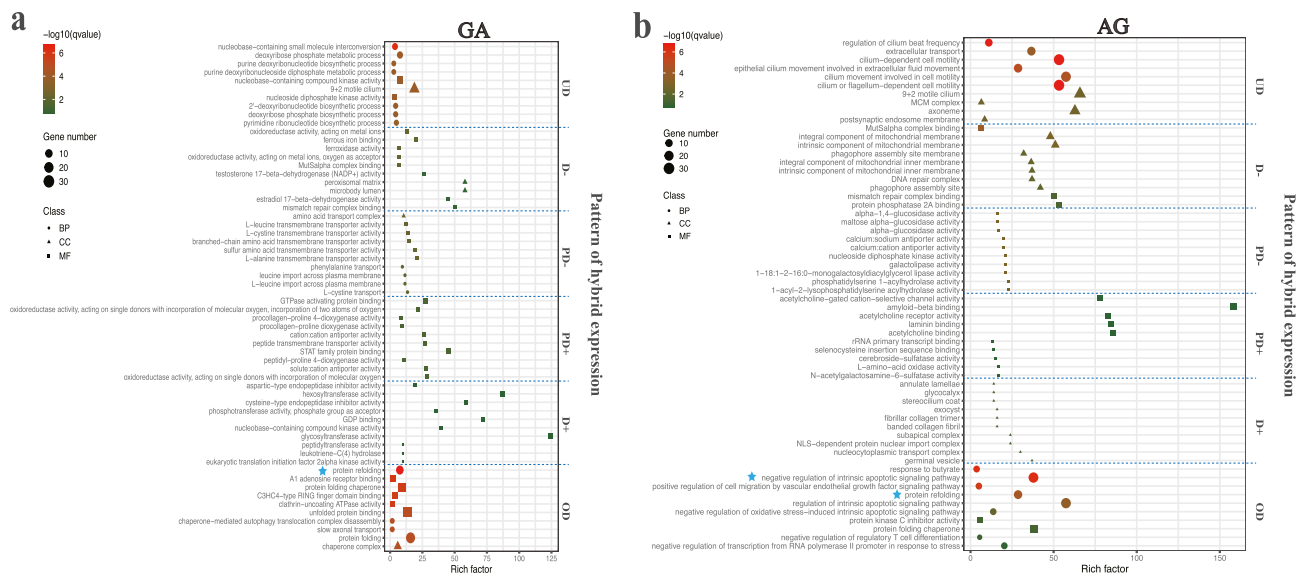
Fig. 3 The top 10 enriched GO terms derived from the GO enrichment analysis of the non-additive DEGs in GA (**a**) and AG (**b**) under different expression patterns. The blue pentagrams indicate common GO terms shared by GA and AG. Genes with OD expression pattern were upregulated in hybrids, and genes with UD expression pattern were downregulated in hybrids

Discussion

Heterosis has been widely utilized in aquaculture breeding practices (Hedgecock et al. 2007; Rawson and Feindel 2012). Despite its vital importance to the aquaculture, a mechanistic understanding of heterosis has not been achieved. Conceptual developments could help increase the production of animals by the manipulation of heterosis (Chen 2013). Here, we preliminarily assessed the relationship between transcriptional profiles and thermo-resistant heterosis in the hybrid oysters.

A total 505 million high-quality reads were obtained from the gills of reciprocal hybrids and two purebreds under heat stress, and 38,210 annotated genes were identified. Over
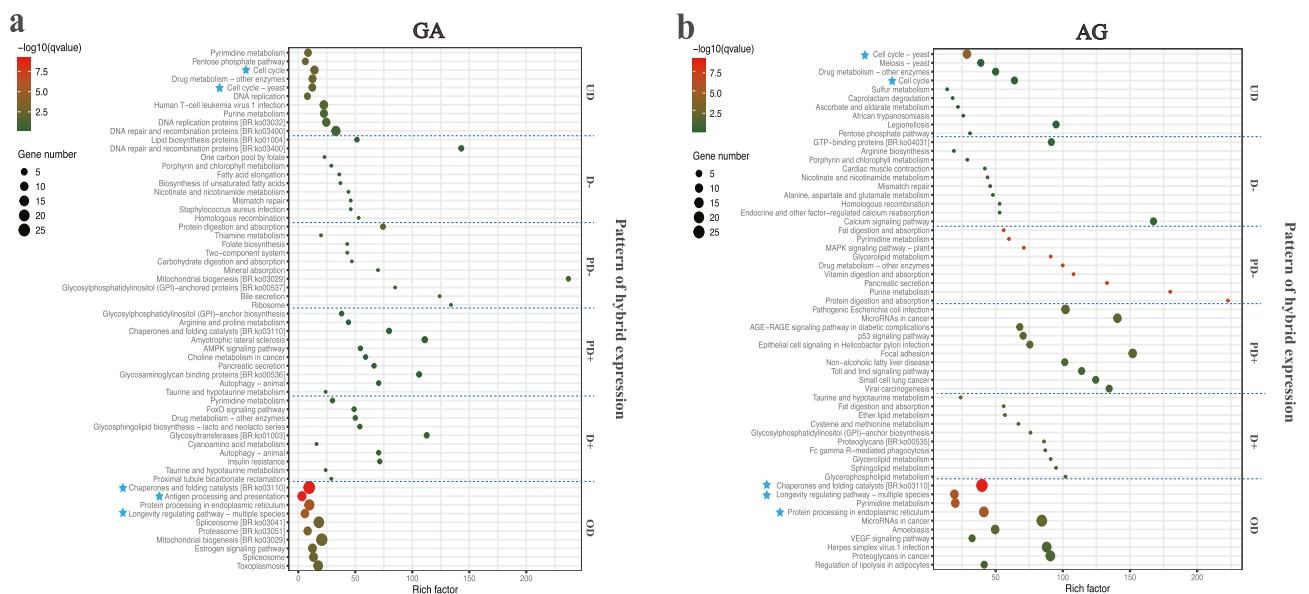

Fig. 4 The top 10 KEGG enrichment pathways of the non-additive DEGs in GA (**a**) and AG (**b**) under different expression patterns. The blue pentagrams indicate common KEGG pathways shared by GA and AG. Genes with OD expression pattern were upregulated in hybrids, and genes with UD expression pattern were downregulated in hybrids

Table 4 Validation of RNA-seq results using qPCR

Group	Gene ID	Gene abbreviation	Log2 fold change	
			RNA-seq	qPCR
GA vs GG	LOC105323261	*GRP78*	2.40	2.68
	LOC105346578	*major egg antigen*	1.37	1.03
	LOC105334580	*BAG3*	1.69	1.59
	LOC105331471	*HSP27*	0.57	0.55
	LOC105326385	*HSP70*	1.48	1.58
GA vs AA	LOC105323261	*GRP78*	4.38	4.11
	LOC105346578	*major egg antigen*	1.96	1.92
	LOC105334580	*BAG3*	2.22	2.68
	LOC105331471	*HSP27*	1.18	1.30
	LOC105326385	*HSP70*	1.45	1.46
	LOC105323151	*dnaJ*	1.24	1.50
	LOC105334815	*Birc3*	2.87	3.05
AG vs GG	LOC105323261	*GRP78*	0.78	0.89
	LOC105346578	*major egg antigen*	1.10	0.97
	LOC105334580	*BAG3*	1.25	1.23
	LOC105331471	*HSP27*	1.27	1.56
	LOC105326385	*HSP70*	0.89	0.95
AG vs AA	LOC105323261	*GRP78*	2.76	2.32
	LOC105346578	*major egg antigen*	1.70	1.86
	LOC105334580	*BAG3*	1.79	2.32
	LOC105331471	*HSP27*	1.89	2.31
	LOC105326385	*HSP70*	0.86	0.83
	LOC105334815	*Birc3*	2.21	2.15

75% of the clean reads of *C. angulata* were mapped to the *C. gigas* genome, indicating high transcriptomic similarity, which may explain why the two oysters can hybridize and obtain fertile F1 offspring. The lowest mapping rate, however, was found in *C. angulata*, which may reflect the species differences. Meanwhile, the mapping rate of GG was slightly lower than that of GA, which might be due to the relatively large variation (such as the presence of SNPs and indels) in GG samples relative to the reference genome under heat stress. Extensive DEGs in aquatic animal purebreds and hybrids has been documented in the past by several studies (Sun et al. 2016; Zheng et al. 2019; Tripp-Valdez et al. 2021). In this study, 3779 DEGs between reciprocal hybrids and purebreds were identified from the annotated genes. It has been hypothesized that DEGs between hybrid and parental species may be responsible for heterosis (Swanson-Wagner et al. 2006; Zhai et al. 2013). In yellow catfish hybrid, several DEGs with important physiological functions for heterosis were identified, indicating genomic instability and generation of the superior phenotype in offspring (Zhang et al. 2019). Also, Guo et al. (2006) suggested a positive relationship between the percent of interparental DEGs and production hybrid vigor. It is interesting to know that the percentage of DEGs between reciprocal hybrids and two purebreds was

asymmetric. DEGs between the GA and purebreds were obviously higher than those of "AG vs AA" and "AG vs GG." Therefore, this asymmetry may explain why GA ($h_p = 2.54$) had higher hybrid potence than AG ($h_p = 2.38$).

One of the objectives of our research was to assess whether transcript levels in reciprocal hybrids resembled one purebred more than the other. In theory, the expression levels of hybrid genes can be close to the mid-parent value (MPV), analogous to either the maternal or paternal genome, or beyond the range of parents. However, we observed that the global transcriptome expression levels of GA and AG were closer to *C. gigas* levels than to *C. angulata* levels under heat stress. Meanwhile, this trend was more evident in the expression levels of DEGs. Usually, hybridization is coupled with uneven gene loss and structural variation, one subgenome possessing more dominant genes than the other (Schnable et al. 2011). Therefore, it is no surprise that genes from the dominant subgenome had higher expression levels. In several hybrid fishes, the maternal genome was dominant compared with the paternal genome, whereas the expression of the paternal genome was considered to be the under-expression of the maternal genome (Debes et al. 2012; Zhou et al. 2015). A possible explanation for the downregulation of subgenomic genes from one parent is genomic imprinting or heterochronic allelic regulation (Guo et al. 2003). Besides this, this expression level bias may also be related to the use of the *C. gigas* genome as a reference genome. In addition, Guo et al. (2006) found that gene expression bias indicated the divergent environmental responses of the parental alleles at multiple loci in maize hybrid: the proportion of paternally biased genes improved with the increase of maize density.

In the classical model of heterosis, there is no genotypic divergence for nuclear genes between reciprocal hybrids, i.e., AB = BA (Hedgecock et al. 2007). We found that although the *d/a* ratio distribution was skewed toward the *C. gigas* parent, the degree varied. The hybrid GA with higher survival rate exhibited a larger proportion of genes with the *C. gigas* genome's biased expression as compared to the hybrid AG. Hybridization is evolutionary and mechanistically interesting, in that the step requires the conciliation of two different sets of genomes and regulatory interactions. In reciprocal hybrids, the divergence in allele expression may be the result of differential regulation in cis-acting elements or trans-acting factors (Springer and Stupar 2007). For example, it was found that the cis and trans effects facilitated the expression of maternal subgenomes in *Oreochromis niloticus* × *Oreochromis aureus*, contributing to the expression advantage of growth hormone. However, there were few pieces of evidence of a significant maternal or paternal effect in the reciprocal hybrids of maize (Swanson-Wagner et al. 2009).

To address the gene expression patterns between hybrid oysters and their parents further, we divided all genes and DEGs with FPKM ≥ 1 into seven categories. We found

a substantial non-additive patterns of gene expression (over 92%), in which $d/|a| < -1.2$ or $d/|a| > +1.2$, a result in line with levels of genome expression modes observed in *Takifugu rubripes* × *Takifugu flavidus* (Gao et al. 2013), *Megalobrama amblycephala* × *Culter alburnus* (Zhou et al. 2015), *Haliotis gigantea* × *Haliotis discus hannai* (Xiao et al. 2021), hybrid *Pinctada fucata martensii* (Yang et al. 2018), and hybrid *C. gigas* (Hedgecock et al. 2007), but differing clearly from that in hybrid maize (Swanson-Wagner et al. 2006; Stupar and Springer 2006) and hybrid mice (Cui et al. 2006). It reckons that the genetic base of hybrid behavior could be primarily or partially attributed to the complementarity of additive and non-additive genetic effects (Zhang et al. 2017a; Xiao et al. 2021). Springer and Stupar (2007) concluded that the combination of two alleles in a hybrid that change due to cis-linked changes would lead to MPV levels (additive effects). This is reminiscent of the metabolic heterosis model, which suggests that hybrid could exhibit best-parent heterosis, with half of the total enzyme between the two parents (Fiévet et al. 2010). On the contrary, our results indicated that non-additive expression genes were closely related to heterosis (Hedgecock et al. 2007; Fujimoto et al. 2012; Yang et al. 2018).

Non-additive expression of genes will result in activation, repression, dominance, or over-expression (Chen 2007). As expected, all possible patterns of hybrid expression, including additivity, partial dominance, high and low dominance, and under- and over-dominance, were observed in GA and AG. Moreover, OD and UD accounted for the highest percentage, more than half of non-additive genes. The over-dominance model was considered to be the basic mechanism of transgressive heterosis, which could create superior heterozygous gene expression pattern (Baranwal et al. 2012; Chen 2013). In tomato, over-dominant expression of a single flowering gene SFT contributes to production heterosis (Krieger et al. 2010). In abalone, the over-dominance was assumed to be associated with heterosis in disease resistance (Di et al. 2015) and heat tolerance (Xiao et al. 2021). We also noted that the hybrid cross with the larger survival h_p, GA, showed a higher percentage of OD modes in DEGs. Meanwhile, more DEGs exhibited OD rather than UD modes in reciprocal hybrids, suggesting that hybridization was usually related to hybrid vigor rather than hybrid weakness in the progeny (Gu et al. 2019). An interesting explanation for the coexistence of OD and UD effects was that miRNAs modulate gene expression through cleavage and transcriptional silencing of target mRNAs (Luo et al. 2009).

DEGs common to both GA and AG were proposed as potential candidates for heterosis (Zhang et al. 2017b). We found, quite interestingly, this only included DEGs with UD or OD expression pattern, implying that under- and over-dominance were involved in thermo-resistant heterosis in oysters. Protein quality control is essential for maintaining cellular homeostasis under adverse circumstances (Zhang et al. 2012a). Among genes with an OD pattern, the most frequently represented biological processes in reciprocal hybrids were protein folding and refolding terms/pathways, including protein refolding (GO:0042026), chaperones and folding catalysts (ko03110), protein processing in endoplasmic reticulum (ko04141), and longevity-regulating pathway in multiple species (ko04213). The major egg antigen belongs to the polygene family of small heat shock proteins (HSP20) (Zhang et al. 2021). GRP78 is an important regulator of endoplasmic reticulum homeostasis, which plays various roles in protein folding, ER calcium binding, and controlling of the activation of transmembrane ER stress sensors (Rao et al. 2002). Two genes were significantly enriched in pathways related to protein homeostasis. Over-dominant expression (upregulation) of these two genes involved in protein homeostasis may well be consistent with the higher survival rate of reciprocal hybrids compared to purebreds. In addition, more than ten GO terms related to protein folding or refolding were significantly enriched in an OD pattern of GA. Accordingly, similar to the results presented in hybrid abalone (Xiao et al. 2021), genes with an OD pattern may play an important role in thermo-resistant heterosis by maintaining protein homeostasis.

The negative regulation of intrinsic apoptotic signaling pathway (GO:1902176) was another most significantly enriched GO term with an OD pattern in GA and AG under thermal stress. The effective anti-apoptosis system may be an adaptive capacity for bivalves to tackle environmental stressors (Hu et al. 2022). BAG protein binds the ATPase structural domain of Hsp70 and triggers conformational alterations, which can strongly respond to anti-apoptosis mechanism (Zhu et al. 2016). Upregulation of intracellular Hsp27 protects cells from TNF-α-mediated apoptosis (Lampros et al. 2022). In this study, BAG, Hsp70, and Hsp27 were OD expressed in both GA and AG, thereby playing a key role in defense under heat stress to prolong hybrid oyster lifespan (Zhang et al. 2012b). Our result parallels the finding in *Crassostrea sikamea* (♀) × *C. gigas* (♂) that inhibition of apoptosis may be a critical cause of hybrid adaptation (Zhang et al. 2022).

Inhibited cell proliferation under stress was shown to preserve energy and prevent cells with damaged DNA from dividing (Buckley et al. 2006). We clearly observed that the DEGs involved in the cell cycle (ko04110) and cell cycle–yeast pathway (ko04111) were UD expressed in GA and AG. MCM6 is an important regulator of DNA replication that carries instructions for partial synthesis of the MCM complex, resulting in the start of eukaryotic genome replication (Zeng et al. 2021). ANAPC4 is a subunit of the anaphase-promoting complex (APC), which facilitates metaphase-anaphase shift by ubiquitinating its specific substrates (Jörgensen et al. 2001). The

UD expression (downregulation) of MCM6 and ANAPC4 may be one of the mechanisms for the reciprocal hybrids to adapt to thermal stress.

Conclusion

Hybrid oysters exhibited higher survival rate than parental species, *C. gigas* and *C. angulata*. Here, we examined the transcriptomic divergence between the reciprocal hybrids and its parents under thermal stress using RNA-seq. The global gene expression levels in reciprocal hybrids were noticeably biased towards the *C. gigas*. The majority of the expressed genes exhibited non-additive expression patterns, in which under- and over-dominant expression levels were the main categories. We proposed that the OD genes contribute to heterosis mainly by maintaining protein homeostasis and inhibiting apoptosis, whereas UD genes contribute to heterosis mainly by reducing cell proliferation. This study enhances our current understanding of thermo-resistant heterosis in hybrid oysters, and several possible candidate genes may prove useful in the efforts to breed thermo-resistant varieties, which might help in reducing production losses due to massive mortality during warm season.

Declarations

Ethics Approval The *C. gigas*, *C. angulata*, and their reciprocal hybrids are neither an endangered nor protected species. All experiments in this study were conducted according to national and institutional guidelines.

Competing Interests The authors declare no competing interests.

References

Baranwal VK, Mikkilineni V, Zehr UB, Tyagi AK, Kapoor S (2012) Heterosis: emerging ideas about hybrid vigour. J Exp Bot 63:6309–6314

Bi Y, Meyer A, Downs GS, Shi X, El-kereamy A, Lukens L, Rothstein SJ (2014) High throughput RNA sequencing of a hybrid maize and its parents shows different mechanisms responsive to nitrogen limitation. BMC Genomics 15:77

Birchler JA, Auger DL, Riddle NC (2003) In search of the molecular basis of heterosis. Plant Cell 15:2236–2239

Buckley BA, Gracey AY, Somero GN (2006) The cellular response to heat stress in the goby *Gillichthys mirabilis*: a cDNA microarray and protein-level analysis. J Exp Biol 209:2660–2677

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol 58:377–406

Chen ZJ (2013) Genomic and epigenetic insights into the molecular bases of heterosis. Nat Rev Genet 14:471–482

Childs LH, Witucka-Wall H, Günther T, Sulpice R, von Korff M, Stitt M, Walther D, Schmid KJ, Altmann T (2010) Single feature polymorphism (SFP)-based selective sweep identification and association mapping of growth-related metabolic traits in *Arabidopsis thaliana*. BMC Genomics 11:188

Cui X, Affourtit J, Shockley KR, Woo Y, Churchill GA (2006) Inheritance patterns of transcript levels in F1 hybrid mice. Genetics 174:627–637

de Lorgeril J, Lucasson A, Petton B, Toulza E, Montagnani C, Clerissi C, Vidal-Dupiol J, Chaparro C, Galinier R, Escoubas JM, Haffner P, Dégremont L, Charrière GM, Lafont M, Delort A, Vergnes A, Chiarello M, Faury N, Rubio T, Leroy MA, Pérignon A, Régler D, Morga B, Alunno-Bruscia M, Boudry P, Roux FL, Destoumieux-Garzón D, Gueguen Y, Mitta G (2018) Immune-suppression by OsHV-1 viral infection causes fatal bacteraemia in Pacific oysters. Nat Commun 9:4215

de la Cruz FL, Gallardo-Escárate C (2011) Intraspecies and interspecies hybrids in *Haliotis*: natural and experimental evidence and its impact on abalone aquaculture. Rev Aquac 3:74–99

Debes PV, Normandeau E, Fraser DJ, Bernatchez L, Hutchings JA (2012) Differences in transcription levels among wild, domesticated, and hybrid Atlantic salmon (*Salmo salar*) from two environments. Mol Ecol 21:2574–2587

Di G, Luo X, You W, Zhao J, Kong X, Ke C (2015) Proteomic analysis of muscle between hybrid abalone and parental lines *Haliotis gigantea* reeve and *Haliotis discus hannai* Ino. Heredity 114:564–574

Doebley J, Lukens L (1998) Transcriptional regulators and the evolution of plant form. Plant Cell 10:1075–1082

Fiévet J, Dillmann C, de Vienne D (2010) Systemic properties of metabolic networks lead to an epistasis-based model for heterosis. Theor Appl Genet 120:463–473

Fujimoto R, Taylora JM, Shirasawab S, Peacocka WJ, Dennisa ES (2012) Heterosis of *Arabidopsis* hybrids between C24 and Col is associated with increased photosynthesis capacity. Proc Natl Acad Sci USA 109:7109–7114

Gao Y, Zhang H, Gao Q, Wang L, Zhang F, Siva VS, Zhou Z, Song L, Zhang S (2013) Transcriptome analysis of artificial hybrid pufferfish Jiyan-1 and its parental species: implications for pufferfish heterosis. PLoS One 8:e58453

Ghaffari H, Wang W, Li A, Zhang G, Li L (2019) Thermotolerance divergence revealed by the physiological and molecular responses in two oyster subspecies of *Crassostrea gigas* in China. Front Physiol 10:1137

Green RD (2009) Future needs in animal breeding and genetics. J Anim Sci 87:793–800

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

Gu H, Qi Y, Jia Y, Zhang Z, Nie C, Li X, Li J, Jiang Z, Wang Q, Qu L (2019) Inheritance patterns of the transcriptome in hybrid chickens and their parents revealed by expression analysis. Sci Rep 9:4043

Guo M, Rupe MA, Danilevskaya ON, Yang XF, Hu ZH (2003) Genome-wide mRNA profiling reveals heterochronic allelic

variation and a new imprinted gene in hybrid maize endosperm. Plant J 36:30–44

Guo M, Rupe MA, Yang X, Crasta O, Zinselmeier C, Smith OS, Bowen B (2006) Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. Theor Appl Genet 113:831–845

Hedgecock D, Lin J, DeCola S, Haudenschild CD, Meyer E, Manahan DT, Bowen B (2007) Transcriptomic analysis of growth heterosis in larval Pacific oysters (*Crassostrea gigas*). Proc Natl Acad Sci USA 104:2313–2318

Hu Z, Feng J, Song H, Zhou C, Yu Z, Yang M, Shi P, Guo Y, Li Y, Zhang T (2022) Mechanisms of heat and hypoxia defense in hard clam: insights from transcriptome analysis. Aquaculture 549:737792

Jiang G, Li Q, Xu C, Liu S, Kong L, Yu H (2021) Reciprocal hybrids derived from Crassostrea gigas and C. angulata exhibit high heterosis in growth, survival and thermotolerance in northern China. Aquaculture 545:737173

Jiang G, Li Q, Xu C (2022a) Growth, survival and gonad development of two new types of reciprocal triploid hybrids between Crassostrea gigas and C. angulata. Aquaculture 559:738451

Jiang G, Zhou J, Cheng G, Meng L, Chi Y, Xu C, Li Q (2022b) Examination of survival, physiological parameters and immune response in relation to the thermo-resistant heterosis of hybrid oysters derived from Crassostrea gigas and C. angulata. Aquaculture 559:738454

Jörgensen PM, Gräslund S, Betz R, Ståhl S, Larsson C, Höög C (2001) Characterisation of the human *APC1*, the largest subunit of the anaphase-promoting complex. Gene 262:51–59

Krieger U, Lippman ZB, Zamir D (2010) The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. Nat Genet 42:459–463

Lai J, Li R, Xu X, Jin W, Xu M, Zhao H, Xiang Z, Song W, Ying K, Zhang M, Jiao Y, Ni P, Zhang J, Li D, Guo X, Ye K, Jian M, Wang B, Zheng H, Liang H, Zhang X, Wang S, Chen S, Li J, Fu Y, Springer NM, Yang H, Wang J, Dai J, Schnable PS, Wang J (2010) Genome-wide patterns of genetic variation among elite maize inbred lines. Nat Genet 42:1027–1030

Lampros M, Vlachos N, Voulgaris S, Alexiou GA (2022) The role of Hsp27 in chemotherapy resistance. Biomedicines 10:897

Langdon C, Evans F, Jacobson D, Blouin M (2003) Yield of culture Pacific oyster *Crassostrea gigas* Thunberg improved after one generation of selection. Aquaculture 220:227–244

Li D, Huang Z, Song S, Xin Y, Mao D, Lv Q, Zhou M, Tian D, Tang M, Wu Q, Liu X, Chen T, Song X, Fu X, Zhao B, Liang C, Li A, Liu G, Li S, Hu S, Cao X, Yu J, Yuan L, Chen C, Zhu L (2016) Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase. Proc Natl Acad Sci USA 113:E6026–E6035

Li A, Li L, Song K, Wang W, Zhang G (2017) Temperature, energy metabolism, and adaptive divergence in two oyster subspecies. Ecol Evol 7:6151–6162

Liang S (2017) Physiological and molecular basis of cold disease resistance heterosis in *Haliotis diversicolor* hybrid. Xiamen University, Xiamen

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}T$ method. Methods 25:402–408

Luo Q, Samanta, MP, Köksal F, Janda J, Galbraith DW, Richardson CR, Ou-Yang F, Rock CD (2009) Evidence for antisense transcription associated with microRNA target mRNAs in Arabidopsis. PLoS Genet 5:e1000457

Meng L, Li Q, Xu C, Liu S, Kong L, Yu H (2021) Hybridization improved stress resistance in the Pacific oyster: evidence from physiological and immune responses. Aquaculture 545:737227

Rawson P, Feindel S (2012) Growth and survival for genetically improved lines of eastern oysters (*Crassostrea virginica*) and interline hybrids in Maine, USA. Aquaculture 25:61–67

Rao R, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, Bredesen DE (2002) Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Lett 514:122–128

Rahman MA, Arshad A, Marimuthu K, Ara R, Amin SMN (2013) Inter-specific hybridization and its potential for aquaculture of fin fishes. Asian J Anim Vet Adv 8:139–153

Schnable JC, Springer NM, Freeling M (2011) Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. Proc Natl Acad Sci USA 108:4069–4074

Shatkin G, Shumway SE, Hawes R (1997) Considerations regarding the possible introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: a review of global experience. J Shellfish Res 16:463–477

Somerville C, Somerville S (1999) Plant functional genomics. Science 285:380–383

Springer NM, Stupar RM (2007) Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res 17:264–275

Stupar RM, Springer NM (2006) Cis-transcriptional variation in maize inbred lines B73 and Mo17 lead to additive expression patterns in the F1 hybrid. Genetics 173:2199–2210

Sun Y, Huang Y, Hu G, Zhang X, Ruan Z, Zhao X, Guo C, Tang Z, Li X, You X, Lin H, Zhang Y, Shi Q (2016) Comparative transcriptomic study of muscle provides new insights into the growth superiority of a novel grouper hybrid. PLoS One 11:e0168802

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents. Proc Natl Acad Sci USA 103:6805–6810

Swanson-Wagner RA, DeCook R, Jia Y, Bancroft T, Ji T, Zhao X, Nettleton D, Schnable PS (2009) Paternal dominance of trans-eQTL influences gene expression patterns in maize hybrids. Science 326:1118–1120

Tan K, Liu H, Ye T, Ma H, Li S, Zheng H (2020) Growth, survival and lipid composition of *Crassostrea gigas*, *C. angulata* and their reciprocal hybrids cultured in southern China. Aquaculture 516:734524

Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, Van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28:511–515

Tripp-Valdez MA, Cicala F, Galindo-Sánchez CE, Chacón-Ponce KD, López-Landavery E, Díaz F, Re-Araujo D, Lafarga-De la Cruz F (2021) Growth performance and transcriptomic response of warm-acclimated hybrid abalone *Haliotis rufescens* (♀) × *H. corrugata* (♂). Mar Biotechnol 23:62–76

Vuylsteke M, Eeuwijk FV, Hummelen PV, Kuiper M, Zabeau M (2005) Genetic analysis of variation in gene expression in *Arabidopsis thaliana*. Genetics 171:1267–1275

Wang C, Liu B, Li J, Liu S, Li J, Hu L, Fan X, Du H, Fang H (2011) Introduction of the Peruvian scallop and its hybridization with the bay scallop in China. Aquaculture 310:380–387

Xiao Q, Huang Z, Shen Y, Gan Y, Wang Y, Gong S, Lu Y, Luo X, You W, Ke C (2021) Transcriptome analysis reveals the molecular mechanisms of heterosis on thermal resistance in hybrid abalone. BMC Genomics 22:650

Xu C (2013) Larch genes mining and mechanism on differential gene expression and heterosis formation. Chinese Academy of Forestry, Beijing

Yang J, Luo S, Li J, Zheng Z, Du X, Deng Y (2018) Transcriptome analysis of growth heterosis in pearl oyster *Pinctada fucata martensii*. FEBS Open Bio 8:1794–1803

Zeng T, Guan Y, Li Y, Wu Q, Tang XJ, Zeng X, Ling H, Zou J (2021) The DNA replication regulator MCM6: an emerging cancer biomarker and target. Clin Chim Acta 517:92–98

Zhai R, Feng Y, Wang H, Zhan X, Shen X, Wu W, Zhang Y, Chen D, Dai G, Yang Z, Cao L, Cheng S (2013) Transcriptome analysis of rice root heterosis by RNA-Seq. BMC Genomics 14:19

Zhang G, Li J, Zhang J, Liang X, Zhang X, Wang T, Yi S (2019) Integrated analysis of transcriptomic, miRNA and proteomic changes of a novel hybrid yellow catfish uncovers key roles for miRNAs in heterosis. Mol Cell Proteomics 18:1437–1453

Zhang H, Xu X, He Z, Zheng T, Shao J (2017) De novo transcriptome analysis reveals insights into different mechanisms of growth and immunity in a Chinese soft-shelled turtle hybrid and the parental varieties. Gene 605:54–62

Zhang C, Lin C, Fu F, Zhong X, Peng B, Yan H, Zhang J, Zhang W, Wang P, Ding X, Zhang W, Zhao L (2017b) Comparative transcriptome analysis of flower heterosis in two soybean F1 hybrids by RNA-seq. PLoS One 12:e0181061

Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, Yang P, Zhang L, Wang X, Qi H, Xiong Z, Que H, Xie Y, Holland PWH, Paps J, Zhu Y, Wu F, Chen Y, Wang J, Peng C, Meng J, Yang L, Liu J, Wen B, Zhang N, Huang Z, Zhu Q, Feng Y, Mount A, Hedgecock D, Xu Z, Liu Y, Domazet-Lošo T, Du Y, Sun X, Zhang S, Liu B, Cheng P, Jiang X, Li J, Fan D, Wang W, Fu W, Wang T, Wang B, Zhang J, Peng Z, Li Y, Li N, Wang J, Chen M, He Y, Tan F, Song X, Zheng Q, Huang R, Yang H, Du X, Chen L, Yang M, Gaffney PM, Wang S, Luo L, She Z, Ming Y, Huang W, Zhang S, Huang B, Zhang Y, Qu T, Ni P, Miao G, Wang J, Wang Q, Steinberg CEW, Wang H, Li N, Qian L, Zhang G, Li Y, Yang H, Liu X, Wang J, Yin Y, Wang J (2012) The oyster genome reveals stress adaptation and complexity of shell formation. Nature 490:49–54

Zhang L, Hou R, Su H, Hu X, Wang S, Bao Z (2012b) Network analysis of oyster transcriptome revealed a cascade of cellular responses during recovery after heat shock. PLoS One 7:e35484

Zhang J, Li R, Chen X, He X, Wang Z, Zheng Y, Hu J, Guo X (2021) Preparation of the major egg antigen recombinant protein of *Echinococcus multilocularis* and its preliminary application in immunodiagnosis. Chinese Veterin Sci 50:982–988

Zhang X, Fan C, Zhang X, Li Q, Li Y, Ma P, Yue S, Huo Z, Wang Z (2022) Transcriptome analysis of *Crassostrea sikamea* (♀) × *Crassostrea gigas* (♂) hybrids under and after thermal stress. J Ocean Univ China 21:213–224

Zheng G, Wu C, Liu J, Chen J, Zou S (2019) Transcriptome analysis provides new insights into the growth superiority of a novel back-cross variety, *Megalobrama amblycephala* ♀ × (*M. amblycephala* ♀ × *Culter alburnus* ♂) ♂. Aquaculture 512:734317

Zhou Y, Ren L, Xiao J, Zhong H, Wang J, Hu J, Yu F, Tao M, Zhang C, Liu Y, Liu S (2015) Global transcriptional and miRNA insights into bases of heterosis in hybridization of Cyprinidae. Sci Rep 5:13847

Zhu Q, Zhang L, Li L, Que H, Zhang G (2016) Expression characterization of stress genes under high and low temperature stresses in the Pacific oyster, *Crassostrea gigas*. Mar Biotechnol 18:176–188

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