Asymmetric Expression of Homoeolog Genes Contributes to Diet Adaption of an Allodiploid Hybrid Fish Derived from *Megalobrama Amblycephala* (♂) × *Culter alburnus* (♀)

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Abstract

Background: Hybridization, which can merge two or more divergent genomes and form a new allopolyploid, is an important technique in fish genetic breeding. However, the coexistence of two subgenomes in a single nucleus may cause subgenome interactions and dominance at the gene expression level; this has been observed in some allopolyploid plants. In previous studies, a new allodiploid hybrid fish derived from the herbivorous *Megalobrama amblycephala* (♂) × the carnivorous *Culter alburnus* (♀) had herbivorous characteristics. It is thus interesting to further investigate whether subgenome interaction and dominance inform dietary adaptation in this hybrid fish.

Results: Differential expression, homoeolog expression silencing and bias were investigated in the hybrid fish after a 70 day adaptation to a carnivorous and herbivorous diet. A total of 2.65×10^8 clean reads (74.06 Gb) from the liver and intestine transcriptome were mapped to the two parent genomes based on the specific SNPs. A total of 4385 and 2538 differentially expressed homoeolog genes (DEHs) in the intestine and liver tissues, respectively, of the two groups of fish were identified. DEHs were highly enriched in fat digestion, carbon metabolism, amino acid metabolism, and steroid biosynthesis. Furthermore, subgenome-selective expression in all tissues was observed, and the paternal subgenome was more dominant than the maternal subgenome during dietary adaptation. Moreover, subgenome expression dominance controlled functional pathways in metabolism, disease, cellular processes, environment, and genetic information processing during the two dietary adaptation processes. In addition, fewer and more robust villi in the intestine, significant fat accumulation, and a higher concentration of malondialdehyde in the liver were observed in carnivorous fish than in the herbivorous fish.

Conclusions: Diet drives phenotypic and genetic variation, and the asymmetric expression of homoeolog genes (including differential expression, selective expression, homoeolog expression silence and bias) may play key roles in dietary adaptation of the hybrid fish. Subgenome expression dominance elucidates the mechanistic basis of heterosis and also provides perspectives on fish genetic breeding, and application.

Background

Hybridization, which can quickly merge two or more divergent genomes and form a new allopolyploid, is a driving force in genomic evolution and speciation[1]. Merged subgenomes must adjust and coexist in a single nucleus; this frequently causes genetic incompatibilities that induce a series of rapid genetic and epigenetic modifications[2]. Such modifications, including sequence elimination, activation of genes and retroelements, homoeologous interactions, and exchanges, may directly affect the establishment of a nascent polyploid and its evolutionary success as a new species [3–5]. The success of newly formed polyploids is evidenced in the variable expression of homeologous genes, including differential expression and subgenome dominance (where one of the parental subgenomes retains more genes and more highly expressed, including expression silence and bias), which have been observed in some

allopolyploid plants and a few animals [2–8]. Subgenome dominance, contributing to the partitioning of certain phenotypic traits, has been reported in cotton [9], wheat [10–11], maize [12] and blueberries[13].

In fish, as in other vertebrates, genetic variability and phenotypic plasticity can promote within-species or within-population diversity of foraging strategies, while dietary shift (adaption) is likely to enhance the overall phenotypic variance and additive genetic evolution in fish populations [14–17]. Genetic variability, including genome evolution (genetic variants, gene duplication, gene family expansion and contraction, allopolyploid, etc.) and gene regulation (epigenetic), contributes to feeding behavior and dietary adaptation and has been documented in humans [18], dogs [19], giant panda [20], bats [21], and fishes [7, 22, 23]. Recently, growing evidence has shown that feeding also drives phenotypic and genetic evolution; this has been demonstrated in perch (*Perca fluviatilis* L.) [15], stickleback [24], and baleen whales [25]. However, the genotype × diet interactions contributing to foraging strategies in fish are poorly studied, especially those in newly-formed hybrid fish species.

Hybridization is an important technique and is widely used in fish genetic breeding. In a previous study, a hybrid fish was derived from the herbivorous blunt snout bream (*Megalobrama amblycephala*, BSB, ♀) × the carnivorous topmouth culter (*Culter alburnus*, TC, ♂) [26]. The hybrid fish has bisexual fertility and contains one set of chromosomes from each parent, providing an ideal model to investigate some interesting biological processes. A comparative analysis of the gut microbiota and gastrointestinal tracts of the hybrid fish and its parents suggested that the hybrid fish is biased towards herbivory [27]. A recent study also observed the asymmetric expression of alleles (including additive and dominance effects and *cis* and *trans* regulations) in the hybrid fish lineage based on the whole genome sequences of BSB and TC [28]. The results provide new insights into the alternative strategies for counteracting the deleterious effects of the subgenomes and improving the adaptability of novel hybrids. It would thus be interesting to further characterize whether subgenome interaction (the expression pattern of homoeolog genes) informs the dietary adaptation of the hybrid fish. In this study, the intestine and liver transcriptome of the hybrid fish were compared after a 70 d adaptation to herbivorous and carnivorous diets. The focus was on the differentially expressed homoeolog genes, as well as the subgenome interaction (selective expression, homoeolog expression silence and bias) between the two groups of fish. This study may provide a basis for the investigation of the signal transduction pathways that mediate food digestion, metabolism, and adaptation and will also provide perspectives for application in fish breeding.

Results

Histological study of the intestine and liver

Histological analysis showed a significant difference between the morphologies of the intestine and liver tissues of the two groups of hybrid fish. The intestinal villi in the carnivorous fish were observed to be sturdy but fewer in number compared to those in the herbivorous fish (which had many, fine villi) (Fig. 1a,b). Normal hepatocytes with regular, round nuclei were observed in the liver of both groups of fish. However, few hypertrophied hepatocytes, without a nucleus were observed in the herbivorous fish,

while a large number of lipid droplets were observed in the carnivorous fish (Fig. 1c,d). In addition, significant fat accumulation in the liver tissues of carnivorous fish was confirmed by the ORO staining method (Fig. 1e,f).

Differentially expressed homoeolog genes (DEHs) associated with dietary adaptation

A total of 2.65×10^8 clean reads (74.06 Gb) were obtained from the intestine and liver tissues. Approximately 90.73% of those clean reads were mapped to the reference genomes based on a total of 2.32×10^6 species-unique SNPs (Table 1, Additional file 1). The complete clean reads of the intestine and liver libraries were uploaded to the NCBI Sequence Read Archive (SRA) website (http://www.ncbi.nlm.nih.gov/sra/) under accession number PRJNAXXXXXX.

Table 1
Basic information of the intestine and liver transcriptomes of the hybrid fish in the two diet groups

Fish group	Total clean reads	Clean reads bases (bp)	SNP Number	Uniq mapped reads	Total mapped reads(%)
C-Intestine1	40875328	6120857148	178115	33,355,355(81.60%)	37659356(92.13%)
C-Intestine2	44897254	6724235532	172520	36,725,040(81.80%)	41284036(91.95%)
C-Intestine3	45432496	6801174866	176720	36,842,441(81.09%)	41430875(91.19%)
H-Intestine1	45623958	6831303448	469045	38,470,050(82.10%)	40048325(87.78%)
H-Intestine2	45240496	6777495390	388932	35,867,617(82.10%)	40857373(90.31%)
H-Intestine3	45129468	6760490246	296917	32,785,787(82.43%)	40738675(90.27%)
H-Liver1	40301740	6031302492	124038	35,510,632(77.83%)	37363894(92.71%)
H-Liver2	50305328	7536275016	134514	36,584,989(80.87%)	46520571(92.48%)
H-Liver3	42994402	6436248490	113674	36,549,783(80.99%)	39787469(92.54%)
C-Liver1	46856834	7010976494	92877	33,170,072(82.30%)	43343128(92.50%)
C-Liver1	43690178	6540010734	92342	41,286,506(82.07%)	35867617(82.10%)
C-Liver1	39773512	5952176384	87825	35,407,930(82.35%)	36940518(92.88%)

Uniq Mapped Reads: the number of reads aligned to the unique position of the reference genome and percentage of reads. C-Intestine1 ~ 3 and C-Liver1 ~ 3: three intestine and liver tissues of hybrid fish from carnivorous diet group, respectively. H-Intestine1 ~ 3 and H-Liver1 ~ 3: three intestine and liver tissues of hybrid fish from herbivorous diet group, respectively.

Based on the number of mapped reads and genome annotation, DEHs between the two groups of fish were detected in the intestine and liver tissues (Additional file 2). A total of 28457 homoeolog genes were expressed in the intestine. Among those expressed genes, 4832 DEHs were detected, including 2446 that were higher expressed and 2386 that were lower expressed in herbivorous fish than in carnivorous fish (Fig. 2a,c). A total of 19218 genes were co-expressed in the liver and 2458 DEHs were detected, including 1244 that were higher expressed and 1214 that were lower expressed in the herbivorous fish compared to the carnivorous fish (Fig. 2b,d). The top 24 DEHs in the intestine and liver tissues are presented in Table 2. Compared with carnivorous fish, some DEHs, such as *sqlea*, *hmgcra*, *pck1*, *ulk2*, and *dio3b*, were detected both in the intestine and liver tissues of the herbivorous fish.

Table 2
Top 24 differentially expressed homoeolog genes in the intestine and liver tissues of herbivorous vs carnivorous hybrid fish

DEHs in intestine			**DEHs in liver**		
Gene symbol	Log2 (fold change)	KO ID	Gene symbol	Log2 (fold change)	KO ID
cyp7a1	8.30	K00489	*sqlea*	9.59	K00511
cxcl8b.3	7.76	K10030	*hmgcra*	9.22	K00021
sqlea	6.75	K00511	*cyp51*	5.91	K05917
col12a1b	5.64	K08132	*mcm6*	5.87	K02542
fgf4	5.59	K04358	*ntd5*	5.76	K17305
rpe65b	5.40	K20991	*am2*	5.47	K03910
clca5.2	4.98	K05030	*mcm2*	5.20	K02540
lamc3	4.95	K06247	*epoa*	5.20	K05437
tubb5	4.89	K07375	*hells*	5.17	K19001
ppp1r3ca	4.79	K07189	*hsd17b7*	5.16	K13373
fuk	4.76	K05305	*mcm3*	5.16	K02541
hmgcra	4.73	K13187	*ebp*	5.12	K01824
mat1a	-7.13	K00789	*klf13*	-5.91	K09208
diabloa	-7.09	K10522	*pck1*	-5.80	K01596
pck1	-6.01	K01596	*lpin1*	-5.30	K15728
ulk2	-5.84	K08269	*lipg*	-5.15	K01046
slc6a19a	-5.79	K05334	*foxo1a*	-5.05	K07201
slc26a6	-5.71	K14704	*arg2*	-5.02	K01476
bco1	-5.28	K00515	*slc25a48*	-4.77	K15124
dio3b	-5.19	K07754	*tdo2*	-4.69	K00453
slc15a1b	-4.97	K14206	*pdzrn3a*	-4.66	K15682
pde9al	-4.63	K13761	*ulk2*	-4.66	K08269
ucp1	-4.58	K15103	*sik2b*	-4.65	K16311
ctrl	-4.50	K09632	*dio3b*	-4.53	K07754

Uniquely expressed genes were detected for each tissue and diet. There were 17 and 11 homoeolog genes identified as uniquely expressed in the liver and intestinal tissues of herbivorous fish, respectively, and

none were observed to be uniquely expressed in carnivorous fish (Table S1). With no apparent correlation to diet, 569 and 48 uniquely expressed genes were found in the intestine and the liver, respectively (Additional file 3). A homoeolog gene, Rho GTPase-activating protein (*arhgap29*), was only expressed in the intestine and liver in the herbivorous fish (Additional file 3).

KEGG function cluster analysis revealed that the intestinal DEHs were enriched in 364 pathways and the liver DEHs were enriched in 345 pathways. The DEHs that were highly enriched in PPAR signaling pathway (ko03320); biosynthesis of secondary metabolites (ko01110); glycine, serine and threonine metabolism (ko00260); cysteine and methionine metabolism (ko00270); glycerolipid metabolism (ko00561); and fat digestion and absorption (ko04975), are associated with metabolism in the two groups of fish (Table 3). Interestingly, genes involved in steroid biosynthesis (ko00100) (including C5 isoprenoid biosynthesis and the mevalonate pathway) (Figure S1) were significantly up-regulated, and genes involved in the insulin signaling pathway (ko04910) (Figure S2) were significantly down-regulated in the liver and intestine of herbivorous fish compared to those of carnivorous fish.

Table 3
Top 15 enriched pathways associated with differentially expressed homoeolog genes related to metabolism in herbivorous vs carnivorous hybrid fish

Pathway in intestine transcriptome	**Gene NO.**	**Reference NO.**	**P value**	**Pathway ID**
Biosynthesis of antibiotics	109	459	1.43E-13	ko01130
PPAR signaling pathway	40	134	1.13E-08	ko03320
Carbon metabolism	59	240	1.60E-08	ko01200
Steroid biosynthesis	19	40	2.02E-08	ko00100
Fat digestion and absorption	27	75	3.65E-08	ko04975
Biosynthesis of secondary metabolites	138	757	4.06E-08	ko01110
Cholesterol metabolism	33	111	2.36E-07	ko04979
Glycine, serine and threonine metabolism	28	86	2.39E-07	ko00260
Cysteine and methionine metabolism	28	87	3.14E-07	ko00270
Biosynthesis of amino acids	42	165	6.80E-07	ko01230
Fatty acid biosynthesis	15	34	2.06E-06	ko00061
Proximal tubule bicarbonate reclamation	19	53	4.09E-06	ko04964
Protein digestion and absorption	53	244	5.08E-06	ko04974
Glycolysis / Gluconeogenesis	32	129	2.46E-05	ko00010
Citrate cycle (TCA cycle)	19	62	5.23E-05	ko00020
Pathway in liver transcriptome	Gene NO.	Reference NO.	P value	Pathway ID
Biosynthesis of antibiotics	80	459	2.85E-15	ko01130
Metabolic pathways	257	2479	8.47E-14	ko01100
Biosynthesis of secondary metabolites	103	757	4.59E-12	ko01110
Steroid biosynthesis	16	40	2.40E-09	ko00100
Glycine, serine and threonine metabolism	22	86	4.08E-08	ko00260
FoxO signaling pathway	47	303	7.83E-08	ko04068
Insulin signaling pathway	49	323	8.44E-08	ko04910
Cysteine and methionine metabolism	21	87	2.42E-07	ko00270
PPAR signaling pathway	27	134	2.68E-07	ko03320
Cholesterol metabolism	24	111	3.14E-07	ko04979

Pathway in intestine transcriptome	Gene NO.	Reference NO.	P value	Pathway ID
AMPK signaling pathway	44	298	8.28E-07	ko04152
Terpenoid backbone biosynthesis	14	46	1.24E-06	ko00900
Glyoxylate and dicarboxylate metabolism	17	68	1.99E-06	ko00630
Carbon metabolism	35	240	1.43E-05	ko01200
Adipocytokine signaling pathway	27	169	2.64E-05	ko04920

Homoeolog expression silencing and bias associated with dietary adaptation

Based on the mapped reads data, parent subgenomes were selectively expressed in all tissues, with homoeolog genes derived from the TC parent significantly more expressed than those derived from the BSB parent. For example, 28457 homoeolog genes were expressed in the intestinal tissue, including 6270 annotated to the BSB genome, 15534 annotated to the TC genome, and 6653 newly assembled genes. In the liver tissues, 19218 genes were co-expressed, including 4093 annotated to the BSB genome, 11220 annotated to the TC genome, and 3905 newly assembled genes (Fig. 3a, Additional file 2).

Homoeolog expression silencing (HES) and Homoeolog expression bias (HEB) were detected in the intestine and liver transcriptome based on a homologous sequence BLAST of the BSB and TC genomes, respectively (Additional file 4). The results showed that the TC subgenome was dominantly expressed in all tissues. The number of BSB-HES genes was higher than the number of TC-HES genes in all tissues ($p < 0.05$). The number of TC-HEB genes was higher than the number of BSB-HEB genes ($p < 0.05$, indicating that the homoeolog genes derived from the parent were unequally expressed in the hybrid fish during dietary adaptation (Table 4). Homoeolog genes also identified cases where subgenome expression dominance was shared or unique to a specific tissue. The results showed that subgenome expression dominance was related to tissue, while it was almost unrelated to diet (Fig. 3b). For instance, the number of shared homoeolog genes (299 in the liver and 277 in the intestine, with BSB subgenome expression dominance) was more than the number of uniquely expressed genes in the liver and intestine, while the number of uniquely expressed homoeolog genes was more than the number of shared genes in the liver and intestine. However, the number of homoeolog genes that showed TC subgenome expression dominance was more than the number of uniquely expressed genes in all tissues. In addition, 140 homoeolog genes that showed parent subgenome expression dominance were shared among all tissues (Fig. 3b).

Table 4
Homoeolog gene expression silencing and bias in tissues in herbivorous vs carnivorous hybrid fish

Type	TC subgenome dominance		BSB subgenome dominance		Normal	Total
	BSB-HES	TC-HEB	TC-HES	BSB-HEB		
C-Intestine	4522(44.92%)	2977(29.57%)	243(2.41%)	254(2.52%)	2070(20.56%)	10066
H-Intestine	4132(37.17%)	3909(35.17%)	233(2.10%)	296(2.66%)	2596(23.35%)	11166
C-Liver	4482(54.95%)	1706(20.91%)	319(3.91%)	198(2.43%)	1452(17.80%)	8157
H-Liver	4334(49.32%)	2315(26.34%)	309(3.52%)	197(2.24%)	1633(18.58%)	8788

COG function analysis revealed that the homoeolog genes with BSB subgenome dominance and TC subgenome dominance were highly enriched in translation ribosomal structure and biogenesis, posttranslational modification, protein turnover, chaperones, carbohydrate transport and metabolism, and signal transduction mechanisms (Additional file 5). KEGG function enrichment analysis revealed that the parent subgenome controlled certain pathways during dietary adaptation. For instance, the BSB subgenome mainly controlled metabolism (including pyrimidine metabolism, glycosphingolipid biosynthesis, glycosaminoglycan biosynthesis, retinol metabolism, glucosinolate biosynthesis, folate biosynthesis) and diseases (epithelial cell signaling in *Helicobacter pylori* infection, inflammatory bowel disease, and antifolate resistance), while the TC subgenome mainly controlled genetic information processing (through ribosomes, spliceosomes, aminoacyl-tRNA biosynthesis, basal transcription factors, and ubiquitin mediated proteolysis) and cellular processes (through peroxisomes, lysosomes, the cell cycle, the mitophagy-animal pathway, and the p53 signaling pathway) (Fig. 4, Additional file 5). The relative expression levels (counts of reads) of the homoeolog genes in the pathways of carbon metabolism, starch and sucrose metabolism, taste, and olfactory transduction are presented in Fig. 5.

qPCR validation

To validate the quality of the RNA-seq data, twelve DEHs involved in the mevalonate pathway and fatty acid biosynthesis pathways (including 3-hydroxy-3-methylglutaryl-CoA reductase (*hmgcra*); glucokinase (*gck*); EBP cholestenol delta-isomerase (*ebp*); Isopentenyl-diphosphate delta isomerase 1 (*idi1*); Cytochrome P450 family 51 subfamily A member 1 (*cyp51a1*), Mevalonate diphosphate decarboxylase (*mvd*); Methionine adenosyltransferase 1A (*mat1a*); Lipase G, endothelial type (*lipg*); Phosphoenolpyruvate carboxykinase 1 (*pck1*); Unc-51 like autophagy activating kinase 1 (*ulk1*); Iodothyronine deiodinase 3 (*dio3*); Beta-carotene oxygenase 1 (*bco1*)) were randomly chosen and subjected to qPCR, with three biological replicates. The relative expression levels of the twelve DEHs are presented in Fig. 6. The trends in the expression levels of the genes detected by qPCR were the same as those of levels determined by RNA-seq data analysis. These results indicate the reliability of the RNA-seq data for the analysis of differentially expressed homoeolog genes during dietary adaptation.

Liver biochemical assays

The concentrations of antioxidants (GSH, MDA, T-SOD and POD) in the liver tissues from carnivorous and herbivorous fish are presented in Table 5. No significant difference was observed between the levels of GSH, T-SOD, and POD between the two groups of fish. However, the concentrations of MDA were significantly higher in carnivorous fish ($p < 0.05$, *t*-test).

Table 5
Biochemical assays of liver tissue of herbivorous vs carnivorous hybrid fish

Type	GSH (g/L)	MDA (nmol/mL)	T-SOD (U/mL)	POD (U/mL)
Carnivorous diet	50.63 ± 2.46	182.21 ± 16.51*	39.23 ± 0.49	27.18 ± 0.67
Herbivorous diet	59.03 ± 2.63	4.02 ± 0.33	41.73 ± 0.44	23.93 ± 0.39
* $p < 0.05$, represent significant different between the two groups.				

Discussion

Diet shifting and adaption affects phenotypic traits

Fish have the potential to change their behavioral and physiological characteristics, such as food selection, food intake, enzyme production, and development of the alimentary tract, to adjust to differences in the quality of available food [27, 29]. Generally, short-term adaptive changes are limited by the range of phenotypic plasticity, and long-term adaptive changes depend on the evolution of the genotype. Previous studies have indicated that differences in the histological structures of the fish gastrointestinal tract are related to feeding habits, food, age, and weight [30, 31]. In this study, the allodiploid hybrid fish that were adapted to carnivorous and herbivorous diets also showed a significant difference in histological structures of liver and intestinal tissues (Figure 1), indicating that food type participates in the developmental process of the gastrointestinal tract of the hybrid fish [14-15].

Genetic adaptation to dietary environments is a key process in the evolution of natural populations and is of great interest in fish breeding, as the ability to adapt to major dietary changes can be used effectively to promote fish welfare and more sustainable aquaculture. Recently studies have focused more on the effect of feeding changes (substituted diets) on the growth performance, metabolism, nutrition, and gene regulation of fishes [32-34]. However, dietary changes and adaptation are more or less affected by the function of hepatic metabolism, which may result in problems related to metabolism and other problems, such as reduced antioxidant capacity, disordered glucose and lipid metabolism, and weakened disease resistance [35-37]. In this study, due to the merge of subgenomes from the carnivorous TC and herbivorous BSB, the hybrid fish seems to have the potential to adapt to carnivorous, herbivorous, or omnivorous diets; in fact, no impact on growth level was observed between the two diet group of fish. Metabolites related to antioxidant protection (GSH, T-SOD, and POD) in the liver were not different between the two groups of fish. A higher concentration of MDA was detected in the carnivorous fish

(Table 5), indicating that adaptation to a carnivorous diet affects hepatic metabolism in the hybrid fish; changes in metabolic enzyme activity need further elucidation.

Diet shifting and adaption affects metabolic pathways

Diet shifting and long-term adaption can affect the digestion and metabolism of major nutrients (carbohydrates, proteins, and lipids) and often leads to significant metabolic changes (such as the modification of certain key metabolic enzymes and hepatic metabolic pathways, including energy metabolism, cholesterol biosynthesis, and proteolytic activity pathways) [35, 38-41]. In the current study, a number of DEHs were identified to be involved in the adaptation of hybrid fish to a herbivorous or carnivorous diet (Figure 2 and Table 2). The DEHs were highly enriched in carbon metabolism, steroid biosynthesis, and amino acid, fatty acid, and protein metabolism (Table 3, Figure S1 and S2), indicating that diet informs genetic adaption, which adjusts to available food. Metabolic adaption of these pathways apparently supports the adaptation of the hybrid fish to a carnivorous or herbivorous diet. In European sea bass (*Dicentrarchus labrax*), genes involved in the cholesterol biosynthetic pathways were upregulated when they were fed a plant diet compared to a fish-based diet [42]. In grass carp (*Ctenopharyngodon idellus*), a herbivorous fish, the genes involved in steroid biosynthesis, terpenoid backbone biosynthesis, and glycerophospholipid metabolic pathways were differentially expressed during the transition from a carnivorous to a herbivorous diet [43]. In BSB, feeding of a high fat-carbohydrate diet for 8 w also caused a significant decline in the number of amino acids entering the TCA cycle, which in turn resulted in elevated levels of seven amino acids [44].

Insulin serves as the primary regulator of the blood glucose balance, regulating the metabolism and storage of nutrients, such as proteins, sugar, and fat, by acting on the liver, muscle, and adipose tissue [45]. In BSB, feeding of a high-carbohydrate diet for 8 w upregulated the genes associated with insulin signaling pathways, which could lead to the development of insulin resistance in hepatocytes, pathological liver changes, and, eventually, non-alcoholic, fatty liver disease [35]. The current study also identified that the insulin receptor, insulin receptor-related receptor, and downstream pathways, including *PI(3)K*, *Akt*, *FOXO1*, *Cbl* proto-oncogene, and *mTOR* pathways, were down-regulated in the herbivorous fish (Fig. S2), suggesting that the adaptation of hybrid fish to a carnivorous diet may also cause insulin unbalance and insulin resistance in hepatocytes [35, 45].

Homoeolog expression silence and bias contribute to dietary adaptation

The merged subgenomes in a allopolyploid must adjust and coexist with one another in a single nucleus; this may cause interactions between diverged regulatory networks due to differential dosage sensitivities and epigenetic alterations. Variable expression of the homoeolog gene, resulting from such subgenome interaction, has been reported in some allopolyploid plants [9-13]. To some extent, investigating the

variable expression of homeologous genes is hindered by at least two major obstacles: (1) lack of genomic resources for extant parental progenitors, and (2) the inability to distinguish the origin of the subgenome. Our previous study completed the whole genome sequencing of the two parents (BSB and TC) of the hybrid fish, providing basic data for the present study [28]. The parent subgenome was observed to be selectively expressed in tissues, and TC exerted a higher subgenome expression dominance than BSB (Figure 3). One possible explanation for this is that the newly formed hybrid must resolve genetic incompatibilities very rapidly within the first generation to adapt to the environment and facilitate reproduction [7, 9]. The subgenome expression dominance may be associated with inherent dominance between subgenomes. Silenced homoeolog genes were unbalanced in tissues, and the number of expression silenced genes was significantly more than the number of expression bias gene during dietary adaptation (Table 4). This may be associated with various regulatory elements, such as *cis* and *trans* regulatory elements, less transposable elements, and DNA methylation [46]. However, the mechanisms to preferentially silence one parental subgenome over other subgenomes and the functions of the subgenomes require further study.

How one subgenome is more expressed and whether the direction of subgenome dominance is externally induced in a polyploid is largely unknown. However, a possible way to resolve genetic incompatibilities is that some certain subset of pathways are controlled by one subgenome, and the rest pathways are controled by another subgenome [11, 47]. Obviously, this would result in the partitioning of phenotypic traits to different subgenomes, and it is of great significance in genetic breeding of plants and animals. Subgenome dominance contributing to heterosis (such as phenotypic bias) has been observed in cotton, wheat, and blueberry plants [9-12]. In the present study, the parent subgenome controlled functional pathways during dietary adaptation. The BSB subgenome mainly controlled the metabolism and diseases pathways, while the TC subgenome mainly controlled genetic information processing and cellular processes (Figure 4). Pathways, such as those involved in the circadian rhythm and digestion and metabolism pathways, were also activated in the grass carp during a transition from a carnivorous to herbivorous diet [43, 48]. Pathways involved in retinal photosensitivity, circadian rhythm, and appetite control show altered expression in mandarin fish (*Sinipercidae*) fed only live prey [49]. Subgenome expression dominance may play key roles in the adaptation of hybrid fish to herbivorous or carnivorous diets. The dynamic expression level of the homoeolog genes may provide a better understanding of the functional pathways that controlled by parent subgenome. However, a limiting factor is that the discontinuity of the SNP site makes it insufficient to design ideal primers. In addition, it will be interesting to investigate whether subgenome expression dominance is heritable and contributes to diet selection in hybrid F_2-F_5 generations.

Conclusions

In summary, the hybrid fish derived from herbivorous BSB (♀) × carnivorous TC (♂) altered the histological structure in the liver and intestinal tissues during adaptation to a herbivorous or carnivorous diet. Furthermore, homoeolog genes involved in metabolic pathways (such as fat, carbon, protein, and amino

acid metabolism) were differentially expressed between the two dietary adaptations. Moreover, subgenome expression dominance was observed and established in the hybrid fish, and the direction of subgenome dominance was almost irrelevant to the dietary adaptation process. In addition, subgenome expression dominance controlled functional pathways (such as pathways related to metabolism, cellular processes, disease, environment, and genetic information processing) during the two dietary adaptations. These results indicate that diet can drive phenotypic and genetic variation, and subgenome expression dominance may play key roles in the dietary adaptation of hybrid fish. This study can provide data for the investigation of signal transduction pathways that mediate food digestion, metabolism, and adaption in fish and may also provide perspectives for applications in fish breeding. However, a better understanding the functional pathways that controlled by parent subgenomes, further studies in homologous recombination, DNA methylation, and the dynamic changing of the expression level of homoeolog genes are need in the future.

Methods

Experimental fish and sampling

Broodstock BSB and TC were originally obtained from Dong Ting Lake (Hunan Province, China). During the reproduction season (May to June in 2019), sexually mature BSB (female = 5) and TC (male = 5) fish were chosen as parents, and the protocols used for fish crossing were as described previously [26]. Approximately 400 larvae from female BSB × male TC were random chosen and reared in two 200 L aquaria. During the breeding process, the water temperature was controlled at 25.5 ± 0.5 °C (with air conditioning) and the photoperiod was natural. The aquaria contained a circulating water system (with a pump), and between 20 and 30% of the water was replaced every three days. The experimental fish were fed fairy shrimp three times a day (08:00, 13:00, and 18:00) until 50 d of age. Then, fish were randomly collected and divided into two groups (each n = 90 fish) and reared in six 200 L aquaria (3 × 30 = 90). One group of fish was fed *Chironomid* larvae (defined as carnivorous) and the other was fed duckweed (*Lemnoideae*) and artificial fodder (100 g fodder containing fish meal 5.00 g, soybean meal 30.00 g, rapeseed meal 20.00 g, rice bran 35.00 g, fish oil 3.50 g, *inter alia*; crude protein = 30.54 g/kg, lipid = 4.88 g/kg, carbohydrate = 28.32 g/kg, moisture = 13.86%) (defined as herbivorous) two times a day (09:00 and 16:00). The amount of the food source was gradually increased according to the body weight of the fish. The two types of fish (n = 6) were sampled at 120 days of age (body weight = 21.75 ± 1.49 g and total length = 11.63 ± 0.46 cm for the carnivorous group; body weight = 20.11 ± 0.96 g and total length = 11.51 ± 0.55 cm for the herbivorous group) after a 12 h fast. All experimental fish were anesthetized with 50mg/L MS-222 before dissection (Sigma-Aldrich, St Louis, MO, USA). Liver and intestine tissues were removed (washed with RNase free water) and immediately placed into RNALater (Ambion Life Technologies, USA) for mRNA analysis. A piece each of liver and intestine was placed into Bouins' fixative for histological analysis, and the rest of the liver tissues were collected and stored in a -80 ℃ freezer for biochemical assays.

RNA extraction, library construction, sequencing, and mapping

Total RNA from intestine tissues and liver were extracted using TRIzol™ Reagent (Invitrogen) following the manufacturer's instructions. After RNA quality assessment, a total amount of 1μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. The library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA) to select cDNA fragments of preferentially 240bp in length. Then, 3μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15min followed by 5 min at 95°C before PCR. Next, PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Finally, PCR products were purified (AMPure XP system) and sequenced on an Illumina Hiseq 2000 platform.

Genome data of the parents, BSB (with 30749 genes) and TC (with 31022 genes), from our previous study were used as the two reference genomes. After the paired-end reads (raw reads) of each sample were obtained, the FastQC software (Babraham Bioinformatics) was used to remove reads containing adapters, ploy-N, and low-quality reads (unknown nucleotides > 5%) [50]. Then, the total clean reads of each sample were aligned to the two reference genomes using the HISAT software, based on the species-unique SNPs (Figure S3) [51]. The number of mapped reads in each gene was calculated using in-house Perl scripts, as previously described [28]. Only reads with a perfect match or one mismatch were further analyzed and annotated based on the two reference genomes. Furthermore, to obtain more comprehensive annotation information, the StringTie software was used to assemble the mapped reads [51]. After removing short sequences (encoded peptide chains > 50 amino acids) or sequences containing only a single exon, 11282 new genes (defined as those assembled sequences that were originally unannotated transcribed regions in the reference genomes) were identified, and 9606 were annotated on at least one database (Nr, Nt, Pfam, KOG/COG, Swiss-Prot, KO and Gene Ontology (GO) terms).

Differential expression analysis

Quantification of gene expression was performed using the fragments per kilobase of transcript per million fragments mapped (FPKM) method, based on the mapped reads [52]. The expression values of three biological replicates were screened using a mean ± 2 standard deviation (SD) threshold for each gene to avoid the interference of expression noise [53]. The total expression value was normalized based on the ratio of the number of mapped reads for each gene to the total number of mapped reads for the entire genome. Finally, DEseq2 in the R software, version 2.13 (R Foundation for Statistical Computing, Vienna, Austria) was used to search for DEHs between the two diet groups, with a false discovery rate

(FDR) < 0.01 and a threshold normalized absolute log 2-fold change >1.0 [54]. Uniquely expressed homoeolog genes in the two diet groups and different tissues were identified. Uniquely expressed genes were defined as those for which the expression level was zero (read = 0 in three biological replicates) in either one tissue or diet group, while the read count was ≥ 3 in the other group or tissue for three biological replicates. GO and KEGG pathway analyses were carried out on the free cloud platform BMKCloud (https://international.biocloud.net/zh/user/login), based on the BSB and TC genome annotation.

Analysis of HES and HEB

Two sets of genome sequences (annotated protein sequences) were aligned using the reciprocal BLAST (BLASTP, v. 2.2.26) hit method, with an e-value cut off of $1e^{-20}$, to identify orthologues [28]. A total of 20130 orthologues were obtained for the next analysis. Then, the mapped reads of each gene were divided into two categories based on the two different parental reference genomes. BSB homoeolog-specific reads (BSB) and TC homoeolog-specific reads (TC) were used to detection HES and expression bias (HEB), respectively [55]. An expressed gene was defined as BSB silenced (BSB-HES) if BSB = 0 and TC ≥ 3 and TC silenced (TC-HES) if BSB ≥ 3 and TC = 0 in three biological replicates. For analysis of HEB, the expressed genes with BSB = 0 and TC ≥ 3 or BSB ≤ 3 and TC = 0 were removed first. The expression level of the gene was considered biased to the BSB subgenome (BSB-HEB) if the ratio of BSB/TC ≥ 2 and biased to the TC subgenome (TC-HEB) if the ratio of BSB/TC ≤ 0.5. All cases, except for these two, were considered “normal.”

Real-time quantitative PCR (qPCR)

Total RNA was isolated from liver tissues of the two diet group of fish using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. First-strand cDNA was synthesized using a PrimeScript RT reagent Kit (RR047A, TAKARA) with PrimeScript RT Enzyme at 37℃ for 15 min and at 85℃ for 5s after genomic DNA removing with DNA eraser. qPCR was performed on triplicate technical replicates and ACTN gene (accession No. GU471241) were used as the internal control for normalization of gene expression. qPCR were performed on LightCycler® 96 (Roche, Switzerland) and the amplification conditions were as follows: 50℃ for 5min, 95℃ for 10min, and 40 cycles at 95℃ for 15s and 60℃ for 60s. Then, relative quantification was performed, and melting curve analysis was used to verify the generation of a single product at the end of the assay. The average threshold cycle (Ct) was calculated for each sample using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers used were given in Table S3.

Biochemical assays

Equal amounts of liver tissue from the two groups of fish were collected, and the homogenized mixture was used to detect the concentration of superoxide dismutase (SOD), glutathione (GSH),

malondialdehyde (MDA), and peroxidase (POD), respectively. The four kits, SOD (A001-1), GSH (A006-1), MDA (A003-1), and POD (A048-2), were purchased from Nanjingjiancheng Bioengineering Institute (Jiangsu, China), and the manufacturer's instructions were followed. Data are presented as the mean ± SD, and SPSS Statistics 19.0 (IBM Corp., NY, USA) was used to analyze the significance of differences between groups (unpaired two-tailed analysis, *t*-test). The level of statistical significance was set at $p < 0.05$.

Abbreviations

BSB: *Megalobrama amblycephala*; TC: *Culter alburnus*; DEHs: Differentially expression homoeolog genes; HES: Homoeolog expression silence; HEB: Homoeolog expression bias.; KEGG: Kyoto Encyclopedia of Genes and Genomes; KO: KEGG orthology; GO: Gene ontology; COG: Cluster of Orthologous Groups of proteins; qPCR: Quantitative real-time PCR; SNPs: Single nucleotide polymorphisms; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutataione; POD: Peroxidase

Declarations

Ethics approval and consent to participate

In this study, all experiments were approved by the Animal Care Committee of Hunan Normal University and followed the stated guidelines of the Administration of Affairs Concerning Animal Experimentation of China. Fish collect and crossing were approved by the Animal Care Committee and Protection Station of Polyploidy Fish of Hunan Normal University. All samples were raised in natural ponds, all dissections were performed with 50mg/L MS-222 (Sigma-Aldrich, St Louis, MO, USA), and all efforts were made to minimize suffering. Specific operation methods were as follows: first, add 4L of water to a 10L glass container, then weigh 200mg of MS-222 solid powder and dissolve in water. After the powder was dissolved, the experiment fish was put into the water. When the body of the experiment fish gradually lost its balance or sank to the bottom of the tank, the frequency of gill motion decreased, and the breathing was weak or stopped. At this time, the experiment fish was under deep anesthesia, so subsequent experiments could be carried out.

Consent for publication

Not applicable.

Availability of data and material

Data sets further supporting the conclusions of this article are included within the article and its additional files. The complete clean reads will submit to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA269572.

Competing interests

The authors declare that they have no competing interests.

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Authors’contributions

This study is conceived and designed by SJL and WHL. Most statistical analyses and write the manuscript by WHL, SW Experimental work: CW, WHL, JH, CFS. Experimental materials collect: WHL, JJD, and JML Manuscript modify: SJL and XY. All authors read and approve the final manuscript.

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Figures

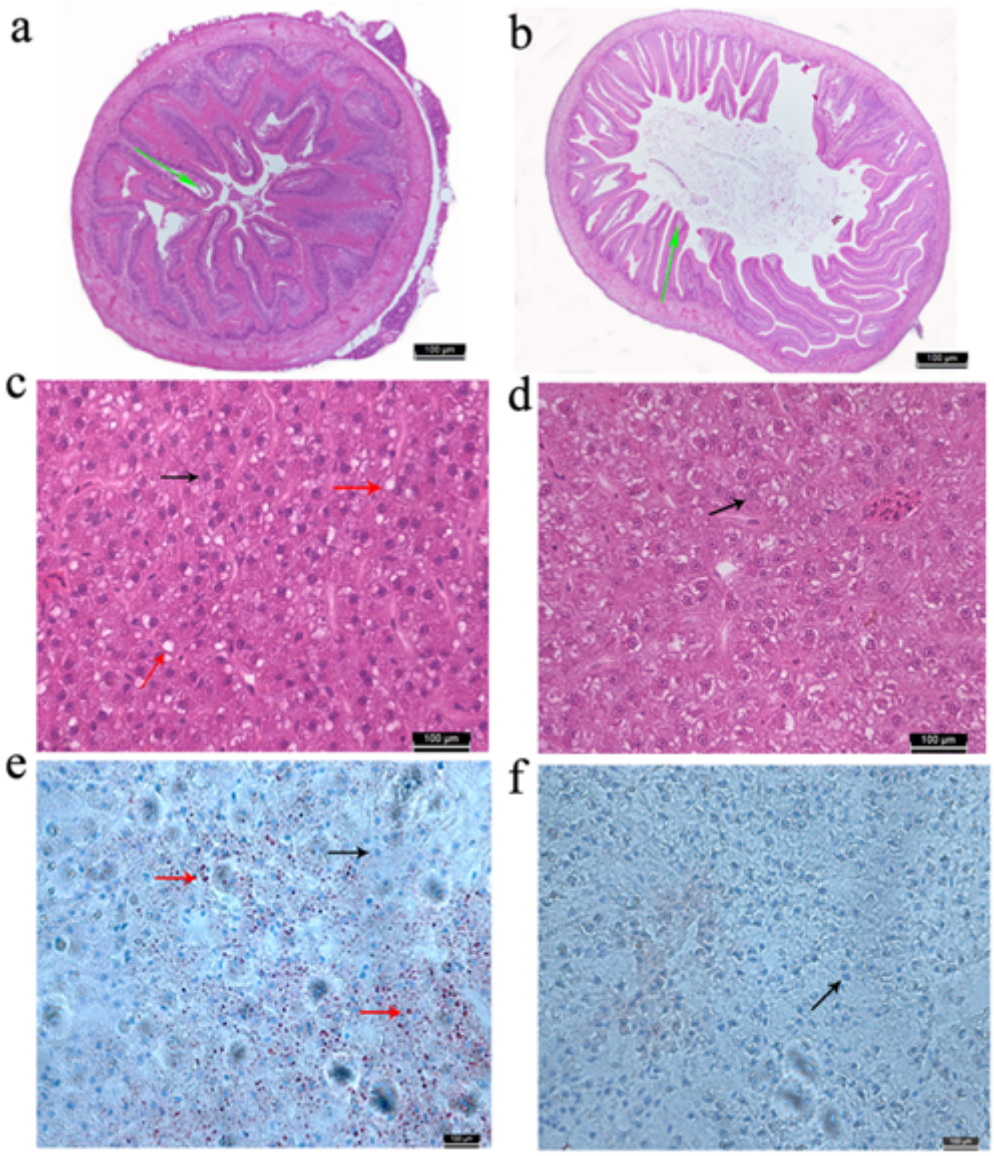

Figure 1

Photomicrographs of the intestine and liver tissues of carnivorous and herbivorous hybrid fish. Intestinal tissues from carnivorous fish (a) and herbivorous fish (b). Liver tissues from carnivorous fish and herbivorous fish, stained by H&E (c and d) and Oil red O-staining (e and f). Green arrows show the intestine villi, black arrows show the hepatocyte nuclei, red arrows show the fat cells and lipid droplets.

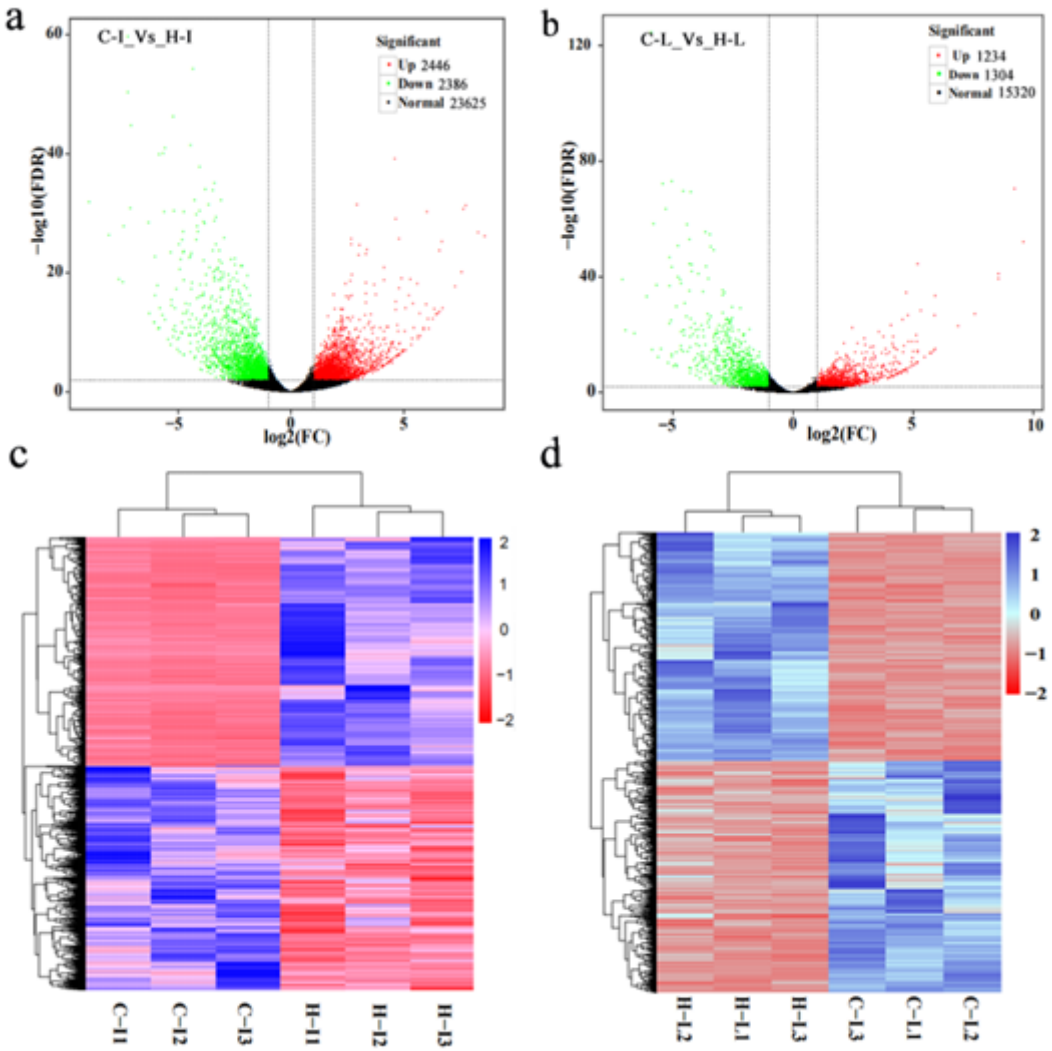

Figure 2

Cluster analysis of differentially expressed homoeolog genes in intestine (a, c) and liver (b, d) transcriptomes of carnivorous and herbivorous hybrid fish. The colors and numbers (log10 fold changes) indicated changes in expression levels. C-I1~I3 and C-L1~L3: intestine and liver tissues from carnivorous diet fish, H-I1~I3 and H-L1~L3: intestine and liver tissues from herbivorous diet fish.

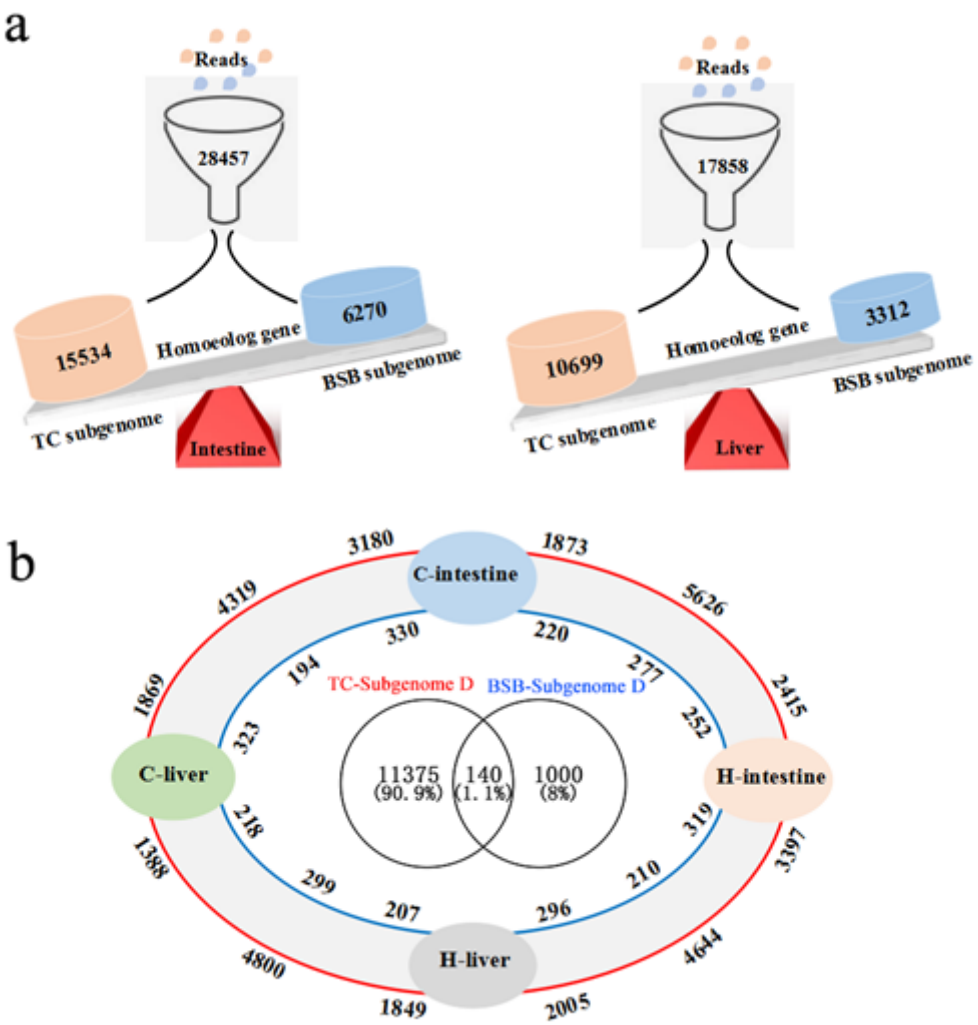

Figure 3

Subgenome expression dominance in intestinal and liver tissues (a), and the shared and uniquely expressed homoeolog genes in different tissues (b). The numbers on the red line represent the shared and uniquely expressed homoeolog genes with TC subgenome expression dominance, the number on the blue line represent the shared and uniquely expressed homoeolog gene with BSB subgenome expression dominance.

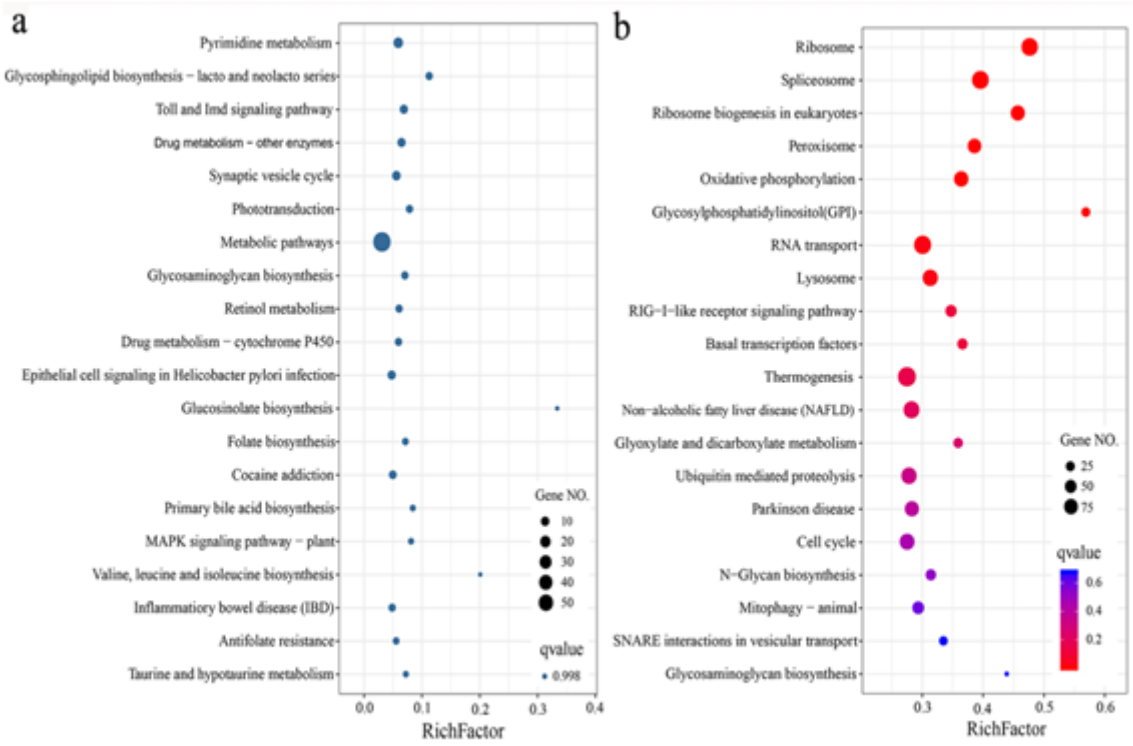

Figure 4

Top 20 enriched pathways of the expressed homoeolog genes with BSB subgenome expression dominance (a) and TC subgenome expression dominance (b) in carnivorous and herbivorous hybrid fish.

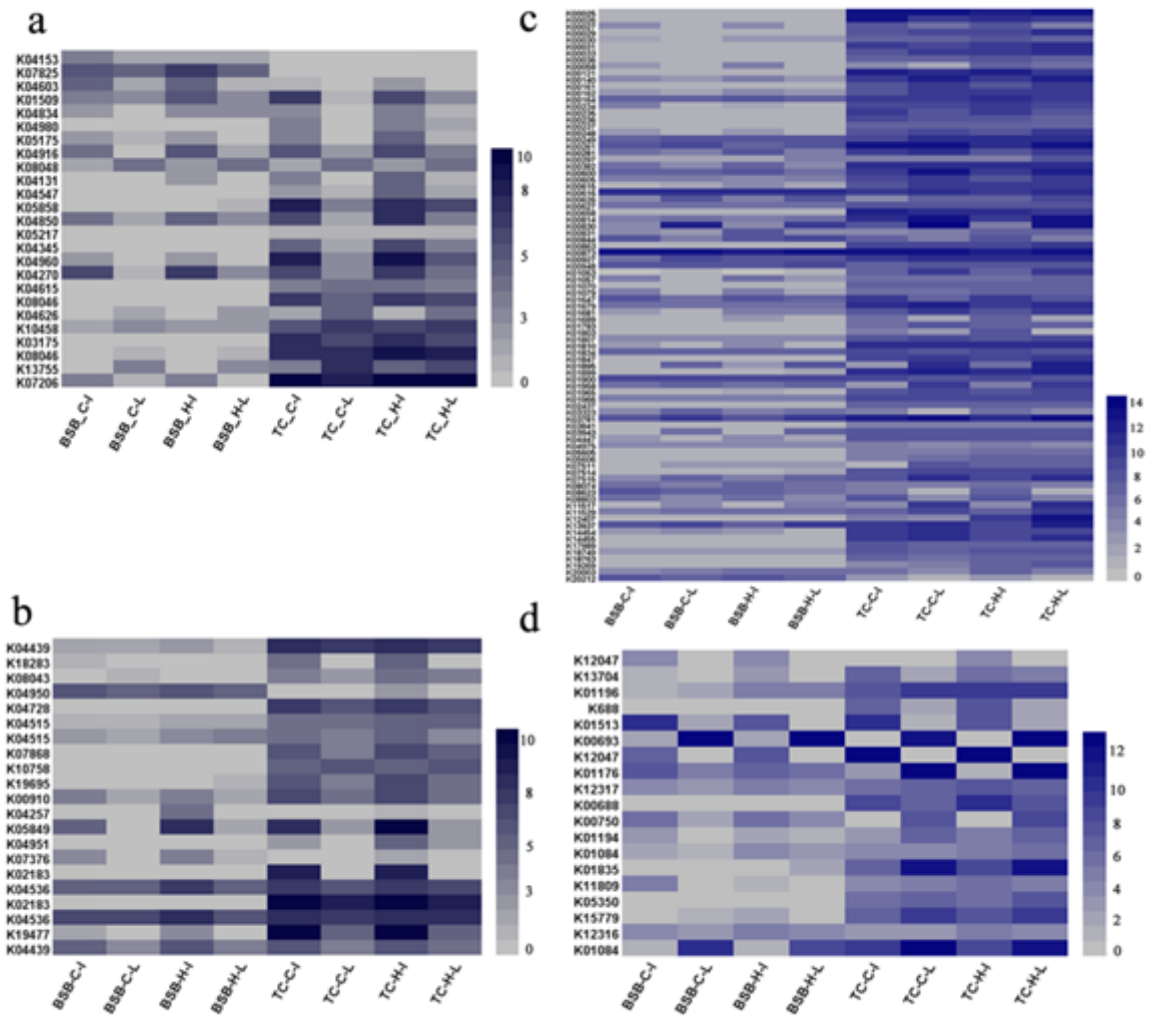

Figure 5

Relative expression level (reads counts) of homoeolog genes that enriched in taste transduction pathway (ko04742) (a); olfactory transduction pathway (ko04740) (b); carbon metabolism pathway (ko01200) (c) and starch and sucrose metabolism pathway (ko00500) (d). The colors and numbers (log2 read counts) indicated changes in expression levels. H-L and C-L: represent liver transcriptome from herbivorous and carnivorous diet group fish; H-I and C-I: represent intestine transcriptome from herbivorous and carnivorous diet group fish, respectively.

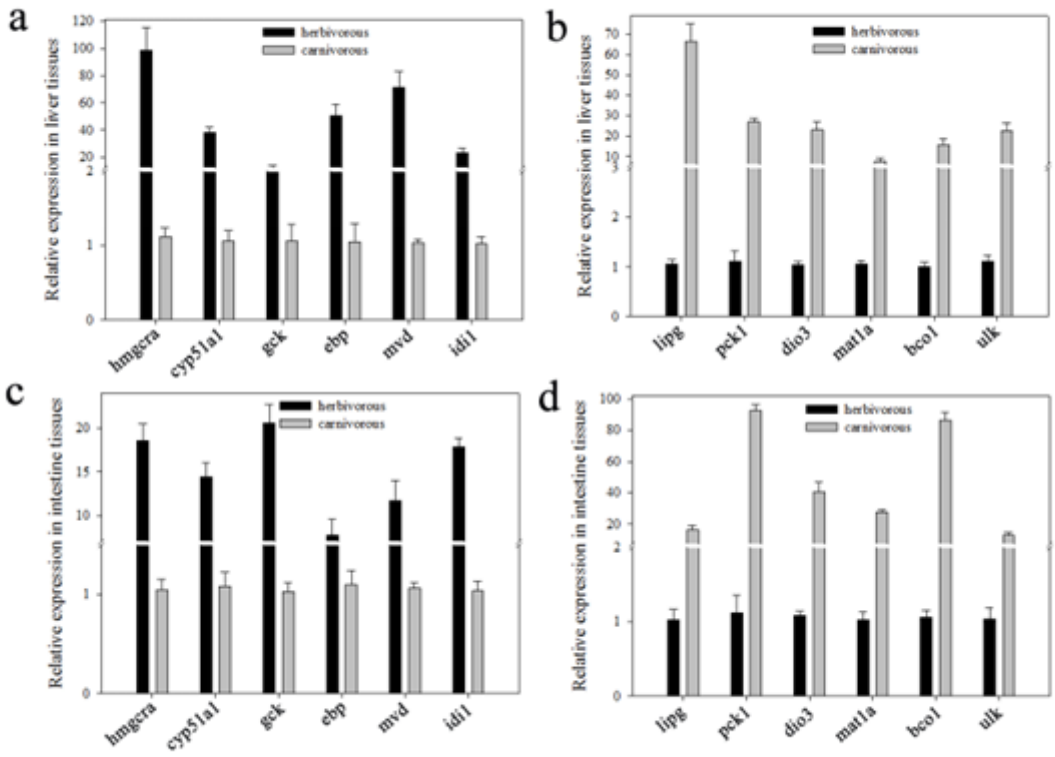

Figure 6

Real-time polymerase chain reaction analysis for 12 differentially expressed genes between the two diet groups fish. The higher expressed homoeolog gene (a and c) and lower expressed homoeolog gene (b and d) in the liver and intestine of herbivorous diet fish compared with carnivorous diet fish, respectively. Data show mean±SD expression of each gene. The genes were including 3-hydroxy-3-methylglutaryl-CoA reductase (hmgcra), Cytochrome P450 family 51 subfamily A member 1 (cyp51a1), glucokinase (gck), EBP cholestenol delta-isomerase (ebp), Mevalonate diphosphate decarboxylase (mvd), Isopentenyl-diphosphate delta isomerase 1 (idi1), Lipase G, endothelial type (lipg), Phosphoenolpyruvate carboxykinase 1 (pck1), Iodothyronine deiodinase 3 (dio3), Methionine adenosyltransferase 1A (mat1a), Beta-carotene oxygenase 1 (bco1) and Unc-51 like autophagy activating kinase 1 (ulk1).

Supplementary Files

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