Relationship between Differential Gene Expression in Ovary and Heterosis of Egg Number Traits in a Chicken Diallel Cross*

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ABSTRACT : In order to understand the molecular basis of chicken heterosis in reproduction traits, mRNA differential display (DDRT-PCR) methods were used to analyze the differential gene expression of ovary tissue between hybrids and their parental lines in a 4×4 diallel cross, involving 4 chicken breeds, which were White Plymouth Rock (E), CAU Brown (D), Silkies (C) and White Leghorn (A). Total of 331 differential displayed cDNA bands from 1,161 were displayed in the 4×4 diallel cross combinations with 30 pairs of primers, which shows the differences of gene expression between hybrids and their parental lines were very obvious in quantity and quality. Seven types of differential expression patterns were found: Co-dominance expressed pattern (T1), under-expression of parental fragments in hybrids (T2), over-expression of parental fragments in hybrids (T3), hybrid-absence expressed pattern (T4), single parent-specific expressed pattern (T5), dominant expression fragments of single parent in hybrids (T6), hybrid-specific expressed pattern (T7). Correlation analysis indicated that there were significant correlations between the pattern of T3 and the heterosis percentage of egg number of 32-week and 42-week old chickens($p<0.01$), while there were negative significant correlations between the pattern of T7 and the heterosis percentage of egg number of 32-week and 42 week-old birds ($p<0.01$). ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 767-771)***

Key Words : Chickens, Egg Number Heterosis, Ovary, mRNA Differential Display, Differential Expression Patterns

INTRODUCTION

Enhancement of performance in important economic traits by use of heterosis (hybrid vigor) is the best bet in poultry breeding, especially for traits with lower heritability such as egg number, Hatchability of fertiles and laying rate. There is, however, no reliable method to predict the level of heterosis that will occur from the mating of individuals from two populations based on the heterosis hypotheses of dominance, over- dominance and epistasis (Haberfeld, 1996). The substantial progress in the research of heterosis had not been made since the beginning of the 20th century to 1990s for the complexity of its genetic basis and the limitations of research methods and means (Tian, 2003). The molecular mechanism of heterosis remains largely unknown. The development in molecular biology and biotechnology, esp. that in QTLs (quantitative trait loci) mapping methods, had made it possible study the relationships among heterosis, QTLs heterozygosity and the modes of QTLs' interactions since the late 1980s. It has been suggested that molecular foundation of phenotypic changes could reside in the variability of genome expression (Tsaftaris, 1995). For a heterozygote, the new internal cellular backgrounds and nuclear-cytoplasm relationship consist of the female parent's cytoplasm and both parents' chromosome sets. The growth and development of the heterozygote is controlled by the new regulatory system. Heterosis is in fact the external exhibition of the gene expression and regulation in the heterozygote (Zhu, 2000). So, it would be necessary to go one step further to research the molecular mechanism of heterosis in terms of hybrids' differential gene expression relative to their parents' other than QTLs heterozygosity and the modes of QTLs interaction on the DNA sequences level (Chen, 1996).

Recently, Differential Display of mRNA or Differential Display Reverse Transcription PCR (DDRT-PCR), a more power, sensitive and rapid method in detecting the differences in gene expression, was used to analyze difference in gene expression patterns between hybrids and their parents (Liang, 1992). Cheng (1996, 1997) detected significant difference in mRNA quantity and patterns between hybrids and their parents in maize and rice. Xiong (1998) reported that differentially expressed fragments that occurred only in one parent but not other parent or in FB_{1B} were positively correlated with heterosis. There are few studies about the difference in gene expression patterns between hybrids and its parents in animal.

In the study reported here, differential gene expression patterns in 32 week-old ovary of 12 hybrids relative to their parental lines were compared by means of DDRT-PCR. In addition the correlation analyses of various differential gene expression patterns with the performance and heterosis of reproduction traits were evaluated in order to provide more

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Table 1. Heterosis percentages of egg number traits (%)

Hybrids	Egg number of 32-week-old	Egg number of 42-week-old
AC	12.2	11.55
AD	7.9	5.08
AE	24.04	13.78
CA	21.61	14.9
CD	11.49	9.11
CE	23.07	15.18
DA	15.85	11.28
DC	36.16	24.77
DE	25.75	17.93
EA	12.47	12.18
EC	33.61	26.82
ED	11.87	9.03

documentation on the molecular mechanism of chicken reproduction traits heterosis.

MATERIALS AND METHODS

Chicken and reproduction traits experiment

4×4 diallel cross involving 4 chicken breeds, which were White Plymouth Rock (EE), CAU-Brown (DD), Silkies (CC) and White Leghorn (AA), and the 12 hybrids, which were ED, DE, DC, CD, DA, AD, AC, CA, AE, EA, CE and EC were used in this study. CAU-Brown is a Brown-egg breed which was high yield and cultivated by researchers in China Agricultural University in 1991. There are 3,084 chickens with the same ratio of male to female in 12 hybrids and 4 chicken breeds. All the chickens were cultivated at the same breeding and management condition in order to eliminate the environment effects. All ovary tissue by random sampling from 16 chicken flocks is collected and stored at -80°C for RNA extraction after recorded the reproduction traits.

3'end anchored primers

$HT_{11}G$: 5'-AAG CTT TTT TTT TTT G-3', $HT_{11}C$: 5'-AAG CTT TTT TTT TTT C-3' and $HT_{11}A$: 5'-AAG CTT TTT TTT TTT A-3'.

5'end oligonucleotide primers

P1: 5'-GAGT\CCCA\GGACATTGAGCAG-3';
P2: 5'-GTT ATC CAC AGA ATC AGG G-3';
P3: 5'-TGC CGA AGC TTT GGT GTC -3';
P4: 5'-TGC CGA AGC TTT GGT ACC-3';
P5: 5'-TGC CGA AGC TTT GGT AGC -3';
P6: 5'-TGC CGA AGC TTT GGT ATG-3';
P7: 5'-TGC CGA AGC TTT GGT CAC-3';
P8: 5'-TGC CGA AGC TTT GGT CAG-3';
P9: 5'-TGC CGA AGC TTT GG T CTG-3';
P10: 5'-TGC CGA AGC TTT GGT CTC-3'.

Total RNA extraction

Total RNA of each material was extracted by the protocols of TRIZOL kit (Invitrogen) and the DNA intermixed in the total RNA was eliminated according to Liang (1992).

Reverse transcription

Reverse transcription was done in a total volume of 40 μl containing 0.4 μg RNA, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 7 mM $MgClB_{2B}$, 10 mM DTT, 20 mM dNTP and 200 pM 3'end anchored primers. 100 U M-MLV reverse transcriptase (Promega) was put together in the containing after 65°C for 5 min. The mixture was incubated at 37°C for 1 h. Then 75°C for 5 min. The reverse transcription product of cDNA was stored at -20°C.

Differential display PCR amplification

In the total volume of 20 μl, the PCR mixture contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgClB_{2B}$, 20 mM dNTPs, 200 pM 3'end anchored primers, 200 pM 5'end oligonucleotide primers, 1 U Taq DNA polymerase (Promega), 2 μl cDNA. The PCR mixture was programmed at 94°C for 2 min, 42°C for 2 min, and 72°C for 2 min, for the frist cycle, and at 94°C for 30 s, 60°C for 60 s, and 72°C for 60 s for 35 cycles. The final cycle was followed by an extension period at 72°C for 10 min.

Fragment isolation and silver staining

The resulting products of DDRT-PCR amplification were separated in a 8% polyacrylamide gel (39:1) electrophoresis with silver staining followed. Gels were then scanned and took pictures for statistics.

Data analysis

Fragments with molecular weight of 1,300-200 bp were calculated for statistics. Difference fragments detected among hybrid FB_{1B} and its two parents were grouped into seven differential expression patterns. Numbers of fragments in every differential expression pattern of each hybrid were calculated. For each differential pattern of all 12 hybrids, the set of numbers of fragments as a whole was used as one variety. Correlation analyses of each differential pattern were evaluated with the percentage of heterosis of egg number traits for 12 hybrids.

RESULTS AND ANALYSES

Heterosis percentage of egg number traits

There was a significant variation in the egg number traits between hybrids and their parents lines.

From the Table 1 we can found that the percentage of hetorosis for egg number traits detected in every hybrid is significant respectively.

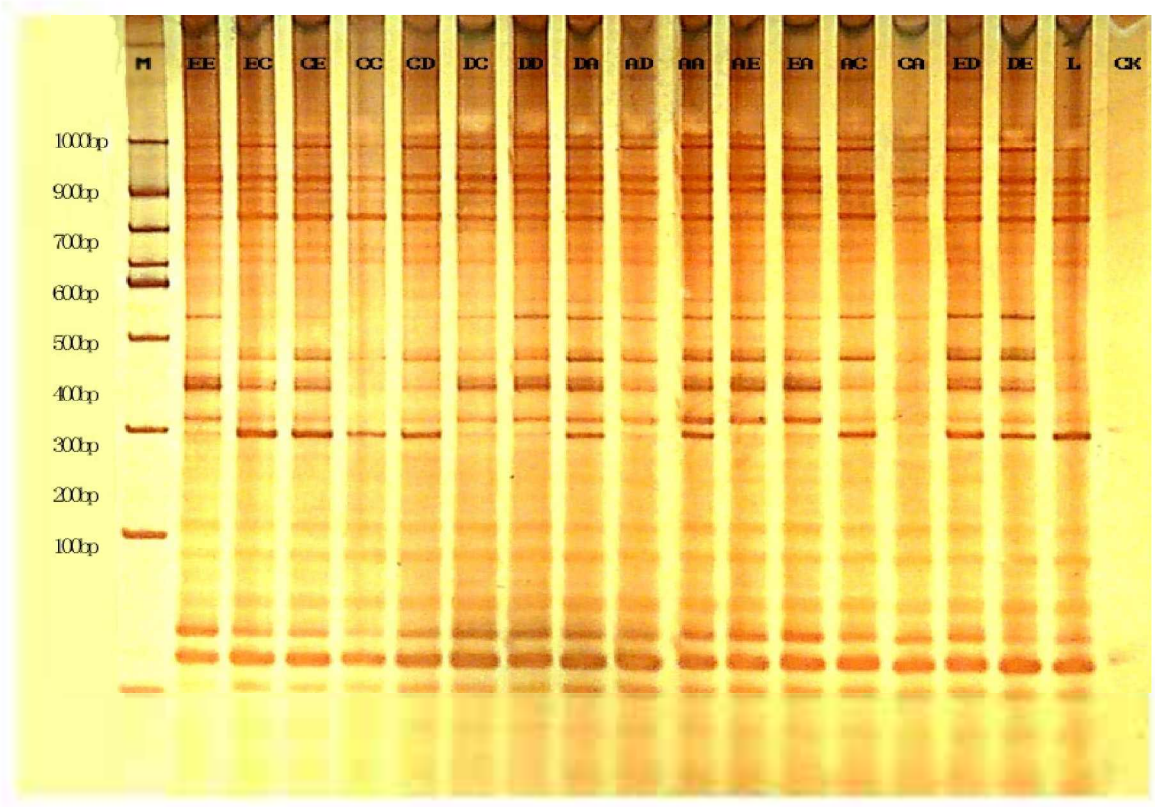

Figure 1. mRNA differential display profiles with primer combination of P1/$HT_{11}G$ in ovary from 16 crosses. Primers: P1\$HT_{11B}G$; M: 100 bp molecular ladder; CK: negative control; Arrowhead: differential expression fragments.

Table 2. The percentage of every pattern of gene differential expression between hybrid and purebred in 32 week-old ovary tissue of chicken (unit: %)

Hybrids	Differential gene expressed patterns						
	T1	T2	T3	T4	T5	T6	T7
AC	19.35	12.90	6.45	9.68	22.58	12.90	16.13
CA	9.52	14.29	4.76	4.76	19.05	9.52	38.10
AE	10.34	17.24	13.79	3.45	31.03	17.24	6.90
EA	11.54	23.08	11.54	3.85	23.08	19.23	7.69
DE	5.26	10.53	15.79	5.26	15.79	21.05	26.32
ED	22.22	3.70	14.81	0.00	33.33	18.52	7.41
DC	9.09	12.12	15.15	6.06	21.21	24.24	12.12
CD	13.16	18.42	15.79	7.89	18.42	23.68	2.63
AD	5.41	18.92	16.22	8.11	24.32	21.62	5.41
DA	8.00	12.00	8.00	4.00	24.00	24.00	20.00
CE	25.00	3.57	21.43	14.29	17.86	10.71	7.14
EC	11.76	5.88	11.76	5.88	17.65	17.65	29.41

T1-T7 denote the seven types of patterns of gene differentially expressed.

Differential gene expression

Using the total RNA in the ovaries of 32-week-old from hybrids and their parents as templates, DDRT-PCR was performed with three eleven-T-base-anchored primes in combination with ten 5'end oligonucleotide primers (P1-P10) and an example was given in Figure 1 to illustrate the resulting products of DDRT-PCR amplification with the primer combination of $P1_B/HT_{11}G$.

From the 30 primer combinations, a total of 1,631 bands were displayed, and 71.18% (1,161 out of 1,631) can be repeated in duplicate DDRT-PCR amplification, in which 331 cDNA bands (28.51%) were found to be polymorphic between 12 hybrids and their parents. For each of the 30 primer combinations, an average of 11.03 polymorphic bands was obtained. Data of differentially expressed fragments based on 30 primer combination of 12 hybrids were analyzed in Table 2.

Patterns of differentially expressed cDNA

When comparing the patterns of differentially expressed genes between hybrids and their parents, it was found that both quantitative and qualitative differences could be

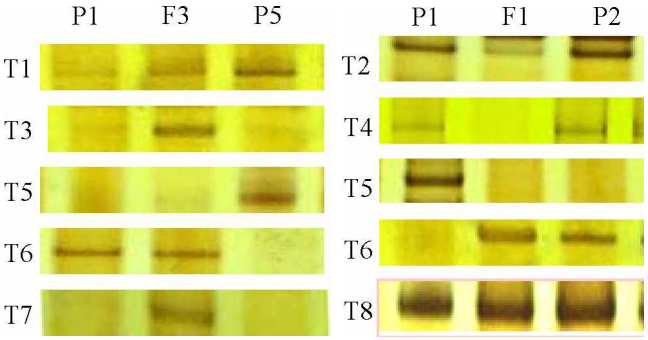

Figure 2. Differential expression patterns of hybrids and their parental lines. T1: Co-dominance expressed pattern; T2: Under-expression of parental fragments in hybrids; T3: Over-expression of parental fragments in hybrids; T4: Hybrid- absence expressed pattern; T5: Single parent-specific expressed pattern; T6: Dominant expression fragments of single parent in hybrids; T7: Hybrid-specific expressed pattern.

observed. Depending on the different in quantitative of gene expression and whether a cDNA fragment could be detected or not among hybrid F1 and its two parents fragments of each hybrid relative to its parents were divided into seven differential expression patterns. The percentage of various patterns of differentially displayed cDNA fragments is as following. Co-dominance expressed pattern (T1 = 12.69%), Under-expression of parental fragments in hybrids (T2 = 13.29%), Over-expression of parental fragments in hybrids (T3 = 13.29%), Hybrid- absence expressed pattern (T4 = 6.34%), Single parent-specific expressed pattern (T5 = 22.66%), Dominant expression fragments of single parent in hybrids (T6 = 18.73%), Hybrid-specific expressed pattern (T7 = 12.99%). In them, the quantitative difference include T1, T2 and T3; as the qualitative difference include T4, T5, T6 and T7 (Table 2), electrophoresis profiles of seven kinds of patterns were in Figure 2.

Correlation analysis

The relationship between gene expression patterns and heterosis of egg number traits were evaluated using data from DDRT-PCR and egg number traits on record. Correlation coefficients between seven differential patterns and the heterosis percentage of egg number traits were listed in Table 3.

Correlation analysis showed that: there is significant correlation between the pattern of T3 and the heterosis percentage of egg number of 32-week and 42-week old ($p<0.01$), while there are negative significant correlation between the pattern of T7 and the heterosis percentage of egg number of 32-week and 42 week-old ($p<0.01$).

DISCUSSION

Differential gene expression and heterosis

Our studies by means of differential display show that for a hybrid relative to its both parents, there are differential gene expressions in quantity and quality. It is these variations in expression that result in variations in phenotype for a hybrid. Although all the genes in hybrid FB_{1P} are derived from its parental lines, hybrid performance is quite different from its parental lines (Table 1). Therefore, differential gene expression between hybrids and their parents should be also responsible for the observed heterosis.

It is clear that the hybrid's genetic performance is not the simply additive product of genetic materials from both parents, but the result of variations in quantitative and qualitative expression of two sets of genes within the hybrid by means of interactions resulting in the occurrence of heterosis (Xiong, 1998; Stuber, 1992). Researches conducted by Romagnoli and his colleagues (Romagnoli, 1992) showed that some proteins and mRNAs are differentially synthesized and expressed between hybrid F_1 and its parental inbreds, and suggested that the occurrence of heterosis was related to the variations of many genes' expression in hybrid. Studies conducted by Dameral and his colleagues (Dameral, 1991) on maize heterosis and protein amount polymorphism of hybrids relative to their parents inbreds showed that regulatory processes were primarily implicated in the morphological variation of hybrids. Although the mechanism underlying these quantitative and qualitative difference in gene expression between hybrids and their parental lines are unknown, the alteration in the patterns of gene expression in hybrid might be associated with heterosis observed in egg number traits.

Moreover, documentations accumulated recently support that there is a close relationship between heterosis and the quantitative gene expression. Thus, it is more noteworthy to research the relationship between heterosis and the regulatory mechanism of gene expression (Wu, 2001; Tian, 2003).

Pattens of differential gene expression and heterosis

It can be seen from Table 2 that the proportions of differential patterns are different for different cross combination lines. Correlation analysis of hybrid

Table 3. Correlation analysis between heterosis percentages of egg number traits and differential expression patterns

Heterosis percentage	Patterns of gene differential expression						
	T1	T2	T3	T4	T5	T6	T7
Egg number of 32 week-old	0.401	0.116	0.736**	0.363	0.150	0.124	-0.845**
Egg number of 42 week-old	0.487	-0.003	0.7201**	0.540	0.010	0.084	-0.795**

Data in the table are correlation coefficients. ** Significant at $p<0.01$.

performance and differential gene expression patterns showed that most differential expression patterns are not related to the performance of egg number traits except for only two patterns which are significant related or negative related to the performance of egg number traits at the 0.01 probability level. These results are basically identical to the conclusions drown by Xiong et al. (1998) and Wu et al. (2001). Whose work supported that little correlation was detected between differential expression patterns and the performance of rice and wheat hybrids respectively.

Negative correlations were detected in the hybrid specific differential expession pattern with the heterosis of 32 week-old and 42 week-old egg number traits. This is coincident with the researches on rice heterosis by Wu (2001) that heterosis may be associated with the inhibition between genes from both parents.

Results in the study revealed that hybrid F1 specific expression genes appeared to restrain the heterosis forming of egg number traits; while Over-expression of parental genes in hybrid F1 appeared to reinforce the heterosis forming of egg number traits.

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