
By

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To study patterns of gene expression

Helianthus annuus

Helianthus petiolaris

Helianthus deserticola
Interspecific hybridization may contribute to biodiversity at both the subspecific level and at species level.

Reproductive isolation is imp. for diploid hybrid speciation.

Allopolyploids - by genome doubling and in diploids – depends on ecological and karyotypic divergence and by sorting of sterility.

Homoploid hybrid speciation as an example of speciation with gene flow.

Annual sunflowers - excellent example for homoploid hybrid speciation.

H. deserticola – Transgressive segregation, reproductive isolation
Genetic and epigenetic changes that alter gene expression

Causes of modified gene expression *H. deserticola* ecological selection and karyotypic divergence.

Causes of Transgressive segregation.

Microarrays for analysing patterns of gene expression in homoploid hybrid species and its progenitors, testing of transgression in *H. deserticola*, contribution of gene expression changes to ecological divergence and fitness effects of some genes in *H. deserticola* habitat.
MICROARRAY GENERATION:

- EST cDNA clones from – *H. annuus* [44,061], *H. paradoxus* and *H. argophyllus* [23,127]. Together they represent 18,031 genes.

- In *H. annuus* – 700,1300,700 cDNAs from envt. stressed roots, shoots and flowers respectively. And 275 cDNAs with known genetic map positions.

- 768 cDNA bacterial clones from salt stressed *H. paradoxus* and drought stressed *H. argophyllus*. 
Differential gene expression and fitness in the wild:

- Candidate gene analysis-5 genes [close to QTLs] with discrimination of alleles based on length.

- Cosegregation bt. candidate gene polymorphisms and fitness were tested in reciprocal second generation back cross hybrids bt. the parents grown in *H. deserticola* envt.

- The head number was tested against candidate gene polymorphisms in each population using one-way ANOVAs.
Results:

- Clone identity rate of 91%. [bt. PCR product and the cDNA inserts.]
- Total 2897 genes printed on array with wide function range.
- No cross species hybridization concern in Helianthus [RT-PCR also validated the results].

Patterns of gene expression in hybrid and parents:
- Parents showed differences for 206 genes [108 in P1 and 98 in P2] [8% on array but 12% in expression]
As predicted, fewer genes showed significant differential gene expression. Hybrid has 151 comparisons with P1 and 174 comparisons with P2. in differential expression.

Among three species 370 genes[12.8% on array] showed significant differential expression.

Gene expression in *H. deserticola* was transgressive for 58 genes and it was diverse.[8% on array but 16% in results-transporters].

*H.deserticola* showed *H.petiolaris* like expression for 99 unique genes and *H.annuus* like expression for 117 unique genes and they had same functions in the hybrid and parents.

When compared the results to real time RT-PCR ,the magnitude of expression changes assessed by the latter was usually greater than those assessed by microarray.
Associations with Karyotypic differences, QTLs and fitness:

- The expression patterns of the 275 mapped cDNAs showed differential expression for 40 unique genes.
- These genes on rearranged chromosomes? –not signi.
- These genes on chromosomes with inversions? –less signi.
- When these 40 genes assessed for QTLs, 5 genes were coincident with QTLs that correspond loosely with the predicted functions.
- When these 5 genes analysed for the fitness effects-1 gene showed fitness association, which has a hit in Arabidopsis.
Low sequence divergence among sunflower species suggest that many phenotypic differences may result from quantitative differences in gene expression, as in human evolution. That’s why it showed significant differential expression for large no. of genes[370].

Genetic and epigenetic changes at hybridization time may contribute to speciation, as in polyploids. For example, the extensive chromosomal rearrangements in the three hybrids are due to transposable elements, which could have a strong effect on gene expression.
This relationship bt. transposons and hybridization, chromosomal rearrangements and gene expression remains to be explored. Although results showed this, sample size is too small to be definitive.

Stacking of alleles may not require change in gene expression.

This expt-Divergence of hybrid may be due to novel gene expression.

Supposed source of changes in gene expression-chromosomal restructuring, transposon activity, other products of hybridization and ecological selection over time.
Gene expression and ecological divergence of *H. deserticola*:

- Negatively transgressive for most traits [in field and GH] but levels of gene expression show a more balanced pattern [26 genes –ve & 32 genes +ve].

- So, the –ve transgression for phenotype is not due to reduced gene expression.

- Even though, *H. deserticola* is phenotypically close to *H. petiolaris* than *H. annuus*, it shows an equivalent divergence from both taxa in no. of genes with differential expression.
Transgression in hybrid was spread across a broad range of functional classes. But the transport related genes are the most striking pattern of expression [16% of genes].

ESTs with differential expression across the three species represent candidate genes for adaptive differentiation in Helianthus. For example, QHB30N12 [head number] in *H. deserticola* was coincident with a QTL. [may be other genes in that QTL control this character].

Reduced ligule size and no., early branching associated with rapid flowering are imp. for desert habitat.

We have to test that gene extensively for it’s role in fitness and ecological divergence.