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Molecular Genetic Response of *Xiphophorus maculatus* - *X. couchianus* Interspecies hybrid skin to UVB exposure

Yuan Lu¹, Mikki Bowswell¹, William Bowswell¹, Kuan Yang¹, Manfred Scharlt², and Ronald B. Walter^{1,*}

Yuan Lu: y_lj54@txstate.edu; Mikki Bowswell: mboswell@txstate.edu; William Bowswell: wb1016@txstate.edu; Kuan Yang: yangkuan81@gmail.com; Manfred Scharlt: phch1@biozentrum.uni-wuerzburg.de

¹Molecular Bioscience Research Group, Department of Chemistry and Biochemistry Texas State University, San Marcos, TX, 78666

²Physiologische Chemie, Universität Würzburg Biozentrum, Am Hubland, and Comprehensive Cancer Center Mainfranken, University Clinic Würzburg, D-97074 Würzburg, Germany

Abstract

The phenotypic and genetic similarities between *Xiphophorus* and human melanoma render *Xiphophorus* a useful animal model for studying the genetic basis of melanoma etiology. In the *Xiphophorus* model, melanoma has been shown to be inducible by ultraviolet light (UVB) exposure among interspecies hybrids, but not in parental line fish similarly treated. This leads to questions of what genes are responsive to UVB exposure in the skin of the interspecies hybrids, as well as how parental alleles in hybrids may be differentially regulated and the potential roles they may play in induced melanomagenesis. To address these questions, we produced *X. maculatus* Jp 163 B × *X. couchianus* (*Sp-Couch*) F₁ hybrid fish, exposed both hybrid and parental fish to UVB, and performed gene expression profiling of the skin using RNA-Seq methodology. We characterized a group of unique UVB-responsive genes in *Sp-Couch* hybrid including *dct*, *pmela*, *tyr*, *tyrp1a*, *slc2a11b*, *rab38a*, *rab27*, *tspan10*, *slc45a2*, *oca2*, *slc24a5*, *ptn* and *mitfa*. These genes are associated with melanin production and melanocyte proliferation. They were also up-regulated in *Sp-Couch* hybrid, indicating their UVB response is hybridization initiated. In the hybrid, several melanin production and pigmentation related genes, including *slc45a2*, *tspan10*, *dct*, *slc2a11b* and *ptn* showed either *X. couchianus* or *X. maculatus* allele specific expression. The finding that these genes exhibit allele specific expression regulatory mechanisms in *Sp-Couch* hybrids, but do not exhibit a corresponding UVB response in either one of the parental fishes, may suggest UVB targets and imply mechanisms regarding the susceptibility of *Sp-Couch* to induced melanomagenesis.

*Corresponding author: RWalter@txstate.edu.

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Keywords

Ultraviolet light; UVB; RNA-Seq; Differential gene expression; melanoma; Allele specific gene expression; hybrid; pigment

Introduction

The most studied *Xiphophorus* melanoma model is the one originally introduced by Myron Gordon and Kurt Kosswig in the late 1920's (Gordon, 1927; Häussler, 1928; Kosswig, 1928). This model, often referred to as the Gordon-Kosswig melanoma model, employs *X. maculatus* and *X. hellerii* interspecies hybrids to produce melanoma in 25% of backcross progeny (Gordon, 1927; Häussler, 1928; Kosswig, 1928). The Gordon-Kosswig model led to the discovery of the *xmrk* oncogene, which is a mutant copy of the fish orthologue of the human Epidermal Growth Factor Receptor (EGFR; Weis and Scharlt, 1998). Recently, the *X. maculatus* derived *xmrk* oncogene, driven by the pigment cell specific *mitf* promoter, has been used to produce a transgenic medaka (Japanese rice fish, *Oryzias latipes*) that develop aggressive melanoma within days of hatching with 100% penetrance (Scharlt et al., 2010; Scharlt et al., 2012). These transgenic Medaka models serve as experimentally tractable melanoma models and are currently being developed for small molecule screening regimen.

In contrast to the Gordon-Kosswig melanoma model, certain hybrids between other *Xiphophorus* species do not develop malignant melanoma spontaneously but have been shown to produce backcross progeny that are susceptible to melanoma induction after exposure to ultraviolet light (UV; Setlow et al., 1989; Setlow et al., 1993; Nairn et al., 1996a,b; Mitchell et al., 2010) or treatment with known mutagens such as *N*-methyl-*N*-nitrosourea (MNU; Kazianis et al., 2001; Rahn et al., 2009). For example, a particularly well-utilized model of UVB inducible melanoma involves the backcross [*X. maculatus* JP 163 B $\times$ *X. couchianus*] $\times$ *X. couchianus* (termed the *Sp-Couch* hybrid) (Setlow et al., 1993). The *Sp-Couch* backcross occurs between two parental species that are quite different in regard to pigment patterns. The *X. maculatus* JP 163 B carries both *xmrk* and the *Spot Side* (termed *Sp*) pigment pattern consisting of black macromelanophore spots distributed on the flanks of the animals (Figure 1), while *X. couchianus* lacks macromelanophore spots (for reviews see: Patton et al., 2011; Walter and Kazianis et al., 2001). Upon interspecies crossing, the *X. maculatus* JP 163 B $\times$ *X. couchianus* F₁ progeny show a greatly expanded macromelanophore (black) pigmentation (Figure 1) that may become even further enhanced in select backcross hybrids. If *Sp-Couch* backcross progeny are exposed to UVB at six days post birth, about 30 to 40% of the heavily pigmented interspecies hybrids may develop melanoma from 6–9 months later (Nairn et al., 1996b; Mitchell et al., 2010). Association between sunlight exposure and melanoma has been well established in humans (Armstrong and Kricker, 2001) and this *Xiphophorus* UVB inducible melanoma model has been employed in attempts to define the specific wavelengths that may be responsible for sunlight induced melanomagenesis (Setlow et al., 1993; Wood et al., 2006; Mitchell et al., 2010). However, there remains controversy on the mechanism of melanoma induction in this model system. Evidence has been reported showing both direct DNA damage by UVB (Mitchell et al., 2004; Mitchell et al., 2010), and indirect cellular damage from oxidative radical

production by photosensitizers (e.g., melanin) may be involved in triggering melanoma induction (Setlow et al., 1993; Wood et al., 2006).

UV radiation is composed of UVA (320–400nm), UVB (290–320nm) and UVC (200–290nm; Luther et al., 2000). Although UVC is highly mutagenic, it is completely absorbed by atmospheric ozone and does not reach the earth surface (Luther et al., 2000). Both UVA and UVB are genotoxic. While UVB is considered a direct cause of mutation due to DNA damage, UVA may produce genotoxicity through indirect mechanisms involving production of reactive oxygen species (ROS; Ananthaswamy and Pierceall, 1990; Kanofsky and Sima, 1991; de Laat et al., 1996; de Gruijl and van der Leun, 2002).

In most studies to date the biology and molecular genetic capabilities of the organism being used to model tumor induction have not been as deeply addressed as have the photochemical properties of the light source being applied to induce tumors. However, contemporary RNA-Seq methods allow one to perform global assessment of alterations in the molecular genetic profiles of cells, tissues and organs after exposure to varied light sources. Accordingly, we have initiated RNA-Seq studies of skin from parental *Xiphophorus* strains utilized in crosses that produce melanoma susceptible experimental genotypes after exposure to various light types and sources (Walter et al., 2014; Yang et al., 2014; and other papers this journal edition). Here we document novel results defining UVB regulated expression signatures and showing patterns of allele specific gene expression in the skin of *Sp-Couch* F₁ interspecies hybrids after exposure to UVB light.

Materials and Methods

Animal model

The *X. maculatus* Jp 163 B, *X. couchianus* and *Sp-Couch* hybrids used in this study were supplied by the *Xiphophorus* Genetic Stock Center (Figure 1. For contact information see: <http://www.xiphophorus.txstate.edu/>). All fish used in this study were 9-month-old sibling males. *X. maculatus* Jp 163 B used in this study were in the 101st generation of brother-sister inbreeding (pedigree JP163B 101.c); *X. couchianus* used in this study were from the 77th inbred generation (Xc.77b). The parents of the *Sp-Couch* F₁ hybrids were from the 100th generation *X. maculatus* (female; pedigree JP163B 100.c) and 76th inbred generation of *X. couchianus* (male; pedigree Xc 76d).

UVB exposure

Two fish were used for each experiment and control condition. UVB exposures were as detailed previously (Yang et al., 2014). Briefly, 9-month-old adult male fish were kept in dark for 12 hours prior to UVB exposure. Immediately before exposure to UVB the fish were transferred to UV-transparent cuvettes containing 95 ml of water. The cuvettes were placed between two banks of two (4 lights total) unfiltered narrow spectrum UVB lamps (Philips UVB TL 20W/01 RS SLV) emitting principally 311 nm light that were mounted horizontally inside of a wooden exposure box. Fluence was determined to be 12.2 J/m²/s on each side of the chamber as measured by an IL-1400A Radiometer/photometer coupled to a SEL 240/UVB detector containing a 280 nm Sharp Cutoff Filter (International Light,

Newburyport, MA). Fish were then exposed in duplicate to either 0 kJ/m² or 8 kJ/m² of UVB by varying the amount of time in the exposure box. After exposure, the fish were returned to dark for 6 hours, to allow time for modulation of gene expression, prior to being sacrificed for dissection and RNA isolation.

RNA isolation and RNA sequencing

Total RNA was isolated as previously detailed (Walter et al., 2014; Yang et al., 2014) using TriReagent (Sigma Inc., St. Louis, MO, USA). Skin from each individual fish was homogenized in Tri-reagent followed by addition of 200 µl chloroform and the samples vigorously shaken and subjected to centrifugation at 12,000 g for 15min at 4 °C. Total RNA was further purified using RNeasy mini RNA isolation kit (Qiagen, Valencia, CA, USA). Residual DNA was eliminated by performing on column DNase digestion at 37°C for 30 min. RNA integrity was assessed by gel electrophoresis (2% agarose in TAE running buffer) and total RNA concentration was determined using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). RNA quality was verified on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) to confirm that RIN scores were above 8 prior to sequencing.

RNA sequencing was performed upon libraries constructed using the Illumina TrueSeq library preparation system that employs a polyA selection. RNA libraries were sequenced as 100bp pair-end fragments using Illumina Hi-Seq 2000 system (Illumina, Inc., San Diego, CA, USA). 70–80 million raw reads were generated for each fish (Table 1). Raw reads were filtered and trimmed based on quality scores by using a custom filtration algorithm that removed low-scoring sections of each read and preserved the longest remaining fragment (Garcia et al., 2012). All raw reads were subsequently truncated by similarity to library adaptor sequences using a custom Perl script (Table 1).

Differentially expressed genes (DEGs) analysis

The trimmed and filtered reads were mapped to *X. maculatus* genome (Xmac_JpA.ens70_genome.fa) from Ensemble using Tophat2 (Kim et al., 2013). Mapped reads were quantified as raw counts in each file by the Subreads package function “featureCounts” and then are converted to Count Per Million (cpm) for each sample (Liao et al., 2013; Liao et al., 2014). Genes with cpm values of no more than 4 in all samples were discarded as low counts transcripts. Differentially expressed genes were analyzed using fisher exact test by R-Bioconductor package edgeR (Robinson et al., 2010). For a gene to be a Differentially Expressed Genes (DEGs), it has to alter at least 2 fold with False Discovery Rate (FDR) less than 0.05 ($\text{Log}_2\text{FC} \geq 1$ or $\text{Log}_2\text{FC} \leq -1$, $\text{FDR} < 0.05$). Since the transcriptome available on Ensembl does not contain *xmrk* sequence, we aligned short read to *xmrk* sequence independently and used the library size to normalize the *xmrk* read counts in different samples. *Xmrk* differential expression was also tested by edgeR (Robinson et al., 2010). For data visualization, the normalized gene expression value in the format of cpm was plotted by R package gplots.

Allele specific expressed (ASE) gene analysis

Herein, we defined ASE genes in the *Sp-Couch* hybrid skin as those genes that exhibit parental allele expression ratios that deviate from 1:1. We used single nucleotide polymorphisms (SNP) and other sequence differences between the two parental alleles to identify transcripts as being derived from different parental alleles. To compare the gene expression from each parental allele, an artificial transcriptome was constructed. *X. maculatus* and *X. couchianus* cDNAs were aligned to each other using Blastn (-evalue 1e-6, -best_hit_score_edge 0.1, -best_hit_overhang 0.1, -num_alignments 1, -max_hsps 1, Shen et al., 2013). Reciprocal-best-hit sequences were kept and gene IDs carried over using a customized Perl script to construct the transcriptome containing both parental allele sequences. Short reads were mapped to the artificial transcriptomes using Bowtie2 (Langmead et al., 2009). Customized Perl script was used to retrieve and quantify the short reads that only aligned to one of the parental alleles. Quantified allele expression values were normalized to the total reads number of each allele and differential expression between two alleles was tested using edgeR (Robinson et al., 2010). Relative Log₂Fold Change (Log₂FC) was used to convert to allele frequency:

$$\text{Allele Frequency}\% = 2^{\text{Log}_2 \text{FC}} / (2^{\text{Log}_2 \text{FC}} + 1) 100\%$$

A gene is determined as *X. maculatus* allele specific if the *X. maculatus* allele frequency is over 60%, or *X. couchianus* allele specific if the *X. maculatus* allele frequency is below 40%.

Gene Ontology analysis

Xiphophorus Ensembl gene IDs were converted to human homolog gene Ensembl gene IDs and gene symbol using R-Bioconductor package BiomaRt. Gene Ontology (GO) term of each gene was retrieved from human genome database (org.Hs.eg.db). All genes that have a GO term were collected as background genes. Over-represented GO terms were tested using hypergeometric test by R-Bioconductor package GStat (ontology = Biological Process, p value cutoff = 0.001; Beissbarth and Speed, 2004).

Quantitative real time PCR

Allele specific expression was verified for selected genes by qualitative RT-PCR analysis as described before (Shen et al., 2013, Walter et al., 2014). QRT-PCR was performed by SYBR Green-based detection with an Applied Biosystems 7500Fast system (Applied Bioscience, Carlsbad, CA, USA). Initially, each set of designed primers was tested for allele specificity in a 20 µL reaction consisting of 1 µL of cDNA, 0.5 µM of each primer, and 10 µL SYBR Green ready mix. Each reaction was subjected to 40 cycles each at 95 °C for 20 s, 95 °C for 15 s, and 60 °C for 30 s, before being subjected to melting curve analysis. The 18S gene was selected for normalization of all samples. The mean CT values from triplicate runs were used to calculate relative expression levels. The allele specific primers were then used to determine efficiency following the same procedure outlined above but using a series of dilutions of cDNA to establish a standard curve (100 ng, 10 ng, 1 ng, and 0.1 ng, respectively). Only primer sets with an efficiency percentage of ±10% of one another were

utilized. Once the primer efficiency and allele specificity were established, the primers were used to test relative expression of each allele in *X. maculatus*, *X. couchianus*, *Sp-Couch* under control condition and UVB exposed *Sp-Couch* hybrid. Primer sequences and real time PCR results are presented in Supplementary Table 4.

Results and Discussion

Unique UVB-responsive genes in the *Sp-Couch* hybrid

To characterize how skin from *X. maculatus* and *X. couchianus* interspecies hybrids (i.e., *Sp-Couch*) respond to UVB exposure, *Sp-Couch* hybrids were exposed to 8 kJ/m² UVB and the skin mRNA was used for gene expression profiling analysis. Compared to unexposed controls of *Sp-Couch* skin samples, we identified 564 genes altered (298 down-regulated; 266 up-regulated) in response to 8 kJ/m² UVB (Figure 2a).

We sought to assess whether these UVB-responsive genes in the *Sp-Couch* hybrid skin exhibit a conserved response in parental fish or are uniquely altered in the hybrid fish. To characterize the gene expression of each parental fish strain in response to UVB exposure *X. maculatus* and *X. couchianus* were also exposed to 8 kJ/m² UVB and the skin mRNA was used for gene expression profiling analysis. Compared to unexposed controls, 425 DEGs were identified in *X. couchianus* (356 down-regulated; 69 up-regulated), while 413 genes were differentially expressed in *X. maculatus* (217 down-regulated; 196 up-regulated), (Figure 2b,c Supplement Table 1). Although *Sp-Couch* shares UVB-responsive genes with each parental fish, there are 341 unique UVB-response genes in the hybrid genetic background, indicating there is extended UVB response in the hybrid compared to either parental fish strains (Figure 2d).

Functional enrichment analysis of UVB-responsive genes for all 3 different fish types (i.e., parental and the hybrid) showed that melanin and pigment related categories are only present in UVB exposed *Sp-Couch* (Supplement Figure 2a,b). The genes that belong to these functional categories include canonical melanocyte pathway genes *mc1r*, *mitf1*, *tyr*, *tyrp1a* and *dct*. *Mc1r*, *mitf1*, *tyr*, *tyrp1a* and *dct* are all consistently up-regulated, indicating that UVB exposure positively induces the melanocyte-signaling pathway only in the *Sp-Couch* hybrids. Melanin, the product of these activities of the encoded genes, acts as a photosensitizer and could induce pre-mutagenic DNA lesions that may account for the susceptibility of melanoma in *Sp-Couch* hybrid.

Hybridization initiated UVB-responsive genes in the *Sp-Couch* hybrid

Since there are 341 genes that responded to UVB exposure only in the hybrid genetic background, we hypothesized the hybridization event disrupted these genes to initiate their UVB response. We compared the gene expression profile of unexposed *Sp-Couch* hybrid control fish to the parental gene expression profiles, also from unexposed fish to assess gene expression that is altered by hybridization. We distinguished 1,079 differentially expressed genes between *Sp-Couch* hybrid and *X. couchianus*, and 706 differentially expressed genes between *Sp-Couch* hybrid and *X. maculatus* (Figure 3a, Supplement Table 2). Functional analysis showed that both sets of differentially expressed genes have melanin synthesis and pigmentation related functions (Figure 3b). Since increased mRNA transcription of specific

genes serves to represent activation of a pathway as a cellular response, we focused on the up-regulated genes in *Sp-Couch* hybrid. By comparing the genes that were up-regulated by hybridization to the 341 *Sp-couch* unique UVB-responsive genes, we found 18 genes were up-regulated in *Sp-Couch* hybrid compared to both *X. maculatus* and *X. couchianus* (Figure 3c). These genes showed a pattern of up-regulation due to hybridization and also further up-regulation effected by UVB exposure. Among these 18 genes, 4 genes are related to melanin synthesis (*mitfa*, *tyr*, *tyrp1a*, *dct*), 3 genes are related to melanosome transport (*rab27*, *rab38a*, *pmela*), 4 genes are related to pigmentation (*slc2a11b*, *slc24a5*, *oca2*, *pax3b*), and 4 genes are melanoma/melanocyte marker (*tspan10*, *slc45a2*, *ptn*, *2,8-sialyltransferase*) (Kobayashi et al., 1994; Kuroda et al., 2005; Kang et al., 2007; Zhang et al., 2013; Haltaufderhyde and Oancea 2014; Kimura et al., 2014; Liu et al., 2014; Morice-Picard et al., 2014; Choi et al., 2015; Eaton et al., 2015; Lindsay et al., 2015). The remaining two genes (*CA10* and *kirrel3l*) are not well studied and their functions poorly characterized.

It is documented that *mitf* can be transactivated by the *Xiphophorus* Melanoma Receptor Kinase gene (*xmrk*), a mutant ortholog of the human EGFR (Delfgaauw et al., 2003; Wellbrock et al., 2002). The *xmrk* gene in the platyfish *X. maculatus* is closely linked to the *Sp* (or *Sd*, see Scharl et al., 2010) pigmentary locus on the X chromosome. The loss of one copy of the *R/Diff* locus, which is thought to play a role in macromelanophore differentiation in *Sp-Couch*, leads to heavy pigmentation of *Sp-Couch* hybrids (Walter and Kazianis, 2001; Patton et al., 2010). This is consistent with the up-regulation of *mitf* genes, and *mitf* target genes, in *Sp-Couch* hybrid. However, it is interesting how *mitfa* might become further up-regulated in *Sp-Couch* hybrids after UVB exposure, as well as the effects of UVB exposure on *mitf* target genes, such as *dct*, *pmela*, *rab38a*, *tyr*, *trypl1a*. It is noteworthy to mention that *mitf* levels are correlated with aggressive stage melanoma malignancy in the transgenic medaka-*Xmrk* melanoma model as well as human melanoma (Koludrovic et al., 2013; Scharl et al., 2010; Scharl et al., 2012). This may suggest that UVB serves to further up-regulate *mitf* and overwhelm normal cellular mechanisms (i.e., a single *R/Diff* gene copy) to keep *xmrk* in check and thereby initiating melanomagenesis.

***Sp-Couch* hybrids show ASE of pigment related genes**

We considered if certain parental alleles would be more favored in response to UVB exposure, and sought to identify genes showing allele specific expression (ASE) responses in the hybrid genetic background. To find ASE genes in the *Sp-Couch* hybrid, we identified sequence variations between the two parental alleles and used them to mark parental alleles in the hybrid genetic background and calculated the ratio of reads emanating from each parental allele. We identified 1,255 *X. couchianus* allele specific genes in *Sp-Couch* hybrid without UVB exposure, and 1,533 *X. couchianus* allele specific genes after UVB exposure. We also identified 1,271 *X. maculatus* allele specific genes in *Sp-Couch* hybrid without UVB exposure, and 1,566 *X. maculatus* allele specific genes in *Sp-Couch* hybrid after UVB exposure (Supplement Table 3). QRT-PCR assay confirmed the allele specificity of selected genes observed by RNA-Seq (Figure 4c). Eight of the hybridization-initiated UVB-responsive genes were identified to be ASE genes (Figure 4). As mentioned before, 7 of these 8 genes are pigment metabolism and melanoma related. *Ptn*, *slc2a11b*, *dct*, *2,8-sialyltransferase-like*, *tspan10* are *X. maculatus* ASE genes in unexposed control skin; *ptn*,

slc2a11b, *tspan10* and *pax3b* were detected to be *X. maculatus* ASE genes after UVB exposure. While *slc45a2* and *CA10* are *X. couchianus* ASE genes in unexposed or the UVB exposed condition, respectively (Figure 4a,b). The dominance of the *X. maculatus* allele in the expression of these genes infers genetic interactions in the hybrid genetic background produces *X. maculatus* bias in allele activation for pigmentation related genes and in the hybrid response to UVB.

Conclusions

By using UVB exposed parental and interspecies hybrids between *X. maculatus* and *X. couchianus*, we found unique gene expression patterns in the hybrid related to both the hybridization event and to UVB induced gene expression. Melanin synthesis and transport related genes were highly expressed in *Sp-Couch* hybrid and further up-regulated after UVB exposure and their expression is contributed more by *X. maculatus* alleles. The expression pattern of these genes suggests they may play a role in UVB induced hybrid melanomagenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

X. maculatus, *X. couchianus* and *Sp-Couch* pigmentation pattern

Figure 2. Differentially expressed genes in response to UVB in the *Sp-Couch* hybrid, *X. couchianus* and *X. maculatus*

Sp-Couch hybrid, *X. couchianus* and *X. maculatus* were exposed to UVB and differentially expressed genes were analyzed in skin. (a) 564 genes differentially expressed in *Sp-Couch* hybrid; (b) 425 genes differentially expressed in *X. couchianus*; (c) 413 differentially expressed genes are identified in *X. maculatus*. (d) Venn diagram shows 56 UVB-responsive genes common in all three fish types (both parental species and the F₁ hybrid). The *Sp-Couch* hybrid shares more UVB-responsive genes with *X. maculatus*, than to *X. couchianus*.

Figure 3. Differentially expressed genes between hybrid and parental fish strains
(a) The *Sp-Couch* hybrid has different gene expression compared to either *X. couchianus* or *X. maculatus* parent. Statistical analysis showed 1079 differentially expressed genes between *Sp-Couch* hybrid and *X. couchianus* and 706 differentially expressed genes between *Sp-Couch* hybrid and *X. maculatus*. (b) Functional enrichment of differentially expressed genes between *Sp-Couch* hybrid and each of the parental fish species is analyzed using Gene Ontology (GO). Of the 257 enriched functions between the *Sp-Couch* hybrid and *X. couchianus*, and 118 enriched functions between the *Sp-Couch* hybrid and *X. maculatus*, 56 functions overlapped. A significant part of the overlapping functions are related to melanin synthesis and pigmentation. (c) 18 genes are identified as hybridization-initiated and UVB-responsive genes. This group of genes does not show a response to UVB in any parental strain. The hybridization event served to up-regulate the expression of these genes while UVB exposure further stimulated the their expression levels.

Figure 4. Allele Specific Gene Expression

(a) Allele specific expressed genes in *Sp-Couch* hybrid where 6 hybridization-responsive genes showed allele specificity. (b) Allele specific genes in *Sp-Couch* hybrid after UVB exposure, 5 hybridization-initiated UVB responsive genes showed allele specificity. (c) Allele specificity of 5 genes (*ptn*, *tspan10* and *dct* showed strong allele specificity, *pomcb* and *tyr* showed no allele specificity) from *Sp-Couch* hybrid in control and UVB-exposed conditions were tested using qRT-PCR. Blue represents unexposed control conditions and red represents the UVB-exposed condition.

Table 1

RNA-Seq result statistics

	X. couchianus		X. maculatus		X. maculatus		SP-Couch	
	control	UVB	control	UVB	control	UVB	control	UVB
raw read counts (in million)	79.3	67.7	81.8	77.5	81.6	72.0		
filtered read counts (in million)	39.6	36.3	38.6	44.1	38.5	37.9		
raw total length (in billion bp)	7.9	6.8	8.2	7.7	8.2	7.2		
filtered total length (in billion bp)	3.4	3.0	3.3	3.7	3.3	3.2		
Transcriptome Coverage	73X	66X	71X	81X	71X	69X		