indication for a possible role of TRPL and arrestin in the phototransduction or synaptic transmission of photoreceptor cells. In order to generate a pure *trpl* mutant without an *ine* second site mutation, we performed a meiotic recombination between the *trpl* and *ine* loci which are 48 cM apart from each other. Among the 13 stocks carrying a recombined second chromosome, five stocks inherited the $trpl^{302}$ mutation (Figure 3A). Among these stocks, one stock was devoid of oscillations (Figure 3B,C) indicating a loss of the *ine* second site mutation. This mutant is now called $trpl^{302NO}$ (No Oscillations) and can be obtained from the authors.

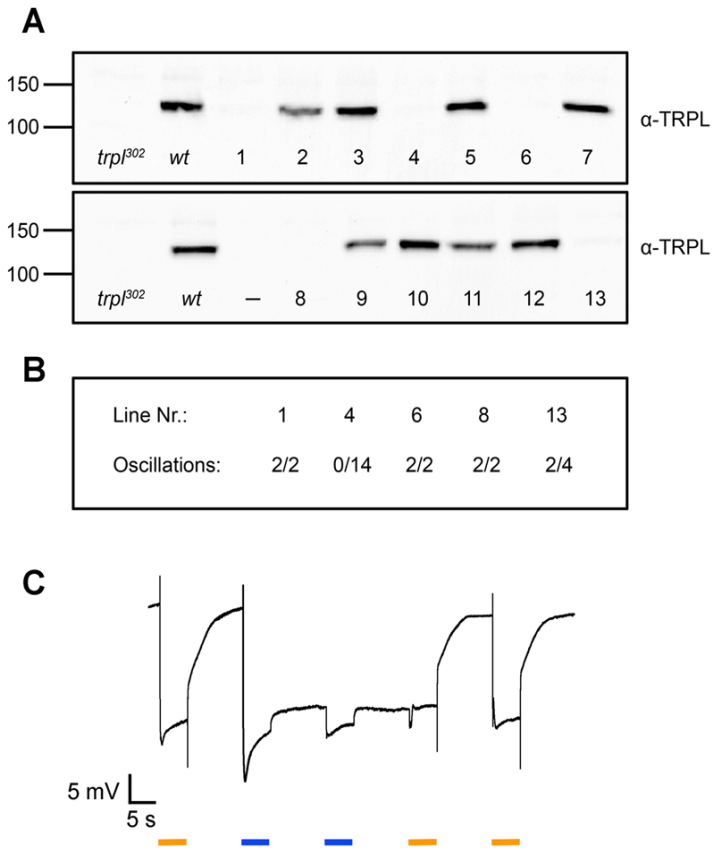

Figure 3. Generation of a pure $trpl^{302}$ mutant. (A) Stocks derived from recombination of the $trpl^{302}$ *cn bw ine* chromosome with a wild type chromosome (see Material and Methods) were analyzed for the $trpl^{302}$ mutation as indicated by the absence of a TRPL signal in Western-Blot analysis. (B) Stocks carrying the $trpl^{302}$ mutation were analyzed for the presence of oscillations in ERG recordings; fraction indicates the number of flies with oscillations/all flies tested. (C) ERG recording of a fly from recombination stock 4 using the OBBOO protocol. No oscillations could be detected.

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Cyp28AI gene variability in *Drosophila eremophila.*

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Abstract

We sequenced and analyzed gene *Cyp28A1* segment in cactophilic fly *Drosophila eremophila* samples obtained from the Mexican states of Guanajuato, Hidalgo, and San Luis Potosi. The results showed high

variability in this gene in *D. eremophila*. This suggests that *D. eremophila Cyp28AI* presents an important role in adaptation to the cactus host.

Introduction

Insects have developed resistance to xenobiotic compounds, including pesticides and allelochemicals ingested from the plants they feed on. The key to this adaptation lies within three detoxification gene super-families: esterases, cytochrome P450 monooxygenases, and glutathione *S*-transferases (Bono *et al.*, 2008).

Cytochrome P450 (CYP) is a family of genes present in all organisms, ranging from *Drosophila* to humans, each coding for an enzyme involved in the detoxification of xenobiotic compounds, as well as in the regulation of various metabolic processes involving hormones and fatty acids (Scott, 2008). Over 20,000 different CYP sequences have been found (Nelson, 2009).

Drosophila melanogaster has 84 different CYP enzymes, and here we evaluated sequence variation in one of these, *CYP28A1,* in the cactophilic species *D. eremophila. D. eremophila* is endemic to Mexico where it breeds in the soil soaked by necrotic cactus (Heed, 1989).

Materials and Methods

Samples and their localities are summarized in Table 1. Total genomic DNA was extracted from individual flies using DNeasy™ (QIAGEN Inc., Valencia, CA) kits. An 1100 bp segment of the *Cyp28AI* gene was amplified by PCR using the following forward and reverse primers: *5'* CTACTACTGCTGGGCCTCTTCTAT *3'* and *5'* CTACTACTGCTGGGCCTCTTCTAT *3'*. The amplifications were performed at 59°C temperature, using Bio Basic Inc. (Canada) *Taq* DNA polymerase. DNA sequencing was performed by Genomic Services, LANGEBIO, Irapuato, Gto., México. Sequences were proofread and aligned in Geneious Pro ver. 4.8.5 software (Biomatters Ltd) followed by manual editing. Analyses were performed on a 352 bp fragment. Phylogenetic analyses were performed using MEGA v. 6.06 software (Tamura *et al.*, 2013), and DNASP ver. 5.10.01 (Librado and Rozas, 2009) software was utilized to measure polymorphism.

Table 1. Summary of number of *Drosophila eremophila* from each locality.

Locality	Number of samples
Barranca de Metztitlán, Hidalgo, Mexico	1
Charcas, San Luis Potosí, Mexico	7
Irapuato, Guanajuato, Mexico	3
San Miguel de Allende, Guanajuato, Mexico	11

Results and Discussion

Cyp28AI is autosomally inherited, found on the third chromosome in *Drosophila mojavensis* (Muller element B). *Cyp28AI* of *Drosophila mettleri*, a close relative of *D. eremophila*, has five introns and with multiple heterozygous sites (Bono *et al.*, 2008). Our 352 bp fragment of *Cyp28AI*, also showed a number of heterozygotes (Bono *et al.*, 2008).

We detected 11 haplotypes and 7 variable sites (Table 2). The only amino acid change associated with these polymorphisms is present in position 279. The 279 residue of CYP28AI of *D. eremophila* can be Asn (10 samples) or Ser (12 samples) (Data not shown). Values for Tajima´s D, Fu and Li´s D, and Fu´s Fs were not significant. Neutrality tests detected no enrichment of rare alleles among the *D. eremophila Cyp28AI* segment ($P > 0.10$).

Table 2. Polymorphism statistics for 352 bp of *Cyp28A1* in *Drosophila eremophila.*

Species	N	S	K	h ± SD	π ± SD	Tajima's D	Fu and Li´s D	Fu'sFs
D. eremophila	22	7	11	0.922± 0.034	0.00685 ± 0.00066	0.81826	0.65029	-4.537

N, number of sequences; S, number of variable sites; K, number of haplotypes; h, haplotype diversity; π, nucleotide diversity.

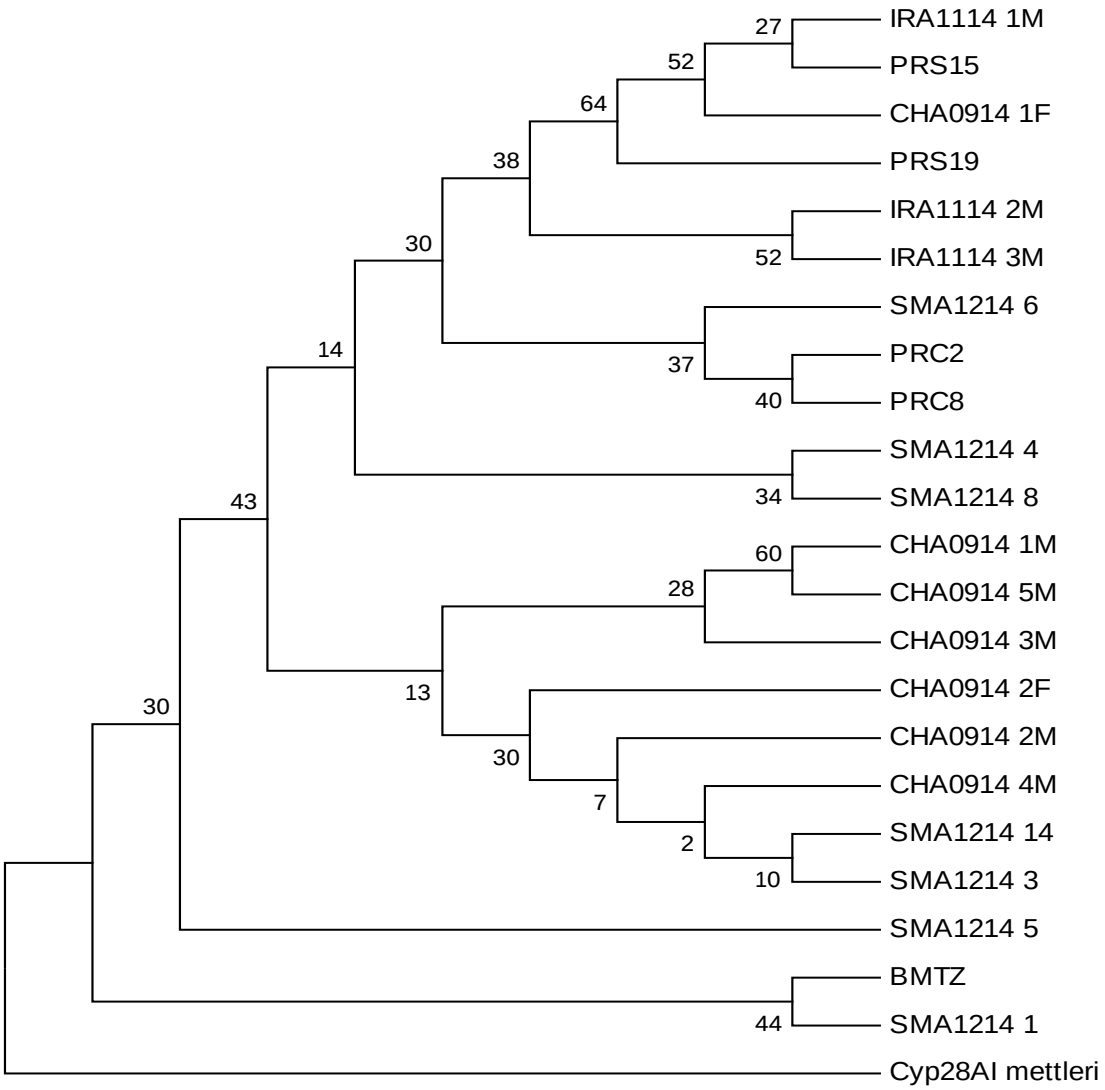

Figure 1. Phylogenetic tree of *Cyp28AI* in *D. eremophila*. (Maximum Likelihood). BMTZ (Barranca de Metztitlán, Hidalgo); CHA (Charcas, San Luís Potosí); IRA (Irapuato, Guanajuato); PRC, PRS, and SMA (San Miguel de Allende, Guanajuato).

We used the Maximum Likelihood method for phylogenetic analysis (Figure 1). The evolutionary model chosen was K80+I (Kimura, 1980). The tree was not well-supported (bootstrap = 2 to 60). We used the *Cyp28AI* of *D. mettleri* (GenBank: EU659006) as the outgroup to root the tree. Our data showed no population structure in the mainland for the 352 bp segment.

While our sample size is small, two things seem clear. One is that the sampled populations of *D. eremophila* comprise a large panmictic population. The other interesting outcome is that one of the substitutions causes an amino acid replacement, which may, upon further ecological work and additional sampling, suggest a degree of adaptation in this gene in *D. eremophila*.

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Co-expression of *Buffy* with *Buffy-RNAi* produces an intermediate phenotype in the *Drosophila* eye.

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Introduction

The use of ribonucleic acid interference (RNAi), a post-transcriptional gene silencing mechanism, to inhibit gene function is widely applied to analyse phenotypic consequences of gene suppression (Izant and Weintraub, 1984; Fire *et al.*, 1998; Ambesajir *et al.*, 2012). RNAi is an evolutionarily conserved cellular mechanism which is present in protozoa, fungi, nematodes, plants, flies and mammals (Agrawal *et al.*, 2003). This method is used in genome-wide screens (Dietzl *et al.*, 2007), functional genomics, genetic therapeutics, crop and animal improvements among many upcoming applications (Ambesajir *et al.*, 2012). In most of the organisms currently being used for studies, RNAi is systemic and cannot, therefore, be restricted to a specific cell type (Dietzl *et al.*, 2007). Using the bipartite UAS/Gal4 expression system (Brand and Perrimon, 1993), RNAi can be triggered in a spacio-temporal manner in *Drosophila melanogaster* (Dietzl *et al.*, 2007). Gene function can be analysed using an appropriate assay by examining the phenotypic effect of the directed inhibition (RNAi) or the overexpression of the gene. To investigate the phenotypic effects of directed overexpression upon the directed RNA interference of an important cell survival gene, we examined the consequences of the overexpression of *Buffy*, the sole pro-cell survival *Bcl-2* homologue (Quinn *et al.*, 2003), and a corresponding RNAi in the developing *Drosophila* eye. We investigated the possibility that an intermediate developmental phenotype can be generated from this interaction that may be subject to modification by other genes.

Materials and Methods

Drosophila stock and culture: *UAS-Buffy* (Quinn *et al.*, 2003) was kindly provided by Dr. Leonie Quinn (University of Melbourne). *UAS-Buffy-RNA*$_i$ (w[*]; P{w[+mC]=UAS-Buffy.RNAi}c3), *GMR-Gal4* (Freeman, 1996) and *UAS-lacZ* flies were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *UAS-Buffy/CyO; GMR-GAL4* line was generated using standard recombination methods and was used to overexpress *Buffy* in the developing eye under the direction of the *GMR-Gal4* transgene. Stocks and crosses were maintained on standard medium containing cornmeal, molasses, yeast, and agar. Stocks were kept at room temperature (23^{o}C ± 2^{o}C) while crosses and experiments were carried out at 29^{o}C.

Biometric analysis of the Drosophila eye: A number of single vial crosses of each genotype were made at 29^{o}C, a cohort of the critical class male flies was collected upon eclosion and aged for three days before being frozen at -80^{o}C. Whole flies were mounted on scanning electron microscope stubs, desiccated overnight and photographed with a FEI Mineral Liberation Analyzer 650F scanning electron microscope. For each cross ten eye images were analysed using the National Institutes of Health (NIH) ImageJ software (Schneider *et al.*, 2012) and biometric analysis performed using GraphPad Prism version 5.04. The percentage