orchard at random positions with equidistance. The trapped flies were carefully extracted after securing the mouth of the traps with a cotton cloth and preserved in 70% alcohol. Four successive collections were done in the following week. The species identification was made according to taxonomic groups by employing several keys of Sturtevant (1927), Patterson and Stone (1952), Throckmorton (1962), and Bock and Wheeler (1972).

Table 1. Sub genus of the species recorded at Taralu.

Sl. No.	subgenus	Species
1		*D. takahashii*
2		*D. melanogaster*
3		*D. ananassae*
4	Sophophora	*D. bipectinata*
5		*D. nagarholensis*
6		*D. malerkotliana*
7		*D. rajashekari*
8	Melanogaster	*D. immigrans*
9	Zaprionus	*Phorticella striata*

Nine species of *Drosophila* were recorded in the present bait trapping studies (Table 1). The trap count of species in the order of percent traps *P. striata* (29 %) > *D. rajashekari* (21%) > *D. malerkotliana* (13%) > *D. bipectinata* (9%) > *D. melanogaster* (8%) > *D. nagarholensis* (7%) > *D. ananassae* (5%) > *D. immigrans* (4%) > *D. takahashii* (3%). *P. striata* and *D. bipectinata* were recorded all through the observation period. The latter is a non-drosophilid genus of family Drosophilidae which is endemic to South India (Sajjan and Krishnamurthy, 1975). A rich diversity index (Simpson_1-D = 0.82) was obtaine; however, moderate richness and the evenness of the community was obtained (Shannon_H =1.94) from the analysis using PAST software (version 3). Ecodistributional analysis of *Drosophila* implies the biodiversity of *Drosophila* of a given locality and also the principles underlying adaptive radiation and central mechanisms involved in speciation (Hegde *et al*., 2001; Guru Prasad and Hegde, 2010). In this regard the present report is a first time record of drosophilid diversity from the selected study area. The results certainly need to be related to the proximity of the study area to the Bannerghatta National Park (BNP) one of the last largest remaining scrub forests of the country, placed on the confluence of the Eastern and the Western Ghats (Varma *et al*., 2009). Hence, future studies on eco-dynamics including drosophild diversity of the entire region is relevant and needs to be undertaken.

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Comparison of *ebony* gene from three *ebony* mutants.

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Drosophila melanogaster ebony mutant is easily recognizable by its black pigmentation instead of the normal brown color of the wild type strains. This mutant is defective for the synthesis of β-alanyl derivatives

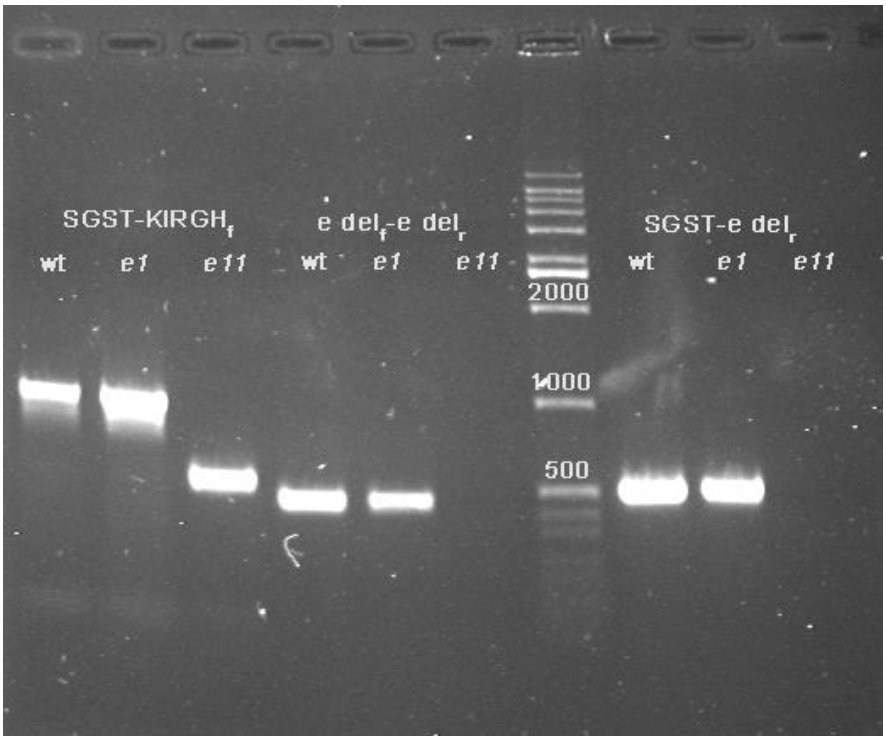

Figure 1. PCR amplification of wt (CS) e^1 and e^{11} cDNA with different primers (see Figure 2). M: DNA markers (numbers indicate the base pairs).

such as N-β-alanyldopamine (NBAD) or N-β-alanyl-histamine (carcinine), which are the products of the conjugation of β-alanine with dopamine or histamine, respectively. Besides body color defect, other well-known features of this mutant are the neurological and behavioral disorders, such as abnormal electroretinograms, lacking "on" and "off" transients (Hotta and Benzer, 1969), reduced phototaxis (Benzer, 1967), and abnormal circadian rhythm (Newby and Jackson, 1991). For many years, Ebony was known as an epidermal enzyme responsible only for tanning and sclerotization of brown cuticles (Wright, 1987). We previously demonstrated that NBAD-synthase is an enzyme induced in epidermis with a narrow window of expression at the beginning of pupariation (Pérez *et al.*, 2002; Wappner *et al.*, 1996a). The enzyme is also induced during the transition from pharate adult to imago and during the first hours after the ecdysis (Pérez *et al*., 2004; Pérez *et al.*, 2010). Recently, we documented the expression of NBAD-synthase in epidermal tissues of *D. melanogaster* embryos (Pérez *et al.*, 2010). In addition to the expression of NBAD-synthase in epidermis, we found that this activity is also expressed constitutively in nervous system (Pérez *et al.*, 2004), suggesting a role in the metabolism of neurotransmitters. Furthermore, it has been postulated that this enzyme functions in a metabolic pathway that may terminate the action of histamine in photoreceptor cells (Borycz *et al.*, 2002; Richardt *et al.*, 2003). Our biochemical and immunohistochemical results demonstrated that NBAD-synthase is widely expressed in the brain. Thus the expression of Ebony in brain regions other than retinas suggests that this enzyme not only plays a role in the metabolism of histamine in visual system but also in the metabolism of other neurotransmitters like dopamine and possibly octopamine and serotonin (Pérez *et al.*, 2002; Richardt *et al.*, 2003; Pérez *et al*., 2004). We previously analyzed NBAD-synthase *in vitro* activity in the *ebony* mutants e^1 and e^4 and we found that they are unable to synthesize β-alanyderivatives (Pérez *et al*., 1997, 2001). We have also cloned and sequenced the e^4 mutant gene, showing that it has a 447 base pair deletion in its first exon, synthesizing a protein without activity due to the lacking of 149 amino acids (Pérez *et al.*, 2001). Some slight physiological differences exist among the different *ebony* mutants, with e^4 being the less drastic phenotype (Newby and Jackson, 1991; Rossi *et al*., data not shown; FlyBase). To address the reason for this discrepancy we characterized molecularly e^1 and e^{11} to better understand their phenotypes.

Flies were from Bloomington stock center, e^1 (stock number 1658), e^4 (stock number 507), and e^{11} (stock number 497); reared on standard corn meal yeast agar medium.

Cell-free synthase activities in slightly purified homogenates from *D. melanogaster* were measured as previously described (Pérez *et al.*, 2002). Briefly, the standard assay for NBAD synthesis contained 2 mM ATP, 5 mM $MgCl_2$, 0.1 mM dopamine, 10 µM β-alanine and 3×10^5 counts/min (cpm) of [14C]β-alanine and 3 µl of enzymatic extract in a final volume of 50 µl, in 50 mM Na-tetraborate-Boric acid buffer, pH 8.3. Catecholamine derivatives were isolated using alumina columns and analyzed by reversed phase HPLC

(Econosphere- C-18, Altech) as previously described (Pérez *et al.*, 2002).

RNA was extracted with TRI-Reagent (SIGMA), purified and used to synthesize cDNA with reverse transcriptase (SuperScript II, Invitrogen) and specific primers. Amplification was with a combination of specific primers named, SGST, KIRGH, EHRQ, (according to the putative amino acid sequence of specific *ebony* regions), eDEL (forward and reverse, which flank the e^4 sequence deletion) and C-term (Figure 2). After electrophoresis, the DNA bands were eluted with Gene Clean (Bio 101), cloned in easy T vector (Promega) and sequenced. Sequence analysis was made with Blast program (NCBI).

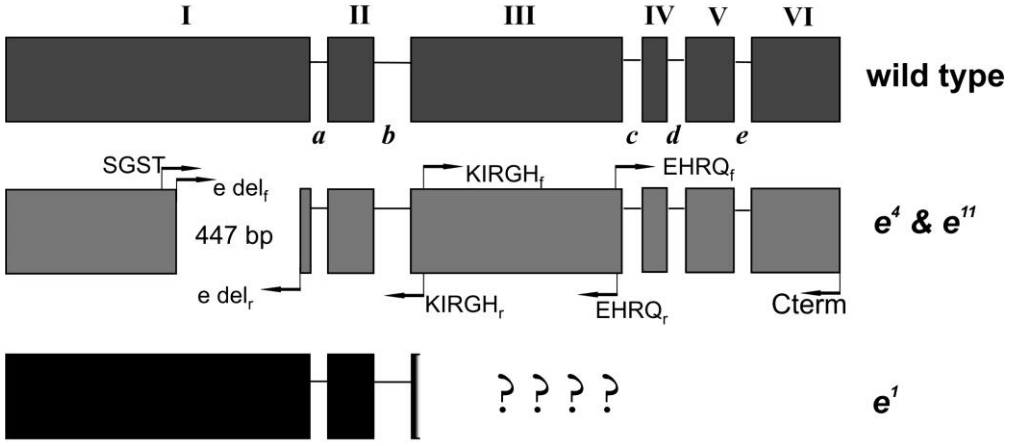

Figure 2. Scheme of e^1, e^4 and e^{11} coding sequence. Boxes represent exons, lines represent introns. Roman numerals indicate exon number, italic lowercase letters indicate introns. Letters and arrows (in e^4 and e^{11} exons) indicate primers and direction of amplification; e del$_f$ and e del$_r$: primers that amplified the sequence deleted in e^4 and e^{11}.

Our results confirm, as expected, that none of these 3 *ebony* mutants were able to synthesize NBAD (not shown). Analyzing the cDNA sequence, we observed that the nature of e^1 defect is different from the previously sequenced e^4 (Pérez *et al*., 2001). The nucleotide sequence of e^1 in the first exon is similar to that of wild type flies. However, we were unable to clone and sequence the e^1 cDNA from the beginning of the 3rd exon to the C-terminal region of the gene. We used different sets of primers (including several poliT primers) and different approaches, but we never were able to amplify and clone this region, thus suggesting that something more complex such as an insertion or inversion occurs. It does not seem a deletion, because otherwise it should amplify the cDNA using a poliT primer. The e^{11} sequence, surprisingly, was similar to that of e^4, with the same deletion of 447 bp in the first exon. As is reported in FlyBase, e^4 flies are the most viable and generally best of the dark alleles, such as e^1 and e^{11}. This suggests that probably e^{11} carries other mutations, in the non-coding sequence of the *ebony* gene or in another site of the genome.

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