intercalating agent, ethidium bromide, acts as a weak mutagen, but a strong toxic chemical. It does not increase clone frequency up to the dose LD40. The strongest effect is registered with DMBA treatment. In comparison with benz(a)pyrene (11.7% clone frequency) (Sidorov *et al*., 2002), DMBA acts as more powerful carcinogen (42.5% clone frequency), that, probably, makes conditional upon methylated structure of DMBA.

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The annotation CG17337 of *D. melanogaster* is the gene *Dipeptidase-A*.

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Supporting Notes and Data

Dipeptidase-A (Dip-A) is one of a cohort of Dipeptidases (-A, -B and -C) which appear ubiquitously in the fly (Hall, 1983; Laurie-Ahlberg, 1982) and provide the final step of protein catabolism (Collett, 1989). The identification of the annotation CG17337 as the gene coding Dip-A reported here emerges from several kinds of information. Its chromosomal region has been defined by the associations of its electrophoretic variants and activities with chromosomal deficiencies (Voelker and Langley, 1978; Hall, 1983; and Table 1), while its physiological characterization in the large dipteran *Calliphora erythrocephala* (Collett, 1989) and in *Drosophila* (Collett, in prep.) has allowed discrimination among the putative proteinases in the annotations of this defined region. Further, these characteristics of Dip-A together with the coding of CG17337 correspond to those of the characterized human proteinase PepA. This information is compiled here as definitive evidence of the identification of the annotation CG17337 as the gene *Dip-A*.

To refine the earlier localization of a gene for Dip-A (Voelker and Langley, 1978) in the proximal region of 2R, Dip-A activity was measured in duplication and deficiency heterozygotes in the F1 progeny of a mating of Oregon R and the stock Df(2R)nap1 (Table 1). The activities of both Dip-A and Dip-C, as a standard within each sample, were measured in samples of adult males of the parental and progeny genotypes and are presented as the ratios of Dip-A and Dip-C. As may be seen in Table 1, these ratios are consistent with the haploid, diploid and triploid copy number of the region of 41D2 – 42B3 in the sampled genotypes. Thus the gene *Dip-A* is restricted to the chromosomal region bounded proximally by 41D2.

Table 1. Localization of Dip-A coding within the chromosomal region 41D2 - 43B3 of *D. melanogaster*.

Activities of Dipeptidase-A and Dipeptidase-C, as ratios (Dip-A/Dip-C)				
Genotype	Oregon-R	Df(2R)nap1/Dp(2;2)BG	Oregon R/Df(2R)nap1	Oregon R/Dp(2:2)BG
Deficiency/Duplication breakpoints	–		– / 41D2 - 42B1-3	– / h38 - 42B
Activities, Dip-A/Dip-C	1.46 ± 0.03	1.35 ± 0.04	0.84 ± 0.01	2.19 ± 0.32

The activities of Dipeptidase-A (Dip-A) and Dipeptidase-C (Dip-C) were measured in at least two samples of 3 to 5 one to two day old adult males of wild-type (Oregon-R), the Deficiency/Duplication stock Df(2R)nap1/Dp(2;2)BG, In(2LR)Gla, wg[$^{Gla-1}$] (BDSC 1006) and their F1 progeny, as indicated. Simultaneous hydrolyses of prolyl leucine (by Dip-A) and leucyl proline (by Dip-C) were assayed and measured spectrophotometrically as optical densities (Collett, 1989) and are presented as ratios (±) differences or standard deviations) among replicate samples.

Table 2. Association of Dip-A electrophoretic variants and activity within the 2R proximal region h46 – 42B3 of *D. melanogaster.*

Source	Deficiency/Duplication	Df/Dp breakpoints	Identification of Dip-A	Breakpoint identification
Voelker and Langley (1978)	Df(2R)bw^{VDe2L} Cy^{R}	h42 - 42A3	electrophoretic variant	Kuroda *et al.* (1991)
	Df(2R)M-S2^{4}	h46 - 42A2	electrophoretic variant	unknown
Hall (1983)	In(2R)bwVDe2LCyR	h42 - 42A3	activity	Kuroda *et al.* (1991)
Collett (Table 1, above)	Df(2R)nap1	41D2 - 42B3	activity	FBab0024067
	Dp(2:2)BG,In(2LR)Gla,wg[$^{Gla-1}$]	h38 - 42B	activity	FBab010165

Compiled in Table 2 are the results of the several associations of Dip-A with this region of 2R. First among these is Voelker and Langley's (1978) association of two naturally-occurring electrophoretic variants with the region of a deficiency defined to have breakpoints in the proximal heterochromatin h42 and in 42A2, thus limiting the gene (or genes') site to the region bounded distally by 42A2. Hall's (1983) analysis of the association of activity hydrolyzing a unique Dip-A substrate within the same deficiency confirmed this earlier finding and further suggests that there is not a regulatory site elsewhere in the genome. The further refinement of the chromosomal region of Dip-A (Table 1) to the proximal region of 2R reported here thus restricts the chromosomal region of *Dip-A* to the well-annotated region of 41D2 - 42A3.

This region contains 45 annotations. Three of these are identified by a Pfam search of its protein database to be proteinases. Characteristics of these putative proteinases and of their annotated genes, together with characteristics of Dip-A, are compiled in Table 3. Of these one annotation has been identified as the gene *scarface* which among the escapers of pupal lethality has a mutant phenotype of an affected head. Since a naturally-occurring and non-leaky *null* allele of *Dip-A* found in *D. pseudoobscura* in standard laboratory conditions does not affect either viability or visible adult phenotype (Jarman and Collett, in prep.), the gene *scarface* is clearly not the source of Dip-A. Candidate annotation CG3107 is also unlikely to be *Dip-A*, since its putative coding is of a protein with a molecular weight that differs substantially from Hall's (1983) estimate of the molecular weight

of Dip-A in a monomeric state. The Pfam protein database also suggests that the CG3107 proteinase is a member of the M16 metallo-proteinase class which encompasses special functions including mitochondrial processing. This also is inconsistent with the observed specificity of Dip-A for hydrolyzing a variety of small peptides (Laurie-Ahlberg, 1982; Hall, 1983; Collett, 1989). Thus a process of elimination brings consideration to annotation CG17337 and its putative coding of a proteinase of the M20 metallo-proteinase class.

Table 3. Characteristics of Dip-A and candidate annotations in chromosomal region 41D2 – 42A3 of *D. melanogaster* (R 5.5).

Character	Dipeptidase-A	CG3107	CG11066 (*scarface*)	CG17337
Locus	41D2 – 42A2	41D3	41F8	41F8
Mutant phenotype	no detected visible* or viability effects	?	pupal recessive sub-lethal/adult head	?
Estimated MW, kDa	44**	120		53.5
Physiological activity	di-, tri-peptidase, broad specificity*** metallo-proteinase****			
Pfam protein		M16	serine protease	M20 metallo-proteinase
Domain identification		metallo-proteinase, mitochondrial processing+ other		mono- and di-homo-polymeric, N-terminal AA hydrolysis
Human 'homologue'				*pepA****** 62% coding identity gene ID: 55748 CNDP2

* Observation based upon a naturally occurring *null* allele of *Dip-A* of *D. pseudoobscura* (Collett, unpublished).

** Hall (1983) estimate of MW by elution of Dip-A prepared in 0.1M Tris-Cl, pH7.1 containing 1% Triton in Sephadex G-200.

*** Laurie-Ahlberg (1982), Collett (1989), Beard and Collett (unpub) substrate testing in electrophoretic assays.

**** Activity of Dip-A is depressed in the presence of EDTA and pepstatin (Collett, unpub.).

***** Teufel *et al.* (2003) characterized pepA as a ubiquitous cytosolic di-peptidase of the M20 metalloproteinase family which hydrolyses N-terminal amino acids non-specifically. Its molecular weight (MW) is 52.7kDa.

Characteristics of Dip-A may be compared with those of the putative protein coded by annotation CG17337 in Table 3. It will be seen that the molecular weight, substrate specificity, and EDTA-chelation (as a metallo-proteinase) of Dip-A do closely match those of the annotation and of the Pfam characterization of its protein. But in addition, characterization of pepA (CNDP2) in humans (Teufal *et al.,* 2003) lends further evidence of identity. With a nearly identical molecular weight, the coding of pepA matches that of CG17337 along 62% of its length, while also sharing substrate specificities. Both enzymes are also sensitive to fungal pepstatin, and both are systemically ubiquitous. Thus, it would be remarkable if the annotation CG17337 is not the gene coding Dip-A in *D. melanogaster*.

One further interest in Dip-A is whether CG17337 is the only gene providing its protein. Electrophoretic separations of Dip-A activities in mobility heterozygotes are consistent with a dimeric protein structure (Voelker and Langley, 1978; Collett, unpublished). This is also consistent with the Pfam identification of a region within the CG17337 (and human *pepA*) which allows activity

in either a monomeric or dimeric form. Although not conclusive, this detail does strengthen the likelihood that only one gene, CG17337, is responsible for the coding of *Dip-A*.

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Distribution of *Drosophila* flies in eight different altitudes of three districts of Karnataka, India.

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Introduction

Drosophila fauna is more abundant in Kodagu, Mysore, and Dakshina Kannada districts of Karnataka, South India (Gai,1985). Studies on cytogenetic aspects of these species were carried out extensively which revealed an interesting situation. However, studies on natural populations of these two sympatric species at different altitude are limited. Here we report the collection of *Drosophila* flies at eight altitudinal regions in three districts of Karnataka state, India at four different months in a year (2001-02) and establishment of isofemale lines of *D. nasuta nasuta* and *D. sulfurigaster neonasuta* to study the morphophenotypic variations and fitness differentiation of these two sympatric species.

Materials and Methods

Collection of flies:

Drosophila flies were sampled from eight geographically and altitudinally distant places in three districts of Karnataka state, South India, namely Dakshina Kannada, Kodagu, and Mysore (Figure 1). The general eco-geographical features, the topographical features, climatic factors and the types of vegetation of these localities are briefly described below.

Dakshina Kannada district (Figure 1; Table 1) is located in the south of Karnataka, which is a coastal belt of Arabian Sea and is situated between 120.25'–130.10' latitude north and 740.50–'750.45' longitude east. The normal annual rainfall during 2001 to 2002 was about 3781 mm and humidity was 82%. The collections were made in two regions. The first spot was near Belma village, which is about 12 km from Mangalore City towards Mangalore University Campus, called as DK-1. The second spot was near Mudipu village located about 25 km from Mangalore City, called as DK-2. The distance between these two spots is 13 km. Both these places are situated at an altitude of 0 MSL and are directly exposed to the southeast monsoon. The main vegetation of this area includes *Nothapodytes foetida*, *Ixora coccinea*, *Zizyphus jujuba*, *Anacardium occidentale*,