ananassae (Tobari, 1993; Singh, 1996). We have initiated studies on multiple mating in *D. ananassae* and report the results of preliminary study on female remating in this species.

Virgin females and males were collected from a wild laboratory stock of *D. ananassae* and aged for seven days. One female and one male were transferred to a food vial without etherization and the pair was observed. Courtship time and duration of copulation were recorded for each mated pair. After termination of copulation, the male was aspirated out and a fresh virgin male was transferred to the vial containing the mated female. The pair was observed and if the remating did not occur until evening, female and male were separated. Again next day in the morning, the mated female was kept in a food vial with a virgin male and the pair was observed. Out of 8 mated females, remating was observed in 5 females. In 3 females, remating was observed on the 6th day and in 2 females remating occurred on the 2nd day. Duration of copulation was recorded for all the females in both the matings. Interestingly, the duration of copulation was shorter in the second mating as compared to the first mating for all the five remated females.

Experiments will be conducted to study the phenomenon of multiple mating in *D. ananassae* with particular reference to its frequency in different populations, reproductive consequences and genetic basis.

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Pérez, M.; N. Castillo-Marín, and L. A. Quesada-Allué. Instituto de Investigaciones Bioquímicas, Fundación Campomar and University of Buenos Aires, Patricias Argentinas 435, Buenos Aires (1405), Argentina. ß-alanyl-dopamine synthase in *Drosophila melanogaster* and *Ceratitis capitata* melanic mutants.

Melanic cuticle mutants have been described and genetically characterized in a number of insects (Czapla *et al.,* 1990; Roseland *et al.,* 1987) including *Drosophila* (Hodgetts and Konopka, 1973; Hodgetts and Choi, 1974). We recently studied a *Ceratitis capitata* mutant, *niger*, that shows a melanic puparium cuticle and was found to be defective for the enzyme conjugating ß-alanine and dopamine to synthesize ß -alanyl-dopamine [NBAD] (Wappner *et al.,* 1996a). This molecule seems to be the main sclerotizing and pigmentation agent in brown insect cuticles (Kramer and Hopkins, 1987). When the NBAD synthase is not functional (Wappner *et al.,* 1996a; this note) or when ß-alanine is not available, like in the *Ceratitis* mutants *Dark pupa* and *Black pupa* (Wappner *et al.,* 1996b), the redundant dopamine substrate (that cannot be conjugated with ß-alanine) enters the melanine pathway, thus giving raise to a black cuticle. *Drosophila* e^1 (*ebony*) (FlyBase FBgn0000527) shows a shining black adult cuticle and is unable to use ß-alanine for tanning the puparium. *ebony* was postulated to be defective in NBAD synthase (Wright, 1987; Hodgetts and Konopka, 1973) but no proof was available, since the data on the direct measurement of the enzyme activity in the wild type were not further substantiated in a publication. Based on both the melanic phenotype and abnormal behaviour (Kyriacou *et al.,* 1978) we previously suggested that *Drosophila* e^1 and *Ceratitis nig* (*niger*) might be mutants corresponding to related genes (Wappner *et al.,* 1991). Here we report confirmative preliminary results related to these questions.

Wild type (Oregon R), *black* and *ebony* strains of *Drosophila melanogaster* were grown in Carolina's blue food. Wild type (Arg.17), *Dark pupae* and *niger* strains of *C. capitata* were grown in carrot-corn-yeast medium (Quesada-Allué *et al.,* 1994). The flies were maintained at 21°C in a D/L regime of 8/16 hs.

The Standard assay for NBAD synthesis contained 1.0 mM ATP, 10 mM $MgCl_2$, 0.1 mM dopamine, 10 µM ß-alanine and 0.01 µCi of [^{14}C]-ß-alanine in 50 mM Na-tetraborate-Boric acid buffer, pH 8.3. The reaction was started with 10 µL of the enzymatic preparation (see Wappner *et al.,* 1996) in a final volume of 50 µL. Radioactive [^{14}C]ß-alanine-containing catecholamines were isolated and measured as previously described (Mason and Weinkove, 1983; Wappner *et al.,* 1995). The different reaction products were separated from substrates and identified in C_{18}-reverse phase HPLC or in silica gel-TLC (Solvent I = Methyl-ethyl-ketone:propionic acid:Water [40:13:11]).

Table 1 shows that crude extracts of *Drosophila melanogaster* wild type and *Ceratitis* w.t. were able to synthesize a [^{14}C]-ß-alanine-containing substance behaving in acid alumina as a diphenolic cathecolamine and further co-

chromatographing in reverse-phase HPLC (not shown) and TLC (R_{fl} = 0.63; see Figure 1) with a standard of ß-alanyl-dopamine. This is the first report on the *in vitro* biosynthesis of this substance in *Drosophila*. The enzymatic activity of NBAD synthase in recently emerged *Drosophila* w.t. adults was found to be similar to that in pupae (Table 1) in spite of the fact that the wild-type puparium of *D. melanogaster* shows a very pale brown coloration whereas the adult cuticle shows the typical brown color generated by NBAD-dependent tanning. In contrast, a strong reddish-brown color develops in *Ceratitis* w.t. puparium. This probably indicates that the synthesis of NBAD in the latter is comparatively higher than in *Drosophila* puparium. This seems to be confirmed by the specific activity data in Table 1. However we cannot discard that, in addition, low levels of one or both of the substrates, dopamine and ß-alanine, might also contribute to the *Drosophila* pale puparium phenotype. Hodgetts and Konopka (1973) reported lower levels of dopamine in *Drosophila* than in other insects at the beginning of pupariation.

The crude extract of the mutants *Dark pupa* of *Ceratitis* and *black* of *Drosophila,* known to be deficient in ß-alanine (Wappner *et al.,* 1996b; Jacobs,1985), were also able to synthesize NBAD (Table 1 and Figure 1), thus showing

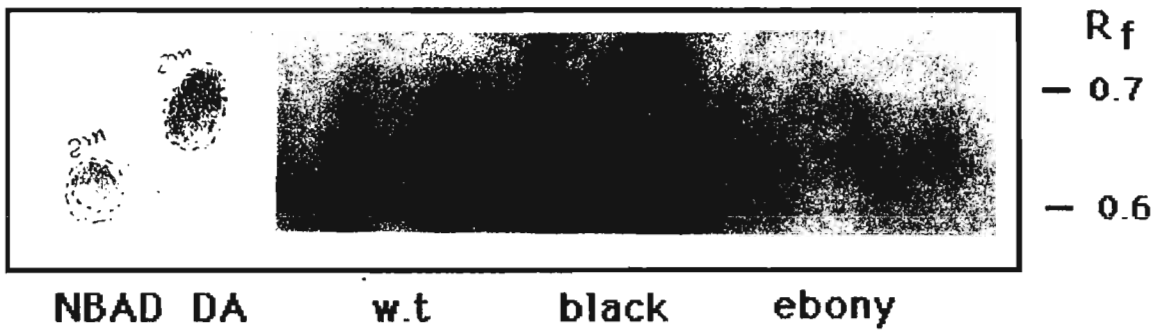

Figure 1. TLC analysis of β-alanyl dopamine synthesized by *Drosophila* extracts (duplicates). Ninhidrin-visualized standards = DA: Dopamine; NBAD: β-alanyl dopamine. Rf: relative mobility in solvent I.

Table 1. Activity of NBAD synthase in wild type and melanic mutants

INSECTS	Specific activity ($pmol.min^{-1}.mg^{-1}$)
Drosophila melanogaster	
- wild type (pupae)	3.26
- wild type (adults)[a]	4.96
- black (pupae)	5.18
- ebony (pupae)	0.56
- ebony (adults)[a]	0.00
Ceratitis capitata (pupae)	
- Arg 17 (wild type)	29.00
- Dark pupa	36.26
- niger	0.72

(a) just emerged exarate adults

that, as expected, the synthase was not affected. Table 1 also shows that the extract of the *Drosophila* mutant *ebony* was unable to synthesize NBAD, thus behaving as the extract of the NBAD synthase-deficient *niger* of *Ceratitis* (Table 1; see also Figure 1). This result represents a direct confirmation of the accepted theory postulating, on the basis of indirect data and unpublished results, that the *ebony*$^+$ locus encodes NBAD synthase (Wright, 1987; Hopkins and Kramer, 1992). It is noteworthy that similar levels of ß-alanine were found in w.t. and in *ebony* pre-pupae as well as in *Ceratitis* w.t. and *niger* prepupae (not shown). Moreover, the enzymatic activities hydrolyzing NBAD to give ß-alanine and dopamine were measured and no differences were found between wild-types and melanic mutants, thus discarding an enhanced hydrolysis of NBAD as explanation for the apparent lack of NBAD synthesis in *ebony* and *niger* mutants (not shown).

From the above data, it can be concluded that the *ebony* mutant of *Drosophila* and the *niger* mutant of *Ceratitis* are deficient for the activity of the NBAD-synthase and therefore seem to be homologous mutants.

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F. Manso, and L.A. Quesada-Allué 1996b, J. Insect Physiol. 42:455-461; Wappner, P., K. Kramer, J.L. Hopkins, M. Merritt, J. Shaefer, and L.A. Quesada-Allué 1995, Insect Biochem. Mol. Biol. 25:365-374; Wappner, P., F. Manso, J. Cladera, and L.A. Quesada-Allué 1991, Dros. Inf. Serv. 70:233; Wappner, P., and L.A. Quesada-Allué 1996a, Insect Biochem. Mol. Biol. 26:585-592; Wright, T.R.F., 1987, Adv. Genet. 24:127-222.

Costas, J.[1], **E. Valade**[1], **and H. Naveira**[2]. [1] Dpto. Bioloxia Fundamental, Facultade de Bioloxia, Universidade de Santiago de Compostela, E-15703 A Coruna, Spain. [2] Dpto. Bioloxia Celular e Molecular, Facultade de Ciencias, Universidade de A Coruna, E-15071 A Coruna, Spain. A preliminary study on the relationship between the blood transposable element and the *Drosophila* genome.

Genetic and molecular studies of the coevolution between transposable elements and the host genome can provide important clues for the elucidation of gene regulation, identifying new loci involved in different aspects of gene expression, from chromatin insulation (*Su(Hw)*, Gerasimova *et al.*, 1995) to RNA stability (*su(f)*, Mitchelson *et al.*, 1993).

In this context, we have initiated an analysis of the relationship between the blood transposable element and the *Drosophila* genome. blood was first described as an insertion into the *white* gene of *Drosophila melanogaster* (Bingham and Chapman, 1986). This mutant, called *white-blood* (w^{bl}), has been included in several studies of modifier genes of the white locus: w^{bl} is partially suppressed by *Inr-a* (Rabinow *et al.*, 1991) and *Mow* (Bhadra and Birchler, 1996), partially enhanced by *Wow* (Birchler *et al.*, 1994) and *Lip* (Csink *et al.*, 1994b) and not affected by *Doa* (Rabinow and Birchler, 1989), $E(w)^a$ (Birchler and Hiebert, 1989), *Msu* (Csink *et al.*, 1994a) and *mw* (Birchler *et al.*, 1989). Nevertheless, a direct interaction between the modifier gene and the blood transposable element has only been demonstrated in the case of *Lip*, which modifies the total transcript abundance of blood.

We tried to search for another modifier of w^{bl} in order to find genes that interact with the blood element. We chose our candidate genes among that than meet these two requirements: i) they modify spontaneous mutations at other loci, but ii) have not been characterized at the molecular level. The selected genes were: $su(t)^1$, $e(dp^v)$, $Su(ss)^2$ and $su(pr)^B$ (Lindsley and Zimm, 1992). We also included $Su(Hw)^3$ because of the proposal of the existence of two potential *Su(Hw)* binding sites in the blood element (Wilanowski *et al.*, 1995).

All of these genes are on the third chromosome. So, males bearing the putative modifier gene were crossed with w^{bl} females over the TM3 *Sb Sr e* balancer chromosome. The F1 males (heterozygous for the tested gene) were screening for modification of the w^{bl} phenotype. The F2 generation allowed us to test for modification of the w^{bl} phenotype in homozygous condition of the tested gene.

w^{bl} is a temperature sensitive allele and the critical period for the w^{bl} eye phenotype is during the first half of pupal development (Ephrussi and Herold, 1945). So, pupae were subjected to two different developmental temperatures during these tests, 24° and 28°C.

No evidence of modification was found in any of these cases, suggesting that these genes do not interact with blood.

When you begin to search for coevolution between a transposable element and the host genome, you must take into account the possibility of a recent horizontal transfer, thus reducing the coevolution period (Kidwell, 1993). The importance of coevolution is clearly shown in the case of the copia transposable element. A transgenic copia element shows 100-700 fold increased expression in cell lines derived from *D. hydei*, which lack copia elements, relative to *D. melanogaster* cells (Cavarec *et al.*, 1994). These data have been interpreted as a result of coevolution between copia and the *D. melanogaster* genome to limit the mutagenic potential of copia.

Using the blood sequence submitted to the Genbank data base by the Berkeley Genome Group (AC: L49394) we designed a PCR to search for the presence of the blood element within the genome of the species of the *D. simulans* complex (*D. simulans*, *D. mauritiana* and *D. sechellia*). We amplified around 800bp comprising the integrase domain of blood in each of these species, using primers:

CAAAAGCCGGAATGCATAAAA and TCTGGGTAGTCTGCCAAATACT.

The comparison of the sequences we obtained revealed eight variable sites. In each site, only one of the species was different from the others. In four cases, *D. melanogaster* was the different one, in two cases, *D. mauritiana*, and in one case each, *D. simulans* and *D. sechellia*.

These data confirm the presence of blood within the genome of the four species of the *D. melanogaster* complex. Besides, they suggest vertical transfer of blood between these species. blood was present in the genome of the common ancestor of these species, thus allowing enough time to expect coevolution between blood and the *D. melanogaster* genome.