In situ hybridization of DNA of the *Su(UR)ES* gene in polytene chromosomes of several species of Dipterans.

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The *Su(UR)ES* (Suppressor of DNA underreplication in polytene chromosomes) gene controls degree of DNA underreplication in both intercalary and pericentric heterochromatin (Belyaeva *et al.*, 1998). When it is mutant, DNA underreplication is suppressed: the polytene chromosomes do not show weak points (constrictions) and ectopic pairing in the intercalary heterochromatin regions. In regions of pericentric heterochromatin, new banded regions appear due to suppression of underreplication. To determine localization of this gene in several other species of *Drosophila*, *in situ* hybridization experiments were performed with salivary gland polytene chromosomes of *Drosophila erecta, D. simulans* (species close to *D. melanogaster*), *D. virilis* (remoted species of *D. melanogaster*), and with nurse cell polytene chromosomes from *Anopheles gambiae*. Salivary gland chromosomes of *D. melanogaster* (Oregon R) were used as a control.

A 3.8 kb fragment of cDNA containing full copy of the *Su(UR)ES* gene was used as a probe (details of cloning and characterization of the gene are in I.V. Makunin, in preparation). The biotin labelled DNA (Gibco-BRL Bio-nick Labelling Kit) in 10% dextran sulfate / 50% formamide was incubated overnight with chromosomes of *Drosophila melanogaster, D. erecta,* and *D. simulans* at 42° C, and with chromosomes of *D. virilis* and *A. gambiae* at 37°C. The washing temperature was set respectively. The signals of hybridization were detected with alkaline phosphotase / NBT + BCIP system (Gibco-BRL Detection Kit).

Only polytene chromosomes of *D. melanogaster, D. erecta* and *D. simulans* showed strong signal in the site of the *Su(UR)ES* localization (68A in *3L* chromosome). Data obtained show that there is no total homology of the *D. melanogaster Su(UR)ES* gene with DNA of remote species; however, this does not exclude possible homology of fragments of the gene. In one of the experiments, 5-year-old squashes of *D. melanogaster* chromosomes were used for the control. Despite the fact that they had been kept for such a long time, they show the signal insignificantly less strongly than those of fresh preparations.

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Back Rows in the Ecological Theatre: different distributions of *Drosophila* in *Opuntia ficus-indica* plantations.

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The prickly pear cactus *Opuntia ficus-indica* was introduced in Europe a few centuries ago. Ecological research has been focussed on the association of *Drosophila buzzatii* with the cladodes of its original host plant *Opuntia* (Fontdevila *et al.*, 1981; Ruiz *et al.*, 1986). We studied *Drosophila* communities breeding in fruits of the cactus *O. ficus-indica* in an area with desert climate in southeast Spain, near Carboneras (37°00'N;1°53'W). The edible and healthy fruit of *O. ficus-indica* is available during the late summer and autumn. The glucose content of fresh fruits is much higher than of green cladodes of *O. ficus-indica*, 23.86% and 3.42% of the dry weight (9%) respectively (Fogleman and Abril, 1990), and once damaged, prickly pears decay readily. *D. buzzatii* is present all year, but as soon as the cactus fruits ripen temporary assemblages of *D. melanogaster*, *D. simulans*, *D. buzzatii*

Table 1. Numbers of *D. melanogaster*, *D. simulans*, *D. buzzatii*, and *D. hydei* captured in banana traps at four sites within the neglected *O. ficus-indica* plantation "Rambla" during five days (19 – 23 September, 1995).

Sites	*Drosophila* species							
	D. melanogaster		*D. simulans*		*D. buzzatii*		*D. hydei*	
	♂	♀	♂	♀	♂	♀	♂	♀
Front Row (A)	68	60	130	102	298	246	1	0
Third Row (B)	61	63	53	58	361	259	0	0
Back Row (C)	7	6	58	87	203	87	0	0
Back Row (D)	4	1	83	146	151	65	0	0
Totals	140	130	324	393	1013	657	1	0
% Species	10.2		27.0		62.8		0.0	

Table 2. Species frequencies and numbers of flies (n), species ratios of various *Drosophila* species, emerging from different numbers of *Opuntia ficus-indica* fruits exposed in the field from 16 (6 p.m.) to 23 (12 a.m.) September 1995, in different locations and sites within the locations.

Population Tray position	Fruit weight g ± SD	Total no. of flies (no. of fruits)	*D. mel.* (n)	*D. sim.* (n)	*D. buz.* (n)	*D. hyd.* (n)	*D. mel. / D. sim.*
"Rambla"	61.0 ± 13.3	7791	0.241	0.703	0.033	0.023	0.343
Front row	66.0 ± 13.4	3376	0.374	0.551	0.022	0.054	0.678
		(18)	(1261)	(1859)	(74)	(182)	
Back row	55.9 ± 11.6	4415	0.140	0.819	0.041	0	0.171
		(46)	(618)	(3616)	(181)	(0)	
"Plantation"	58.8 ± 15.0	2700	0.373	0.549	0.035	0.042	0.679
Front row	64.3 ± 15.1	1207	0.442	0.424	0.039	0.094	1.043
		(10)	(534)	(512)	(47)	(114)	
Back row	53.8 ± 13.5	1493	0.317	0.650	0.033	0	0.487
		(12)	(473)	(971)	(49)	(0)	
"El Algarrobico"	53.3 ± 11.2	1444	0.235	0.413	0.141	0.211	0.570
		(12)	(340)	(596)	(204)	(304)	

and *D. hydei* build up quickly. Typical frugivorous species *D. melanogaster* and *D. simulans* develop much faster than *D. buzzatii* and *D. hydei* in *O. ficus-indica* fruits (approximately 11 days against 18 days). We placed prickly pear baits in two theatre-like locations with rows of cactuses in semi-abandoned plantations of *O. ficus-indica* on the slopes of the dry ravines, called ramblas, which are enclosed on three sides by steep hills. The "Rambla" was a neglected *O. ficus-indica* plantation with many bramble-bushes in a wide ravine (Ruiz *et al.*, 1986). The "Plantation" was a tidy *O. ficus-indica* plantation at the banks of the dry river bed of Rio Carboneras. A third location was completely

different, a parking place near the beach, mainly deserted in September. The main plants at this location were *Algabarro* trees, one big cactus and a decaying *Agave*.

Samples of flies present in the area were captured in traps consisting of mashed bananas for five days in September 1995. Traps were placed at several sites in the "Rambla" and emptied at dusk and dawn (Table 1). The ratio of *D. melanogaster* relative to *D. simulans* was 0.377, which was similar to the ratio determined by the numbers of flies that emerged from the fruits. *D. buzzatii* formed by far the majority of the captured flies of which apparently more males than females were attracted, but *D. buzzatii* is not dependent on the cactus fruits and emerge mainly from rotting cladodes. Only one specimen of *D. hydei* was caught with banana traps (see also Atkinson, 1977; Nunney, 1990). Very few *D. melanogaster* were attracted to the traps in the Back Row. Sampling errors can be caused by the bait, but also by the length of the trapping period. Fermenting banana traps became gradually more attractive toward *D. melanogaster* (Eisses and Santos, 1997).

A sample of seventy-two prickly pears were weighed and placed on moist vermiculate in rings of polyvinylchloride (PVC; 7 cm in diameter), which at one side was closed with fine meshed gauze. Each plastic plant-breeding tray covered with vermiculate contained twelve rings with fruits. The trays were placed at different sites in the three locations. Six more trays were placed at different sites of the "Rambla": two in the Front Row (A), Third Row (B), and Back Row (C and D, i.e. left and right corner respectively). Additional 36 prickly pears were placed in six open boxes, which were placed in the vicinity of the trays. All fruits were recollected after 6 3/4 days. The PVC-rings with fruits were covered with a transparent 250 ml plastic beaker with a ventilation hole, stoppered with foam-rubber. The trays were transported to the laboratory in Barcelona and left at ambient temperature. Emerging flies were collected every day and examined for species and sex the following days. In contrast to earlier years we distinguished females of *D. melanogaster* and *D. simulans* (Eisses and Santos, 1998; Gallo, 1973).

Fruit - Fly interactions: *D. melanogaster*, *D. simulans* and *D. buzzatii* colonized damaged prickly pears from the first day of exposure in the field. *D. hydei* was attracted after 4 days (H. Laayouni, unpublished results), which was similar to its late colonization of decaying oranges (Nunney, 1990) and mashed bananas (Atkinson, 1977). Individual *O. ficus-indica* fruits were colonized in different numbers by the four species: *D. simulans* colonized 93.1 ± 5.0% of the fruits, *D. melanogaster* 80.1 ± 12.3%, *D. buzzatii* 37.5 ± 8.9%, and *D. hydei* 17.3 ± 7.2%. These differences in percentages of colonized fruits are also reflected in the clumping parameter k of the negative binominal distribution (Bliss, 1953) as referred to by Southwood (1971), because k changes the shape of the distribution of flies over the fruits. For *D. simulans* values of k ranged between 1.825 and 5.159 between various locations, sites and years, for *D. melanogaster* between 0.818 and 3.925, for *D. buzzatii* the value of k ranged between 0.074 and 0.366, and for *D. hydei* between 0.041 and 0.247. Relatively high values for k of *D. buzzatii* and *D. hydei*, 0.366 and 0.247 respectively, were obtained in the location "El Algarrobico", with one tray of fruits placed under an *Algabarro* tree and not much substrate available nearby.

Table 2 presents the grand totals of the fly emergences of the three locations, together with a subdivision in sites per location. Unfortunately, one tray with twelve fruits near a large decaying *Agave* had disappeared at the parking place. Large differences in relative species abundances can be noticed between and within the locations. Three types of community structures within relatively short distances might be identified: 1. Within tidy and more or less neglected *O. ficus-indica* plantations the frugivorous species ratio was different between the Front Row areas and Back Row areas further up the slopes of the ramblas, close to the precipitous mountain ridges. *D. melanogaster* was 1.4 times more abundant in the Front Row than in the Back Row of the "Plantation". A more extreme figure turned up in the neglected "Rambla" plantation: 2.7 times more abundant in the Front Row than in the Back Row, where also very few *D. melanogaster* were trapped (Table 1). These ratios were for *D.*

simulans 0.67 and 0.65 respectively. In the "Plantation" Front Row *D. melanogaster* was even more abundant than *D. simulans*; 2. *D. hydei* was absent in the Back Rows of the two plantations; 3. The area close to the beach with only a few cactus plants scattered around, differed from the plantations in the high percentages of emerging *D. buzzatii* and *D. hydei*: 14 and 21% respectively in the "El Algarrobico" fruits, compared with 2 to 4% in prickly pears placed in the plantations. A similar high emergence of *D. buzzatii* was observed in fruits collected in a Tunisian oasis, where the regular substrate in the form of decaying cladodes was lacking (Haouas *et al.*, 1984). The input of some prickly pears in an area which was otherwise poor in substrate, probably invited the species of the *repleta* group to stay longer at one particular spot or to be less fastidious.

D. buzzatii and *D. hydei* were aggregated in much smaller numbers of fruits than *D. melanogaster* and *D. simulans*, and they formed only a minor fraction (about 2% to 4%) of the total number of emerged flies per fruit placed in the two plantations. The species of the *repleta* group have much longer developmental times in *O. ficus-indica* fruits compared with the frugivorous species, which turns a relatively fast decaying fruit into an unfavourable resource for the *repleta* group species. In addition, competitive numbers of *D. melanogaster* and *D. simulans* and the presence of indirect "predators" such as fruit-eating birds and rabbits reduce the chances for slowly developing insects to complete development. The slow developing species do maintain in the community: *D. hydei* by colonizing relatively old fruits, and *D. buzzatii* has its main breeding site in rotting *O. ficus-indica* cladodes.

Differences in microgeography result in differences in microclimate. Within short distances, i.e. meters, real temperatures vary largely depending on plantation, soil structure, and landscape (Stoutjesdijk and Barkman, 1992). Allele and inversion frequencies fluctuate annually within natural populations of *D. melanogaster* (Muñoz-Serrano *et al.*, 1985; Sanchez-Refusta *et al.*, 1990). When polymorphic populations do show rapid changes in genetic composition with consistent macroclimatological factors (seasons), it is conceivable that interspecific differences can and will be affected by consistent microclimatological factors within short distance and time. The more as various life-cycle-stages of *D. melanogaster* and *D. simulans* are at the same time-scale as day-night cycles. However, there is a specific lack of information about temperature profiles over the day at various sites of the plantations, in the sun and in the shade of the cactuses, in the vicinity of the mountain ridges or at a distance. Close vicinity of the Back Rows to the mountain ridges possibly has a quenching effect on temperature variation over the day. Although explaining local species diversity seems to be a problem with too many solutions (Shorrocks, 1996), this study shows some consistent differences in *Drosophila* species distributions within short distances in the field. Microclimatic studies are necessary to unravel the relationships between local temperature profiles and species ratios, fly-sizes, oviposition and migration behaviour. Relative abundance of substrate might also influence species distributions.

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RAPD as genetic marker in taxonomic and evolutionary studies in the *Drosophila buzzatii* cluster.

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Introduction

Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic variability within and between species. One of these methods, the RAPD technique, requires only small amounts of DNA, is easy and rapid to perform, and exploits the use of universal primers (Williams *et. al.*, 1993). The great differentiation found with the use of different markers in the populations of the *Drosophila* species included in the *buzzatii* cluster (Baimai *et al.*, 1983; Tosi and Sene,1989; Silva and Sene, 1991; Tidon-Sklorz and Sene, 1995; Madi-Ravazzi, *et al*, 1997 and Lapenta *et al*, 1998) and the intriguing aspects of its speciation process led to the present study, elaborated mainly with the purpose of evaluating the taxonomic potential of the RAPD assay to discriminate among strains and species of *Drosophila*.

Material and Methods

Strains and geographic distribution: Three strains of *D. buzzatii*, two of *D. seriema*, two of *D. koepferae*, one of *D. serido* type B, and one of *D. serido* type D were used in the present study. As an outgroup, one strain of *D. mulleri* and one of *D. simulans* were utilized. Strains and geographical origins are shown in Table 1.

DNA extraction method: Genomic DNA was extracted from single individuals, randomly sampled from isofemale lines. Samples were stored with TE (10mM Tris, 1 mM EDTA, pH 8). Each fly was homogenized in a microtube with 160µl of cold solution I (10mM Tris, 5% sucrose, 60mM NaCl, 10 mM EDTA, pH 7.8). Then, 200 µl of solution II (300mM Tris, 5% sucrose, 1.25% SDS, 10 mM EDTA, pH 8) were added, the homogenate was mixed by inversion of the tube and the tube was placed in a water bath at 65°C for 30 min. A total of 60 µl of 3M sodium acetate was added, and the tube was cooled at -20°C for 20 min. After centrifugation for 15 min, the supernatant was transferred to an eppendorf and the sample centrifuged for another 15 min. To the pellet was added an equal volume of isopropanol at - 20°C; rinsing was with 70% ethanol. DNA was air dried overnight and resuspended in 100 µl of TE (10 mM Tris, 1mM EDTA, pH 8) and stored at - 20° C.