Variability and differentiation of genomic DNA in the *Drosophila melanogaster* populations of Russia and Ukraine.

Vaulin, O.V., T.Yu. Zharikov, L.I. Gunderina, and I.K. Zakharov. Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090, Russia; e-mail: Oleg.v.vaulin@mail.ru

The genetic diversity of cosmopolitan species including *Drosophila melanogaster* presents an unfailing interest for the genetics of populations and ecology studies. The level and character of genetic diversity of the populations are determined by the combination and correlation of such factors as mutation processes, selection, genetic drift, and migration. The purpose of this study is the investigation of genomic DNA differentiation in the natural populations of *Drosophila melanogaster* of Russia and Ukraine, and the characterization of temporal dynamics of genomic DNA diversity in Uman population (Ukraine). We used the RAPD-PCR and ISSR-PCR methods for the analysis of inter- and intra-population genomic DNA diversity (Williams *et al.,* 1990; Zietkiewicz *et al*., 1994). The RAPD-PCR method was used to estimate interpopulation genomic DNA diversity between the populations of *Drosophila melanogaster* from Russia and Ukraine (refer to Table 1 for the geographic coordinates of the populations). The flies were collected in the years 2002 (Izhevsk) and 2003 (other populations). 17-21 isofemale lines for each population under study were analyzed. A single fly from each line was

Table 1. Locations of *Drosophila melanogaster* collection.

Collection location	Designation of population	Geographic coordinates
Village of Chemal, Republic of Gorny Altai, Russia	Chemal	$51^0$26′N $86^0$00′E
City of Izhevsk, Republic of Udmurtia, Russia	Izhevsk	$56^0$50′N $53^0$10′E
Town of Zvenigorodka, Cherkassy region, Ukraine	Zvenigorodka	$49^0$5′N $30^0$53′E
Town of Uman, Cherkassy region, Ukraine	Uman	$48^0$46′N $30^0$14′E
Town of Nikopol, Dnepropetrovsk region, Ukraine	Nikopol	$47^0$35′N $34^0$24′E

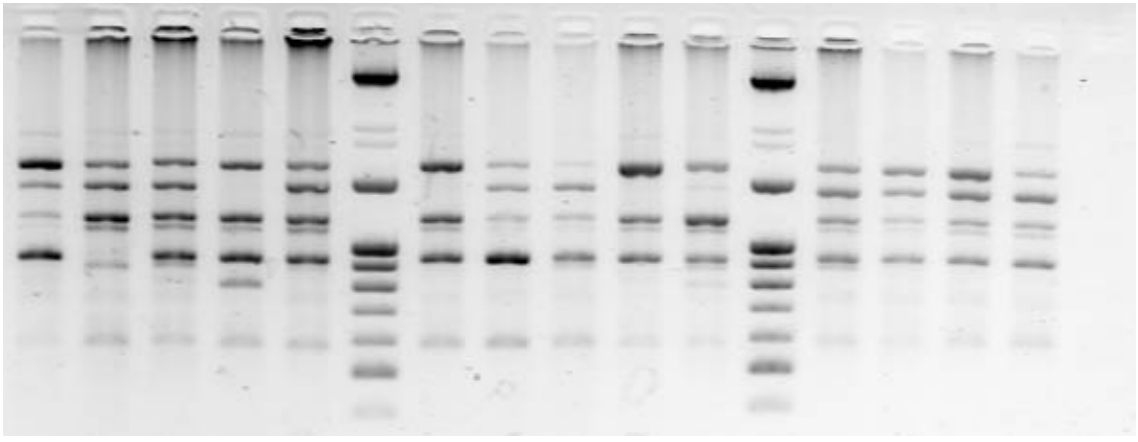

Figure 1. Electrophoretic pattern of *Drosophila melanogaster* DNA amplified by $(AG)_8G$ primer by ISSR-PCR method, Uman population: 1-3 – year 2004; 4, 5, 7, – year 1990; 8, 9 – year 2003; 10, 11 – year 1991; 13, 14 – year 1984; 15, 16 – year 1988; lanes № 6 and № 12 are the MW references λ *Hin*dIII and 100 bp+1.5 kb; lane № 17 – negative control.

Table 2. Polymorphism of genomic DNA amplified with primers P1, P2, and P3 in the studied *Drosophila melanogaster* natural populations.

Primer	Characters	Chemal 2003, N=20	Izhevsk, 2002, N=17	Zvenigorodka, 2003, N=20	Nikopol, 2003, N=21	Uman, 2003, N=20	Joint data for the samples, N=98
P1	N_{mean}	14.2±1.4	14.4±1.6	14.4±1.5	14.5±1.6	14.5±1.6	14.4±1.5
	N_{lim}	12-17	12-18	12-17	12-18	12-17	12-18
	∑N	22	23	22	23	23	28
	N_m	8	8	9	9	9	8
	P	0.500	0.536	0.464	0.464	0.500	0.714
	H	0.156	0.162	0.143	0.149	0.152	0.162
P2	N_{mean}	12.1±2.8	11.9±2.7	12.3±2.0	12.3±2.2	12.8±2.3	12.28±2.3
	N_{lim}	5-15	5-15	7-15	7-15	6-16	5-16
	∑N	16	16	16	16	17	17
	N_m	2	5	5	3	4	1
	P	0.845	0.688	0.688	0.813	0.765	0.941
	H	0.358	0.284	0.239	0.318	0.281	0.333
P3	N_{mean}	6.8±1.1	6.5±0.8	6.5±0.7	6.5±0.9	7.3±1.6	6.7±1.1
	N_{lim}	6-10	6-8	6-8	5-9	6-12	5-12
	∑N	11	10	11	13	12	17
	N_m	6	5	6	4	6	4
	P	0.455	0.500	0.455	0.692	0.500	0.765
	H	0.057	0.081	0.041	0.111	0.089	0.09
Overall data	N_{mean}	33.1±3.4	32.9±3.8	33.2±2.1	33.2±3.1	34.5± 3.6	33.4±3.2
	N_{lim}	26-39	24-39	29-37	28-39	28-40	24-40
	∑N	49	49	49	52	52	62
	N_m	16	18	19	16	19	13
	P	0.548	0.500	0.468	0.580	0.532	0.790
	H	0.195	0.182	0.152	0.195	0.179	0.202

Note: N_{mean} – mean number of DNA fraction per individual in the population, N_{lim} – minimal and maximal numbers of DNA fractions in the population, ∑N – total number of DNA fractions, N_m – number of monomorphic DNA fractions, P – proportion of polymorphic DNA fractions, H – mean heterozigosity.

Table 3. Genetic distances between the populations of *Drosophila melanogaster* estimated by RAPD-PCR markers.

Genetic distance measure	Population, year of collection	Izhevsk, 2002 г.	Zvenigorodka, 2003 г.	Nikopol, 2003 г.	Uman 2003 г.
D_{Nei} *	Chemal, 2003 г.	0.011	0.013	0.010	0.012
	Izhevsk, 2002 г.		0.007	0.013	0.021
	Zvenigorodka, 2003 г.			0.017	0.018
	Nikopol, 2003 г.				0.012
GD **	Chemal, 2003 г.	0.078	0.115	0.179	0.179
	Izhevsk, 2002 г.		0.115	0.135	0.164
	Zvenigorodka, 2003 г.			0.113	0.182
	Nikopol, 2003 г.				0.143

* (Nei, 1972)
** (Link *et al*., 1995)
Note: Mean interpopulation genetic distance according to D_{Nei} estimate is 0.017±0.008; according to GD estimate – 0.135±0.033.

Table 4. Genetic distance (GD) values (Link *et al*., 1995) between the samples of *Drosophila melanogaster* from Uman population collected in different years and estimated by ISSR-PCR markers.

Year of collection	1988	1990	1991	2003	2004
1984	0.077	0.111	0.000	0.080	0.040
1988		0.172	0.077	0.148	0.038
1990			0.111	0.179	0.143
1991				0.080	0.040
2003					0.115

Note: Mean GD is 0.094±0.052.

used for DNA isolation. The temporal dynamics of *Drosophila melanogaster* genomic DNA variability in Uman population over the years 1984, 1988, 1990, 1991, 2003, and 2004 was studied by ISSR-PCR. As many as 14-31 flies (each representing a single isofemale line) were studied for each year.

Three decanucleotide primers (CCCAGCTGTG (P1), CTCCCTGCAG (P2), and GACGGATCAG (P3)) were used for RAPD-PCR reaction. The amplified DNA fragments were fractionated in the agarose gels and UV images were taken (Figure 1). The amplification with the use of those three primers yielded the 62 amplicons, 13 out of which were monomorphic in all populations. The proportion of polymorphic loci (amplified fragments) in the populations varied between 0.468 (Zvenigorodka, 2003) and 0.580 (Nikopol, 2003), and the mean heterozygosity varied from 0.152 (Zvenigorodka, 2003) to 0.195 (Chemal, 2003, and Nikopol, 2003) (Table 2). The genetic distances between the population assayed by the methods of Nei (D_{Nei}) or Link *et al.* (1995), (GD) varied from 0.007 to 0.021, or from 0.078 to 0.179, respectively (Table 3). The obtained results demonstrated that the there were no direct correlations of the genetic and genetic distances between *Drosophila melanogaster* populations. The gene flow values calculated by G_{st} value (Slatkin and Barton, 1989) using POPGENE software package (Yeh *et al*., 1999) varied from 9.2 to 25.0, thus indicating a significant degree of genetic exchange between the populations regardless of the geographic distance between the populations.

The analysis of temporal dynamics of the genetic structure of the Uman population of *Drosophila melanogaster* was performed by the ISSR-PCR method (Zietkiewicz *et al*., 1994). Amplification was performed by three primers of the following sequences $(AG)_8G$, $(CT)_8A$, and $(CA)_8G$ annealing to the dinucleotide repeats termini. Those primers allowed us to amplify the total of 30 DNA fragments, 6 of which were monomorphic. The mean heterozygosity varied from 0.097 (1991) to 0.127 (1990) with the proportion of polymorphic loci varying from 0.333 (2003) to 0.633 (1990). The genetic distances D_{Nei} and GD characterizing the genomic DNA differentiation in the Uman population varied from 0.004 to 0.048 and from 0.000 to 0.179, respectively (GD values are presented in Table 4). According to those two measures of genetic differentiation, the highest similarity of the genetic structure of population was shown to be in the years 1984 and 1991. The obtained results indicate stability of genetic structure of the Uman population of *Drosophila melanogaster* over the period of 1984-2004.

The estimates of genomic DNA polymorphism and differentiation of natural populations of *Drosophila melanogaster* from Russia and Ukraine were similar to that observed in other Insecta species, such as *Drosophila buzzatii, Aedes aegypti, Anticarsia gemmatalis,* and *Chironomus* species (Laayouni *et al.*, 2000; Apostol *et al.*, 1996; Sosa-Gomez, 2004, Gunderina *et al*., 2005).

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Yeh, F.C., R. Yang, and T. Boyle 1999, POPGENE. Version 1.31, University of Alberta and Centre for International Forestry Research; Zietkiewicz, E., A. Rafalski, and D. Labuda 1994, Genomics 20: 176-183.

Anecdotal example of a newly-eclosed female caught in copulation.

Hallman, Clayton. Department of Zoology, University of Oklahoma, Norman, OK 73019.

During routine collection of virgin female *D. melanogaster*, a recently eclosed female was found in copulation with a more mature male (Figure 1). All of the standard indicators used to distinguish a very young fly from all others are clearly present: very light cuticle color, elongated body, and the meconium spot.

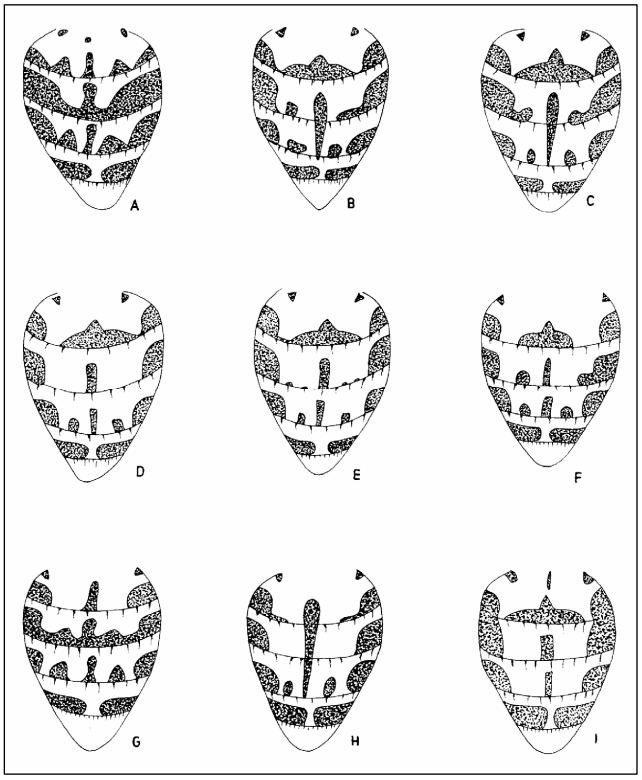
Figure 1.

Variation in abdominal pigmentation pattern of *Leucophenga angulata* Singh, Dash and Fartyal from Kumaon region, India.

Upadhyay, Kamla, and B.K. Singh. Cytogenetics Laboratory, Department of Zoology, Kumaon University, Nainital-263002, India. e-mail: kamla_zoo@yahoo.com

Sturtevant (1919) discovered that *Drosophila melanogaster* has a closely resembling sibling species, *Drosophila simulans*. Both the species are cosmopolitan and coexistent (Lachaise *et al*., 1988). The two species are distinguished by checking the male offspring of isofemale lines, because of different genital arches (Coyne, 1983; Sorrocks, 1972). It is possible to make a distinction between the females of *Drosophila melanogaster* and *Drosophila simulans* based on the measurements of eye sizes (Burla, 1951; Gallo, 1973; Mc Namee and Dytham, 1993), but it is a laborious job where a large number of flies are involved. Based on a paper by Eisses and Santos (1997), we decided to examine the abdominal pigmentation pattern of *Leucophenga angulata,* which is a new species described by Singh, Dash and Fartyal (2002) from Paharpani in Nainital district of Kumaon region, India.

The Kumaon region includes six border districts of the state Uttaranchal, *viz*., Nainital, Almora, Pithoragarh, Bageshwar, Champawat and Udham Singh Nagar and located at an elevation of just below 2000m. The area is characterized by having dense evergreen coniferous forest with medium to very steep slopes and extremely moist condition due to heavy rainfall. The flies were collected from several collecting stations *viz*., Dunagiri, Dwarahat and Chaukhutia in Almora district,



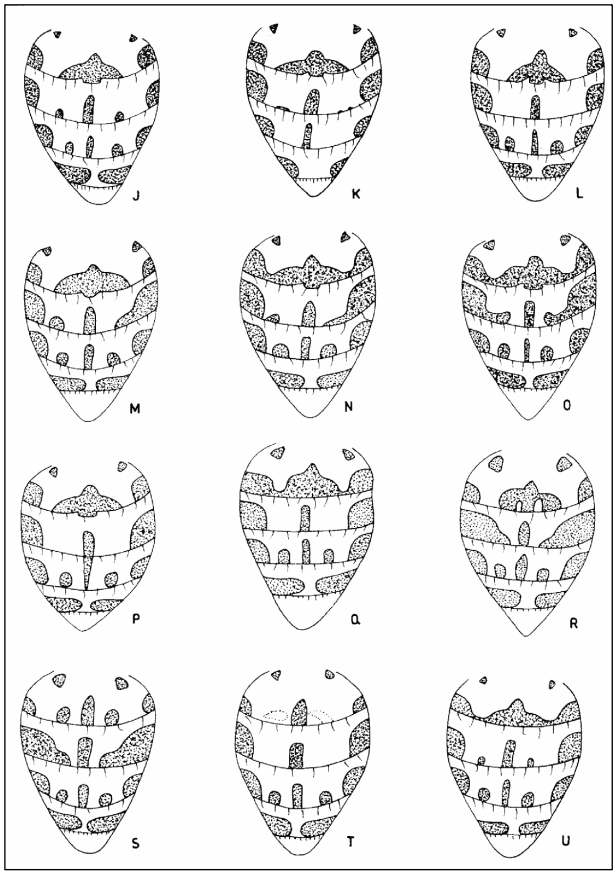
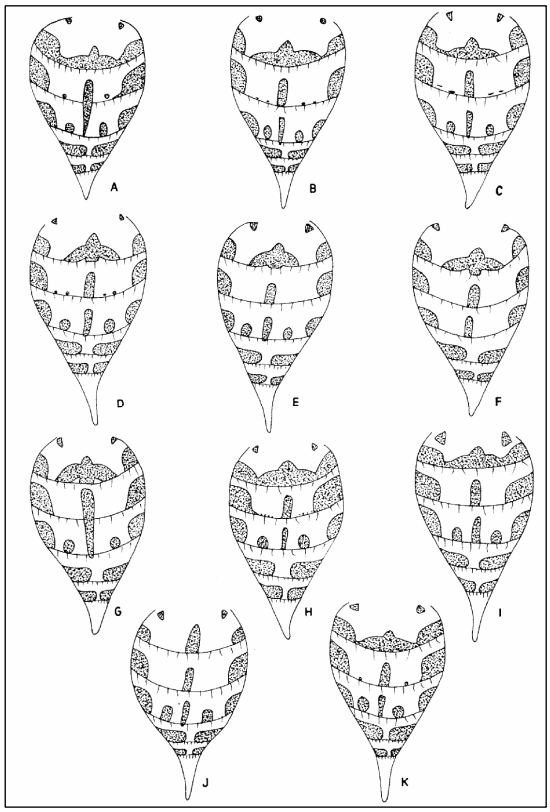


Figure 1 (above), Figure 2 (right), Figure 3 (below).



Amsyari, Kausani and Pinath in Bageshwar district and Gangolihat in Pithoragarh district, and different methods were employed to collect Drosophilid flies like (i) sweeping through undergrowth or above debris on the forest floor; (ii) sweeping above rotting native fruits and artificially yeasted fruit baits and (iii) from fungi growing on decaying logs. About 650 flies of Drosophilidae were collected out of which *Leucophenga angulata* was predominant on all collecting stations. Males and females of this species were separated and examined for abdominal pigmentation pattern of 1st, 2nd, 3rd, 4th, 5th, and 6th abdominal segments. A total of 21 types of abdominal pigmentation patterns were observed in males (Figure 1 and Figure 2; A-U), and 11 types of abdominal pigmentation patterns were observed in females (Figure 3; A-K).

Looking into the types of abdominal pigmentation pattern variation in *Leucophenga angulata* males and females, it is interesting to note that no other Indian *Leucophenga* species has this much abdominal pigmentation pattern variation.

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Hyperexpression of the C subunit of CCAAT-box binding factor NF-Y specifically blocks *ci* transcription in the *Drosophila* wing imaginal disc.

Ramos, Rosio, and Jym Mohler. Barnard College, Department of Biological Sciences, 3009 Broadway, New York, NY 10027 USA; Author for correspondence: Jym Mohler; e-mail: jmohler@barnard.edu

Introduction

The CCAAT box was originally identified as a common structural motif in many eucaryotic RNA PolII promoters (Bucher, 1990) and is commonly located 60 to 100 bp upstream of the major start site of transcription. Transcription factors that bind these sequences, termed "CCAAT binding factors", also interact with the TFIID initiation complex to stabilize its interaction with the core promoter. Of the three principal classes of CCAAT binding factors that have been identified, only the recognition sequence of NF-Y matches the CCAAT consensus found upstream of the core promoter (Bi *et al*., 1997), indicating that NF-Y has the potential to interact with every promoter CCAAT box and that NF-Y is the factor that functions consistently through this element. NF-Y is a trimeric DNA binding protein (Sinha *et al*., 1995; Ronchi *et al.,* 1995). The B and C subunits of NF-Y (NF-YB and NF-YC, respectively) fold to form a tight dimer and A subunit (NF-YA) associates with this dimer forming the trimeric protein, which has the potential to bind DNA.

CCAAT boxes can be found in a substantial proportion of promoters from most eucaryotic organisms (Mantovani, 1998). An exception is *Drosophila*, whose promoters are virtually devoid of this element. Despite this apparent paucity of CCAAT boxes, *Drosophila* retains sequences encoding conserved versions of each of the three NF-Y subunits (*CG3891* = NF-YA, *CG10447* = NF-YB, *CG3075* = NF-YC, Adams *et al.,* 2000), suggesting some conserved role for the NF-Y protein in *Drosophila*. In addition to the conversed *nf-yc* gene, *Drosophila* also has a divergent version of NF-YC, *mes4*, which is involved in regulating mesoderm-specific transcription and has been hypothesized to form a mesoderm-specific NF-Y factor (Stathopoulos *et al*., 2002).

To investigate the role of the NF-Y factor and specifically of its conserved NF-YC subunit, we overexpressed the *nf-yc* gene in the wing imaginal disc of the fly. The *nf-yc* coding region, including the 5' leader and 483 bp of 3' trailer, was removed as a 2.4 kb EcoRI-BglII fragment from BDGP EST clone, SD06336, and inserted into the pUAST transformation vector to generate the *nf-yc* UAS expression construct. A single transformant located on the X-chromosome was recovered and used for the analysis.

Results and Discussion

nf-yc was expressed strictly in the wing pouch using two Gal4 driver lines: MS1096, which expresses predominantly in the dorsal half of wing pouch as well as the most ventral region, and 71B, which expresses most strongly close to the anterior-posterior midline of the wing pouch region. Both drivers are not active during most of larval development and become active only during the late third instar after growth of the disc is complete.

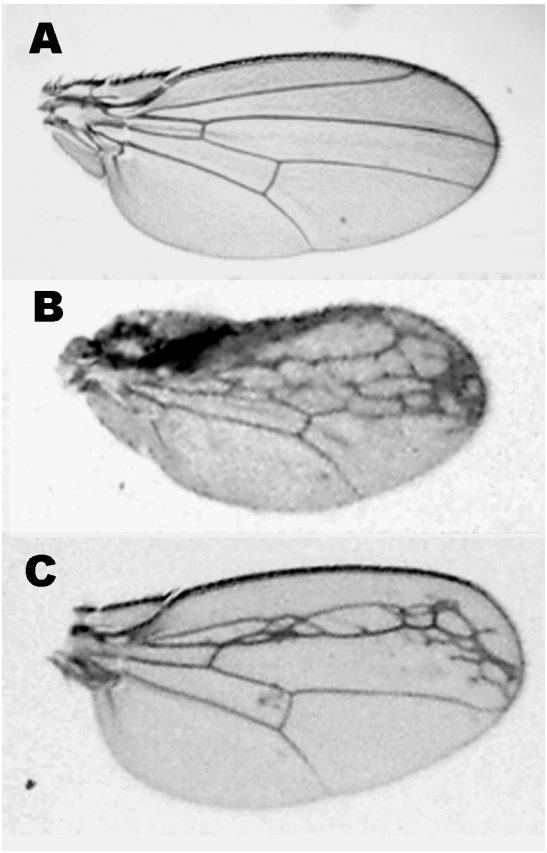

Figure 1. Venation pattern in wings with *nf-yc* overexpression, from specific Gal4 expression lines. (A) wild-type, (B) MS1096::*nf-yc*, (C) 71B::*nf-yc*.

Expression of *nf-yc* with either of these drivers results in gross disruption of vein patterning in the anterior compartment of the wing but leaves venation in the posterior compartment essentially unchanged (Figure 1). When *nf-yc* is driven by MS1096, the entire anterior compartment is a plexus of veins and the overall size of the wing is slightly reduced (Figure 1B). When *nf-yc* is driven by 71B, the central region of the anterior compartment, essentially that portion of the wing that would normally be between veins 2 and 3, is a plexus of veins without clearly defined longitudinal veins that would correspond to either veins 2 or 3 (Figure 1C).

Because *nf-yc* overexpression affected only the patterning of the anterior wing, the genes whose transcription was affected must be active in a process specific to the anterior compartment. Hh-signalling is a process limited to the anterior compartment of the wing, because the receptor Ptc is expressed only in the anterior compartment (see reviews by Aza-Blanc, 1999; Vervoort, 2000). Disruption of Hh-signalling has a profound and well-characterized effect on vein patterning. Therefore, we examined the effect of Gal4-driven *nf-yc* expression on known targets of Hh-signalling in the wing.

Overexpression of *nf-yc* had a profound effect on the expression of all known targets of Hh-signalling (Figure 2). *ptc* (Figure 2G-I), *dpp* (Figure 2J-L), and *vn* (Figure 2M-O) all exhibited ectopic expression within the anterior compartment in response to *nf-yc* overexpression. With the MS1096 Gal4 driver, ectopic expression was observed in much of the dorsal wing pouch and to a lesser extent in the ventral region. With the 71B Gal4 driver, ectopic expression of these three genes was in a region just anterior to the normal expression domain along the anterior-posterior compartment boundary. Both regions of ectopic expression correspond to the domains in which Gal4 is activated to the highest levels in these lines. In contrast, the target gene *kn* was inactivated in its normal domain along the anterior-posterior boundary in response to MS1096-driven *nf-yc* expression (Figure 2P-Q).

These alterations in the expression patterns of most of the Hh-target genes can be accounted for by ectopic expression of the Hh signal. Overexpression of *nf-yc* causes ectopic transcription of the *hh* gene in the anterior compartment of the wing in patterns that mimic the altered patterns of expression of the Hh-target genes (Figure 2D-F). The altered expression of *hh* itself can be

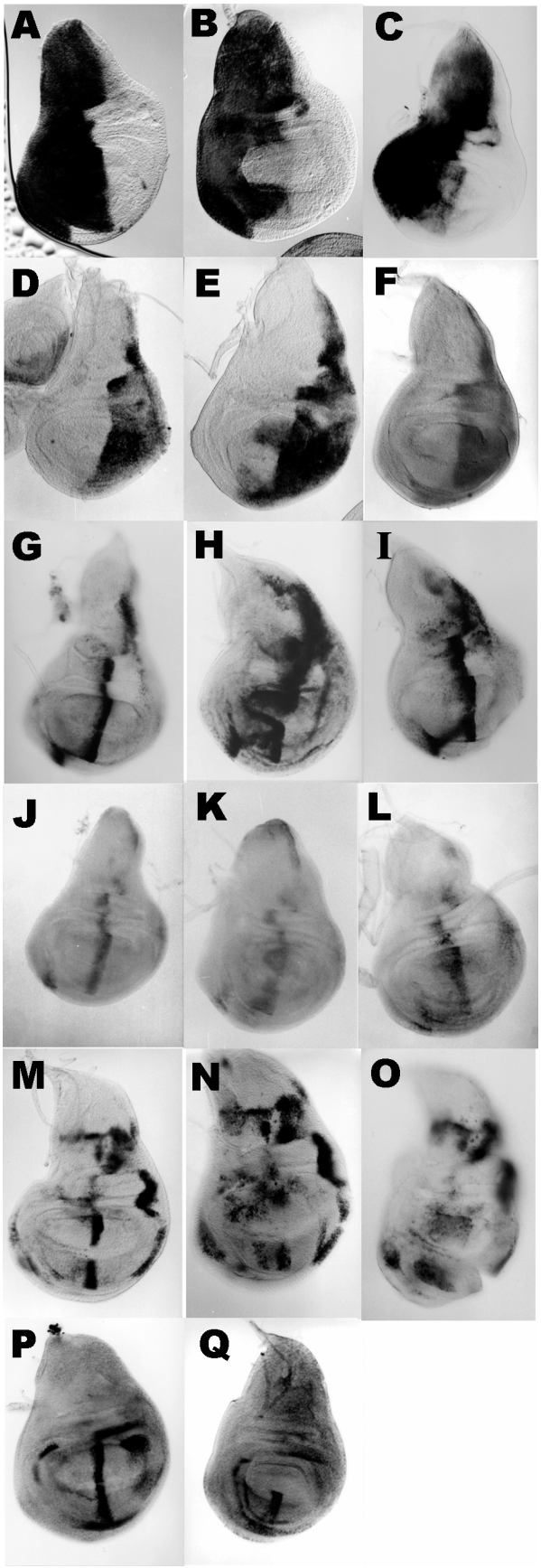

Figure 2. Target gene expression in late third instar wing imaginal discs in which *nf-yc* has been driven from specific Gal4 lines as detected by *in situ* hybridization. Gal4 lines include MS1096 (middle column), which drives expression strongly in the dorsal wing pouch and more weakly in the ventral wing pouch, and 71B (right column), which drives expression most strong in medial portions of the wing pouch. (A-C) *ci*, (D-F) *hh*, (G-I) *ptc*, (J-L) *dpp*, (M-O) *vn* and (P-Q) *kn* transcript distribution. (A, D, G, J, M, P) wild-type, (B, E, H, K, N, Q) MS1096::*nf-yc* and (C, F, I, L, O) 71B::*nf-yc*.

accounted for by lower expression of the anterior compartment selector gene *ci*, which represses *hh* transcription in the anterior compartment. In regions of the wing pouch where Gal4 drives high levels of *nf-yc*, transcription of *ci* is notably reduced though rarely absent (Figure 2A-C). In contrast, overexpression of *nf-yc* driven by either of these two drivers had no effect on the pattern of expression of the posterior compartment selector gene, *en*, (not shown) indicating that the effect of overexpression of *nf-yc* is specific to levels of *ci* transcription within the anterior compartment.

Unlike most other *Drosophila* genes, *ci* does have CAATT boxes associated with its promoter. Within the 1.4 kb region that includes the characterized *ci* promoter are 6 CCAAT boxes; there are no other CCAAT boxes in the DNA 5 kb upstream or downstream from the promoter region. Two of these are located in proper position with respect to one of two TATA boxes (90 and 55 bp upstream) in the promoter region, although neither of these TATA boxes corresponds to start point of transcription mapped by Schwartz *et al*. (1995) by primer extension. While the exact significance of this clustering of CCAAT boxes in the *ci* promoter region is unclear, it does suggest that the effect of *nf-yc* overexpression on *ci* transcription is most likely direct.

The effect of *nf-yc* overexpression on wing patterning may be all ascribed to the lowered expression of *ci*. The activation of most of the Hh-target genes can be accounted for by the lowered *ci* expression, resulting in insufficient Ci-repressor to

suppress *hh* activation. The ectopic Hh signal would then activate its target genes in regions adjacent to the region of *nf-yc* expression or where levels of Ci-activator remain sufficient. Consistent with this is the observation that expression of the target genes is often most robust at the margin of the region of ectopic expression (compare the expression of *ptc* in Figure 2H with that of *hh* in Figure 2E), where cells unaffected by *nf-yc* expression may respond to Hh signal expressed from adjacent tissue. An apparent exception is *kn*, which is apparently inactivated rather than activated by *nf-yc* and ectopic Hh expression; however, because *kn* requires the highest levels of Ci-activator of all Hh-targets its expression may be more sensitive to the loss of Ci-activator than the other Hh-target genes. It is highly likely, therefore, that the effect of *nf-yc* expression in the wing disc is mediated solely through *ci*. This argues that NF-Y may have specific roles, not a general one, in the regulation of gene transcription in *Drosophila*.

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Molecular investigation of *white* - like mutants in *Drosophila willistoni.*

D'Ávila, M.F., E.L.S. Loreto, F.P. Torres, R.N. Garcia, and V.L.S. Valente. Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Bloco B, Prédio 43323, Caixa Postal 15053. CEP 91501-970. Porto Alegre, RS, Brazil. E-mail: vera.gaiesky@ufrgs.br.

Drosophila willistoni is one of the most abundant species in Neotropical fly communities. The species raises special interest because of its great ecological versatility expressed by the wide ability to explore several environment types (Da Cunha *et al.,* 1950, 1959; Da Cunha and Dobzhansky, 1954; Dobzhansky and Powell, 1975; Valente *et al.,* 1989, 1993; Santos and Valente, 1990; Valiati and Valente, 1997; Goñi *et al.,* 1997, 1998), and substrates (Carson, 1965; Valente and Araújo, 1986). Besides its ecological opportunism, *D. willistoni* also presents high genetic variability detected at the levels of chromosomal and enzymatic polymorphisms. The polymorphism for paracentric inversions in *Drosophila,* and in other Diptera, is clearly adaptive (reviews in Krimbas and Powell, 1992). More recent evidence points to an important role of the transposable elements in the genetic variability of the organisms, being considered as *materia-prima* for evolution (Kidwell and Lisch, 1997). Sometimes, the mobilization of transposable elements in *D. melanogaster* can result in alterations as, for instance, changes in eye color (review in Lindsley and Zimm, 1992) and hybrid dysgenesis (Kidwell and Kidwell, 1976; Petrov *et al.,* 1995). The objective of this study was to investigate putative sources of variability in the genome of *D. willistoni*, starting with a spontaneous *white*-like mutant that appeared in the 17A2 strain. Such types of mutants generated by transposable elements were described for *D. melanogaster* (review in Lindsley and Zimm, 1992) and

for *D. simulans* (Torres *et al*., 2006).

The fly stocks were maintained by mass mating in culture medium described by Marques *et al.* (1966) in chambers with controlled temperature and humidity: 17°C ± 1°C and 25°C ± 1°C and 60% R.H.. Two populations were used: 1) *D. willistoni* strain 17A2 with white-type phenotype and another with wild phenotype; 2) *D. simulans* ELD strain with wild phenotype and a *white* strain (control in the molecular biology experiments). Cytological analyses of polytene chromosomes in third instar larvae of *D. willistoni* strains were made applying the protocol described by Ashburner (1967) with the aid of photomicrographs.

Genomic DNAs of strains used were prepared from adults (approximately 100 flies) according to the protocol described by Jowett (1986). DNA samples were digested with the appropriate restriction enzymes following the manufacturer's directions (Invitrogen). The enzymes employed were *Eco*RI, *Bgl*II and *Sst*I, according to the restriction map of the *D. simulans white* locus (Inoue and Yamamoto, 1987; Inoue *et al.*, 1988). DNA fragments were separated by electrophoresis on 1% agarose gels and transferred to nylon membranes (Hybond N+/Amersham Biosciences). Fragment sizes, in kilobase pairs (kb), are indicated by 1 kb Plus DNA Ladder (Invitrogen). The membranes were hybridized to a random primer-labeled probe at 60°C in 5× SSC; 0.1% SDS; 5% dextran sulfate, and 20-fold dilution of liquid block. The filters were washed twice with 0.2× SSC and 0.5% SDS for 15 min at 60°C. Hybridization and detection were performed using the Gene Images Kit (GE Healthcare) according to the manufacturer's instructions. The pCaSpeR-hs plasmid (GenBank U59056) that has the *D. melanogaster* mini-*white* gene (Thummel and Pirrota, 1992) was used as probe for hybridization. RNA extraction of *D. willistoni* followed the protocol by Trizol™ (Invitrogen), and cDNA was obtained through the protocol as defined in the BD SMART™ cDNA synthesis kit (BD Biosciences). Both procedures were carried out according the manufacturers' specifications. Three pairs of oligonucleotide primers for the *white* locus were used for the PCR analyses: 1) WS-5' TTGGGGTGGTGATTGGTT 3' and 5' AGCAAGATACGCACGCAG 3' (Torres *et al.,* 2006), which the expected length of amplification for *D. simulans* is 721 bp. 2) WT-5' CGGTGAGTTTCTATTCGCAA 3' and 5' CGGATTGTAGTTGGTGGGAC 3' (Torres *et al.,* 2006) producing an amplified fragment with expected length of 800 bp in *D. simulans*. 3) A direct WS sequence with the reverse WT sequence to amplify a higher fragment of the locus.

Afterwards, another pair of degenerated oligonucleotide primers was drawn using Oligo 5.0, starting from the conserved regions, based on the sequence alignment of *white* locus available in *Genbank* (NCBI) of *D. simulans* (U64875), of *D. melanogaster* (X02974) and of two other diptera: *Ceratitis capitata* (AF315648) and *Bactrocera tryoni* (U97104) - WD- 5' CAAAAAYTAYGGCACRCTCY 3' and 5' AAGTCSACSGCTTCRCYGG 3'. Those sequences were constructed in order to anneal in regions of the second and third exons of the locus, interspersed by the second intron, and to produce a fragment with an expected length of around 1000 nucleotides in *D. melanogaster*. The amplification conditions were: 94°C for 2 min followed by 35 cycles of 30s at 94°C, 40s at 55°C and 1.5 min at 72°C. The *D. willistoni* product amplified with the pair of *primers* WS obtained by purification of the agarose gel using the kit GFX™ PCR DNA and Gel Band Purification® (Amersham Pharmacia Biotech) was also employed as probe in the Southern blot experiments, as recommended by the manufacturers.

As a first step, we tried to verify whether the white-eye phenotype that appeared in the 17A2 strain of *Drosophila willistoni* was or was not the result of a chromosomal rearrangement, by analyzing the left arm of the X (XL) polytene chromosome in the larval salivary glands. This is the chromosomal arm of *D. willistoni* corresponding to the acrocentric chromosome X of *D. melanogaster* where the *white* locus is mapped in this species, according to Muller (1940) and Lakovaara and Saura (1972). In all slides prepared from 10 individuals, no arrangement different

from that described for this strain was found: all slides presented the XL-B arrangement, characteristic of the populations of southern Brazil (Rohde *et al.,* 2006). Thus, the possibility of occurrence of an inversion able to carry the *D. willistoni white* locus to heterochromatic regions (as the chromocenter, for instance) promoting the silencing of the gene expression was, therefore, discarded.

In the first attempt to characterize the *D. willistoni white* locus, we used the plasmid p-CaSpeR-*hs* as probe in Southern blot experiments, resulting in no hybridization pattern. The results are suggestive of low homology between the genomic DNA of *D. willistoni* and the *D. melanogaster* (Figure 1). The following attempt was made to find a specific probe for *D. willistoni* using two pairs of specific primers for the *white* locus (WS and WT) of *D. simulans*, kindly provided by Torres *et al.,* (2006). By such an approach, we obtained PCR amplification only with the first pair of primers (WS) in *D. willistoni*. The amplification product, however, has around 650 nucleotides, being smaller than the 721 nucleotides expected for *D. simulans* (Figure 2). This product was hybridized, using the pCaSper-*hs*, with the *D. melanogaster white* locus by Southern Blot, resulting in positive hybridization with the *white* locus, but probably corresponding to a sequence with no complete homology with that of *D. melanogaster* (Figure 3). The use of the purified 650-nucleotide fragment as probe in the genomic DNA of *D. willistoni* resulted in low specificity of the probe, although submitted to considerable time of X-rays film exposure. No banding pattern was revealed, suggesting that the probe hybridized with no specific regions of the total DNA (data not shown).

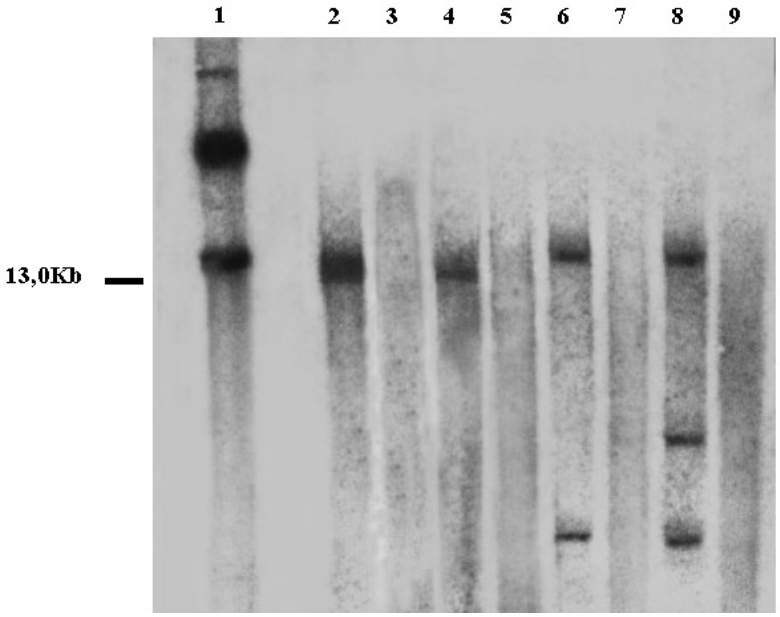

Figure 1. *Southern blot* with genomic DNA of *D. simulans* and *D. willistoni* using p-CaSpeR-*hs* (lane 1) as probe; lanes 2 and 6 are *D. simulans* wild-type, ELD strain; lanes 3 and 7 are *D. willistoni* wild-type, 17A2 strain; lanes 4 and 9 are *D. simulans white* and lanes 5 and 9 are *white*-like *D. willistoni* mutants. In lanes 2, 3, 4, and 5 DNA were digested with *Eco*RI; lanes 6, 7, 8, and 9 DNA were digested with *Sst*I. Fragment sizes are indicated on the left, in kilobase pairs (kb).

Another pair of degenerated primers (WD) was then constructed considering the sequences of *white* from other Diptera. These primers were used in PCR analyses with the genomic DNA of *D. willistoni*, *D. simulans*, *D. melanogaster*, and *Zaprionus indianus*, in order to test its efficiency. A sole fragment around 1000 nucleotides long, corresponding to part of the second exon and almost all

the third exon, was expected for each species. Three fragments with approximately 850, 1000 and 1500 nucleotides were obtained in the species assayed (Figure 4). These fragments were then analyzed by Southern blot using the p-CaSper-*hs* plasmid as probe revealing homology of the three fragments with the probe. The fragment amplified of the genome of *D. willistoni* was the most divergent, as compared with those of the other species (Figure 5). All fragments were then purified from the agarose gel, and the 1500-nucleotide fragment was used as probe in the genomic DNA of *D. willistoni*. Once again, no satisfactory result was obtained.

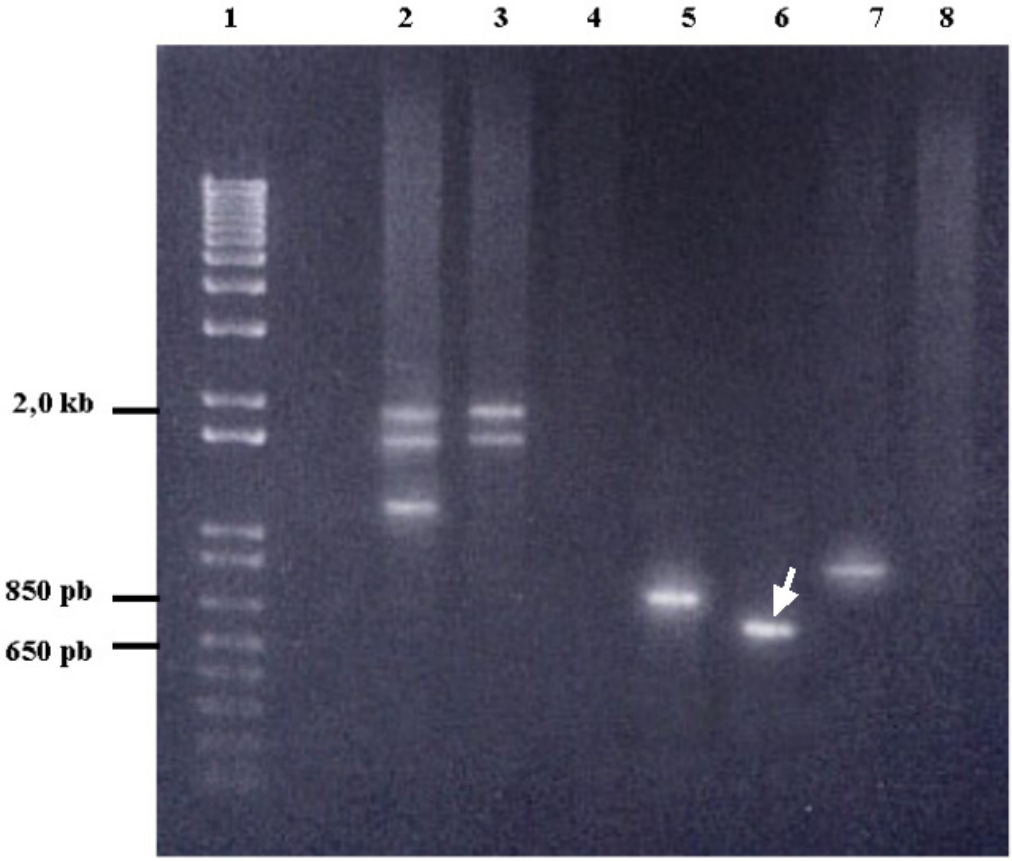

Figure 2. Analysis of the PCR products obtained with WS and WT primers. Lanes 2 and 3: products obtained with direct sequence of WS primer and reverse sequence of WT primer amplified in the *D. simulans* (wild-type, ELD strain); lane 4: Negative control; Lanes 5 and 6: products obtained with WS primers in *D. simulans* (wild-type, ELD strain) and *D. willistoni* (wild-type, 17A2 strain), respectively. The arrow indicates the 650-bp *D. willistoni* fragment (lane 7). Results obtained with pairs of primers WT in *D. simulans* (wild-type, ELD strain) and *D. willistoni* (wild-type, 17A2 strain) in which no amplification occurred (lane 8). Fragment sizes are indicated on the left.

Trying to obtain better results with the degenerated primer and to restrict its annealing exclusively with the exons, we started a new approach using *D. willistoni* cDNA. Figure 6 shows the result of PCR amplification with the three pairs of primers (WS, WT and WD), besides the primer for ß-Actin, used as positive control. Considering that the primers WS and WT anneal in introns of the *D. simulans white* locus, we did not expect amplification with the cDNA of *D. willistoni*, but fragments with around 350 nucleotides were amplified with both the primers. The pair WD generated a product with approximately 200 nucleotides, also different from the expected, since the

expected length for the exons 2 and 3 was around 1 kb. After analyses by Southern blot, we saw that these fragments diverge from the p-CaSpeR-*hs* probe. This finding and the fact that WT did not amplify any sequence of the *D. willistoni* genomic DNA, as also shown in Figure 2, suggests that these fragments do not correspond effectively to the *white* locus. They may indicate, for instance, the amplification of unspecific products creating new sites of annealing of the primer, in the spliced mRNA.

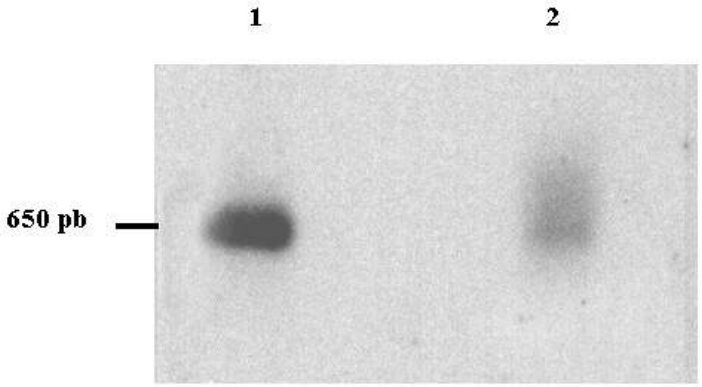

Figure 3. *Southern blot* analysis of PCR products obtained with WS primers. Lane 1: *D. simulans* (wild-type, ELD strain) and lane 2 *D. willistoni* (wild-type, 17A2 strain). The probe is the product obtained from *D. simulans* (wild-type, ELD strain) with WS primers.

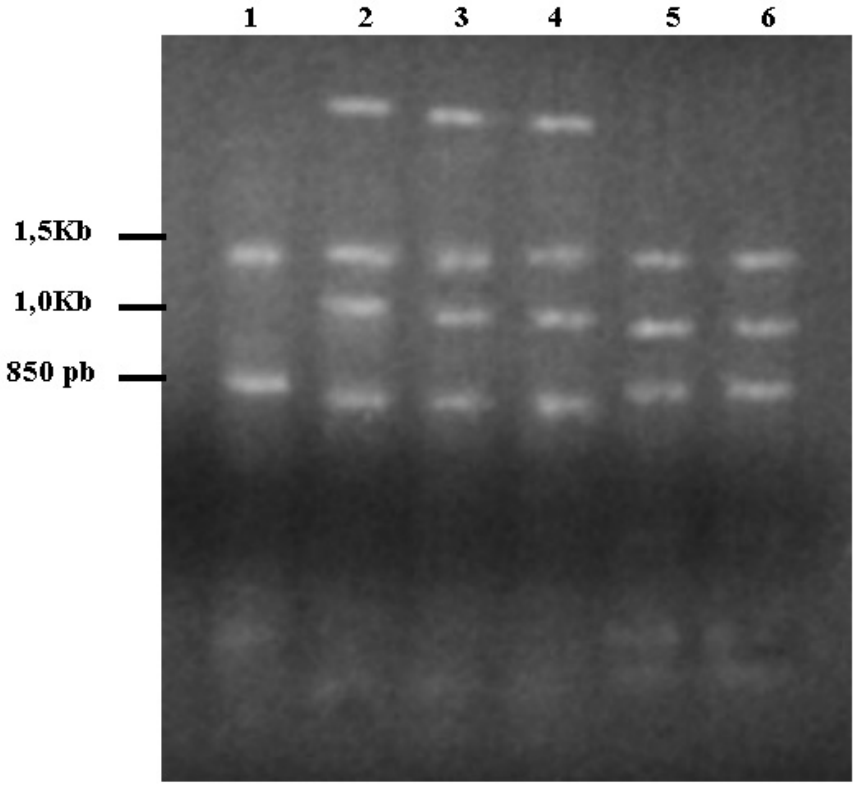

Figure 4. PCR products obtained with degenerated oligonucleotides primers WD. Lane1: *Zaprionus indianus*; lane 2: *D. melanogaster*; lane 3: *D. simulans* (wild-type, ELD strain); lane 4: *D. simulans* (*white*); lane 5: *D. willistoni* (wild-type, 17A2 strain) and lane 6. *D. willistoni* (*white*-like mutants). Fragment sizes are indicated on the left.

A possible explanation for the low similarity found between *D. melanogaster* and *D. simulans* (species used as controls) and *D. willistoni* may lie in the differences accumulated along the time of divergence between the *willistoni* and the *melanogaster* groups. Estimates by Powell and DeSalle (1995) and Russo *et al.,* (1995), with several data sources, place this divergence around 36-53 million years ago. Another possibility is that the *white* locus has not been subject to a strong selective pressure, allowing the accumulation of changes in the exons, though without affecting the viability of

the mutant individuals. This occurred, for instance, in the locus *Adh*, critical for the survival of *Drosophila* in alcohol-rich substrates, in which a remarkable variation in structure and organization was found (Anderson *et al.*, 1993). These authors detected at least three different organizations of this locus in the Genus *Drosophila.*

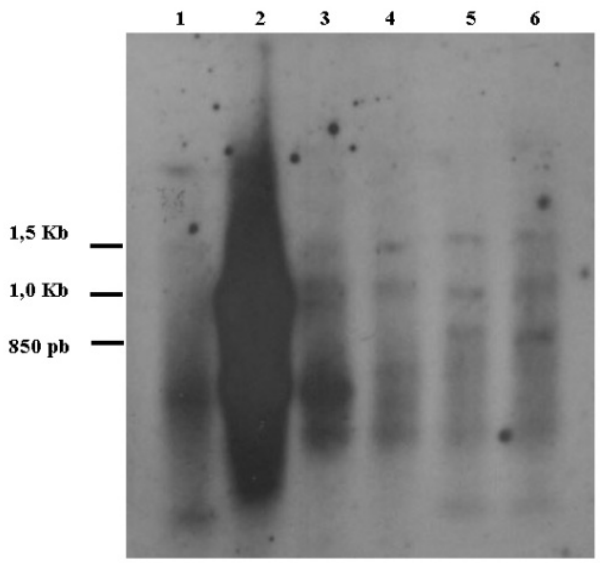

Figure 5. *Southern blot* analysis of the PCR products obtained with WD primers, using p-CaSper-*hs* plasmid as probe. Lane 1. *Zaprionus indianus*; lane 2. *D. melanogaster*; lane 3. *D. simulans* (wild-type, ELD strain); lane 4. *D. simulans* (*white*); lane 5. *D. willistoni* (wild-type, 17A2 strain) and lane 6. *D. willistoni* (*white*-like mutants). The sizes of fragments are indicated on the left.

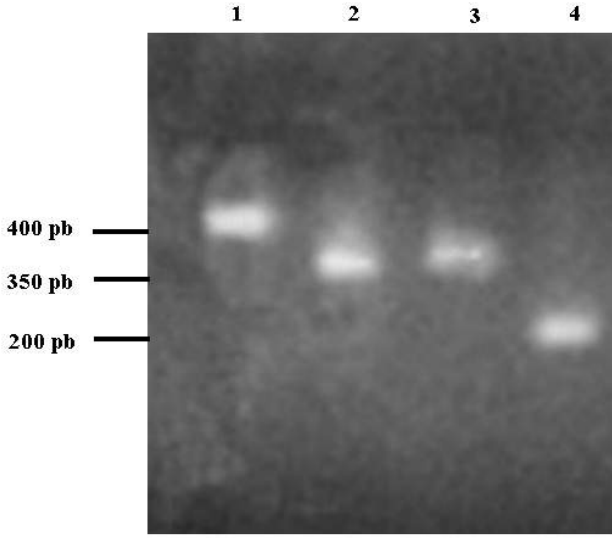

Figure 6. PCR with cDNA of *Drosophila willistoni*, using the primers β-actin (lane1); WS (lane 2); WT (lane 3) and WD (lane 4). Fragment sizes are indicated on the left.

The findings of the present study indicate the possibility of high divergence between the *white* locus of *D. willistoni* and that of *D. melanogaster*, resulting in the inefficiency of the methods here employed for its characterization in *D. willistoni*. The information obtained after the conclusion of the Genome project will hopefully support our findings.

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Reference photomap of the salivary gland polytene chromosomes of *Drosophila neomorpha* (Streisinger, 1946).

De Toni, D.C.[1], M.A. Loureiro[1], P.R.P. Hofmann[1], and V.L.S. Valente[1,2].
[1]Departamento de Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina; [2]Departamento de Genética, Instituto de Biociências-UFRGS: Universidade Federal do Rio Grande do Sul. detoni@ccb.ufsc.br.

Throughout the years, populations of the four species of the *cardini* subgroup (*D. polymorpha, D. cardinoides, D. cardini,* and *D. neocardini*), that are commonly distributed throughout Brazil, have been studied to elucidate the characteristics that allow them to exploit various habitats, such as chromosomal inversions (De Toni *et al.*, 2001b,c) and abdominal pigmentation (Da Cunha, 1949; Heed, 1962; Heed and Krishnamurthy, 1959; Heed and Russel, 1971; Rohde and Valente, 1986a; Machado *et al.,* 2001; Brisson *et al*., 2005).

Studies of the genetic characteristics of marginal populations make it possible to test the idea that gene flow is a cohesive force that holds a species together and allows it to evolve as a unit. Such populations may yield information on the role of heterozygosity at the chromosomal level, as in the case of many *Drosophila* species.

In De Toni *et al.* (2005), we report the movement of two species of the *cardini* group, *D. parthenogenetica* and *D. neomorpha*, into Brazil. Previous reports indicated that both of these species were distributed from central Mexico to northern South America (Heed, 1963). It appears that *D. parthenogenetica* and *D. neomorpha* are now sympatric with these four species that have traditionally been found throughout Brazil. *D. parthenogenetica* and *D. neomorpha* occupy very similar, if not identical, ecological niches that overlap with *D. polymorpha* and *D. neocardini*, and

both exhibit similar abdominal pigmentation adaptations to temperature and humidity (Penton *et al.*, 2006, unpublished data). This raises questions regarding how and when this territory expansion occurred and if these expansions are due, or not, to a new karyotype evolution.

These species, particularly *D. neomorpha* and *D. polymorpha*, are very similar with respect to their morphology and ecological requirements and have been very hard to tell apart. The specific differentiation is until now made through the analysis of the external and internal male genitalia, since, in the south of Brazil, *D. neomorpha* and *D. polymorpha* have the same pattern of abdominal pigmentation. With this new map, the species recognition will also be possible by the chromosomal elements.

Although these four native species share some environments in nature, *D. cardinoides* is more frequently found in drier places, whereas *D. polymorpha* is a typical forest fly as *D. neocardini,. D. neomorpha* and *D. parthenogenetica* that have also been collected in forest. *D. neomorpha*, however, is much less frequent than *D. polymorpha* and *D. cardinoides*.

As a starting point to study the inversion polymorphism in *D. neomorpha*, we constructed a reference photomap of the polytene chromosomes of third instar larvae salivary glands. The slide preparations were made through the method of Ashburner (1967). Approximately 25 individuals (about three nuclei per gland) from Panama, at Gamboa (9º123"N; 79º 242"W) were analyzed and photomicrographed to reach a consensus on the identity of the elements.

Figure 1 corresponds to the photomap of *D. neomorpha*. The chromosomal complement of the salivary glands of this species consists of 4 chromosome pairs: two submetacentric chromosomes: II and III, the sexual pair (composed of the acrocentric XX and the Y chromosome which is heterochromatic and not distinguishable from the chromocenter), and the fourth, a dot pair.

This chromosomal complement was deduced, initially, by comparison of the polytene banding patterns of with those of *D. cardinoides, D. polymorpha* (Rohde and Valente, 1996b), *D. neocardini* (De Toni *et al.*, 2001), and *D. parthenogenetica* (De Toni *et al.*, 2006; unpublished data), four closely related species.

Five chromosomal arms linked to the chromocenter can be observed in the salivary gland squashes. The shorter arm is the X chromosome (even though it is bigger than the *D. polymorpha* one), followed by left arm of chromosome III (IIIL) and the left arm of chromosome II (IIL), also observed in *D. polymorpha*, *D. parthenogenetica,* and *D. cardinoides* karyotypes, which have both IIL and IIIL arms, equivalent in size. The right arm of chromosome II (IIR) and the right arm of chromosome III (IIIR) are the longest, respectively, as was found for the other species of the *cardini* subgroup already mapped (Rohde and Valente, 1996b, De Toni *et al.*, 2001; De Toni *et al.*, 2006; unpublished data). The X chromosome (pair 1) was subdivided into 20 sections (from the tip to the base) and is distinguished from the remaining chromosomes by the following characteristics: its distal part (section 1) stays permanently puffed during the third instar showing a fan shaped puff.

The basal section (20) of this chromosome remains attached to a big portion of the chromocentric heterochromatin. These two X characteristics are noticeable, again in all mapped species from this subgroup.

The left arm of the chromosome II (IIL) was subdivided into sections beginning with the section 21 (tip) and ending in the section 40 (base). This chromosomal arm has, on the tip, at the proximal region (section 21), two strong colored bands separated by one clear interband, the section 22 has several bands and with approximately the same width. In the proximal region of the section 24 there is a pair of strong dark bands with a clear interband. This arm is relatively free of puffed bands in the third instar larvae, but close to the tip, in the distal portion of the section 25 it has a constantly puffed region in hexagonal format, which could help in the chromosomal identification. Close to the heterochromatic base (section 40) it shows a puff edged by two densely colored bands,

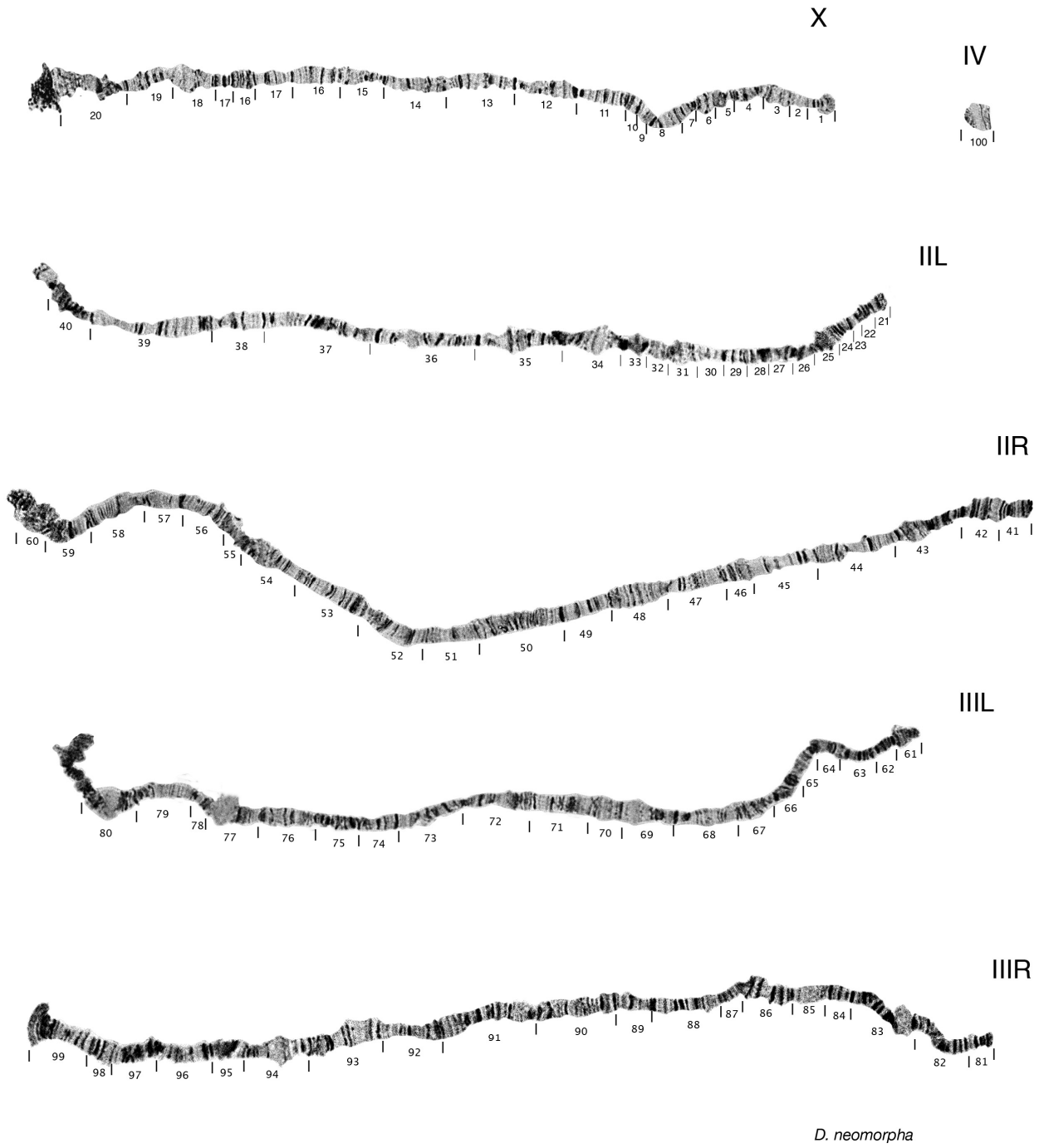

Figure 1.

characteristic of this larval instar. Diagnostic constrictions were observed especially on the distal part in the sections 34 and 35.

The right arm of the chromosome II (IIR) is the longest and was subdivided into sections numbered from 41 to 60. The tip in section 41 has a straight aspect**,** showing eventually a sharp end. On the section 42 we can find two very strong bands edging a clear interband, just before the section 43. On this last section 43 is possible to observe a constant puff that has, on the proximal region, a dotted band. In the more proximal section (60), we observed a puff, also characteristic of this larval instar. This puff can be observed in many other species of this subgroup as *D. cardinoides* (Rohde and Valente, 1996b), *D. neocardini* (De Toni *et al.*, 2001) and *D. parthenogenetica* (De Toni *et al.*, unpublished data), but not in *D. polymorpha,* showing, again, commonality trait of the karyotypic evolution of this subgroup.

The left arm of chromosome III (III L) was subdivided into sections numbered from 61 to 80. In the sections close to the tip, between the proximal median part of section 61 and the distal median of 62, there are several bands and interbands with approximately the same width, characteristic of this chromosome [as in *D. neocardini* (De Toni *et al.*, 2001)]. The tip (in section 61) always shows the same arrow shaped aspect. In the proximal section 80 we can frequently verify a puff, attached to the chromocentric heterochromatin. On the other hand, the remaining sections present neither particular constant "landmarks" or characteristics that could be used to identify this chromosomal arm. The size of this arm, therefore, is a good diagnostic characteristic, easily told apart from the IIL arm by the arrow shaped tip.

The right arm of chromosome III (III R) was subdivided into sections numbered from 81 to 99. Its tip in section 81 has a straight form, with several bands and interbands with approximately the same width. The section 82 in *D. cardinoides* and *D. neocardini* is smaller then here, where we observe a higher number of bands and interbands. The distal part of section 83 conserved a puff that has as boundaries, in the both sides, two strong colored bands. This puff is also observed in *D. cardinoides* and *D. neocardini* but not in *D. polymorpha,* pointing to a discrepancy between the karyotypic and molecular evolution of this subgroup (Penton *et al.*, 2006, unpublished data). The proximal part of section 86 is very frequently puffed too, in this developmental phase.

The 93 is another section very frequently puffed in this developmental phase, with two pair of bands separated by interbands with almost the same width at the distal region. In the basal section (99) we can frequently find another puff fan shaped (similar to the X tip, but less circularly puffed). Finally, the small (dot) chromosome IV, frequently attached to the chromocenter, comprehends the section 100.

Highly conserved areas occur in the chromosomes of some *cardini* species such as *D. neomorpha, D. neocardini, D. cardinoides,* and *D. parthenogenetica,* in the X chromosome, IIR, and IIIR arms.

The sections of the polytene chromosomes of *D. neomorpha* were compared to the photomap of *D. cardinoides* and *D. neocardini* to determine areas of apparent homology. Some recent phylogenies, based on nuclear and mitochondrial genes, showed that *D. polymorpha* is more closely related to *D. neomorpha* than these other species (Penton *et al.*, 2006, unpublished data), even though the comparison of the band patterns was performed mainly based on the photomap of *D. cardinoides.* One exception is the IIL arm that was based on *D. neocardini* map, because the chromosomes of these two species were more similar to *D. neomorpha* than with those of *D. polymorpha* with respect to their banding patterns, especially considering the length of the sections, the width and the aspect of bands or groups of bands ("marker bands"). This fact points to a divergence between molecular and cytogenetic data, as mentioned before. The homology between sections of polytene chromosomes of the *cardini* group species studied was simple to establish, since they have a conserved karyotypic

complement. In the few cases, where we could not find the homology, we subdivided the sections in the chromosomes of *D. neomorpha*, maintaining lengths approximately similar to that correspondent in the related species**.**

Even with reduced inversion polymorphism in the *D. neomorpha* population searched (no inversion was found), the absence of conspicuous chromosomal polymorphism is not necessarily conclusive evidence of reduced genetic variability in this population. Molecular studies could help to resolve this uncertainty.

We are working on the chromosomal photomap of other species from *cardini* group and we hope to improve considerably all of them when this work is completed, since we will have the whole panorama of the *cardini* subgroup karyotype evolution.

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A new incipient polymorphic inversion, In(2R)O, in *D. melanogaster* Japanese populations.

Inoue, Yutaka[1], and Masayoshi Watada[2]. [1]Osaka University of Foreign Studies, E-mail: inoueyu@post01.osaka-gaidai.ac.jp; and [2]Department of Biology and Earth Science, Faculty of Science, Ehime University, Mastuyama, Ehime 790-8577, Japan.

D. melanogaster natural populations show a high degree of chromosomal polymorphism which entirely consists of the paracentric type of inversions on the four major autosome arms. They were classified into two categories: Common Cosmopolitans and Rare Cosmopolitans (Inoue and Igarashi, 1994). The Common Cosmopolitans are usually maintained in most populations all over the world with the higher frequencies, on occasion being more frequent than the standard sequence. Four inversions each of which exists in the right and left arms of the two major autosomes, respectively, In(2L)t, In(2R)NS, In(3L)P, and In(3R)P, are in this category. The Rare Cosmopolitans are also distributed all over the world, but their frequencies are usually low at around a few percent, or absent in some populations. Two inversions in the right arm of the third chromosome, In(3R)C and In(3R)Mo, are in this category.

In this note we report the results of a survey for inversion frequencies in a total of 26 populations distributed all over Japan, from Hokkaido to Iriomote of the Southwest Islands. Also a

Table 1. Inversion frequencies in the recent Japanese populations of *D. melanogaster*.

Locality	Year	No.*	Common Cosmopolitans				Rare Cosmopolitans		Average**	Others	
			2Lt	2RNS	3LP	3RP	3RC	3RMo		2RO	3RK
1. Toyotomi	2000	38	0	0	0	0.079	0	0.132	0.053	0	0
2. Sapporo	2000	48	0	0	0	0.042	0.021	0.021	0.042	0	0
3. Aomori	2000	44	0.046	0.046	0	0.023	0	0	0.028	0	0
4. Akita	2003	40	0.050	0	0	0.100	0.025	0.025	0.050	0	0
5. Iwate	2000	28	0	0.036	0	0.107	0	0	0.036	0	0
	2003	58	0.052	0.035	0	0.086	0	0	0.043	0	0
6. Yamagata	2000	48	0	0	0	0.021	0	0.021	0.010	0	0
7. Niigata	2000	22	0	0.046	0	0.136	0	0	0.046	0	0
8. Fukui	1999	70	0.029	0	0	0.043	0.029	0.114	0.054	0.071	0
9. Shizuoka	1999	200	0.250	0.050	0.020	0.145	0	0.050	0.129	0.025	0
10. Yamanashi	1981	244	0.156	0.152	0.004	0.225	0.045	0	0.154	0	0
	1999	200	0.080	0.015	0	0.210	0	0	0.076	0	0
	2003	200	0.135	0.010	0.010	0.150	0	0	0.076	0	0
	2004	200	0.140	0.020	0.010	0.205	0.005	0.010	0.098	0	0
11. Shiga	2001	96	0.260	0.031	0.031	0.083	0.010	0.021	0.109	0.021	0
12. Kyoto	2001	76	0.158	0.066	0.013	0.053	0.053	0.053	0.099	0.053	0
	2002	386	0.127	0.086	0.008	0.132	0.098	0.054	0.126	0.073	0
	2004	200	0.105	0.155	0.020	0.060	0	0.060	0.100	0.020	0.005
13. Osaka	1994	168	0.161	0.101	0.048	0.161	0.066	0.030	0.141	0	0.007
	2000	200	0.270	0.100	0.045	0.170	0.020	0.075	0.170	0.090	0.005
14. Hyogo	1993	130	0.254	0.162	0.085	0.200	0.038	0.031	0.192	0	0.008
	2001	56	0.268	0.179	0.036	0.089	0.054	0.071	0.174	0	0
15. Nara	2000	44	0.091	0.159	0.023	0.091	0.046	0.046	0.114	0	0
16. Okayama	1994	200	0.075	0.035	0.035	0.215	0.005	0.030	0.099	0	0
	2001	60	0.117	0.050	0.017	0.150	0	0.033	0.092	0	0
	2004	200	0.155	0.085	0.075	0.295	0.015	0.035	0.165	0	0
17. Tottori	2001	106	0.160	0.057	0.057	0.113	0.057	0.028	0.118	0.019	0
18. Yamaguchi	2001	54	0.019	0	0	0.019	0.019	0.019	0.019	0.019	0
19. Ehime	2001	60	0.017	0.033	0.050	0.033	0	0	0.033	0	0
20. Kochi	2001	58	0.155	0.017	0.121	0.328	0	0.017	0.155	0.035	0
21. Fukuoka	2001	60	0.017	0	0.050	0.017	0	0.017	0.025	0	0
22. Ogasawara	2000	30	0.367	0	0	0.533	0	0	0.225	0	0
	2004	54	0.241	0	0	0.315	0	0	0.139	0	0
23. Okinoerabu	2005	20	0.350	0.200	0.350	0.400	0	0.050	0.338	0	0
24. Okinawa	2003	34	0.088	0.235	0.118	0.265	0	0	0.177	0.029	0
	2004	80	0.500	0.113	0.113	0.125	0.075	0	0.231	0.063	0
25. Kumeshima	2001	62	0.323	0.194	0.065	0.145	0.032	0	0.190	0.113	0
	2002	162	0.414	0.142	0.086	0.222	0.068	0	0.233	0.117	0
	2003	58	0.241	0.121	0.086	0.207	0.086	0	0.181	0.086	0
26. Iriomote	2001	446	0.496	0.339	0.339	0.726	0.061	0.011	0.493	0.020	0.002
	2003	218	0.427	0.381	0.362	0.821	0.055	0	0.511	0.009	0
	2004	200	0.415	0.295	0.375	0.690	0.090	0	0.466	0.030	0

* Number of chromosomes analyzed .

** Average frequencies of total cosmopolitan inversions per major autosome arm.

new paracentric inversion named 2RO was first reported, which appeared in many Japanese localities in the present surveys. Inversions were identified through direct observation of single F1 larva chromosomes from each isofemale which was established immediately after collection. Frequency of each inversion was calculated per each chromosome arm. Average frequencies of total Cosmopolitan inversions per major autosome arm were also calculated to compare the degree of inversion polymorphisms, because significant positive correlations were observed among all four major autosome arms (Inoue *et al.,* 1994).

On the Cosmopolitan inversions: In Hokkaido (Locality No. 1 and No. 2), which is the northern part of Japan, inversion frequencies were very low to be 0.053 and 0.042 on average. Three of the four Common Cosmopolitans, In(2L)t, In(2R)NS, and In(3L)P, were not observed in this region in a total of 86 genomes analyzed. The similar low frequencies were also observed in the eastern Mainland populations (No. 3, 4, 5, 6, 7, and 8) to be 0.010 – 0.050 on average. Among the four Common Cosmopolitans In(2L)t and In(2R)NS were observed, but In(3L)P was still absent. In the middle Mainland (No. 9 – 15) all populations showed higher average values than those of the eastern Mainland populations, being from 0.076 (No. 10) to 0.192 (No. 14). The four Common Cosmopolitans were all observed in all populations, except the Yamanashi (No. 10) 1999's sample. Recently in the Yamanashi population In(2R)NS drastically decreased from 0.152 (1981) to 0.015 (1999), 0.010 (2003), and 0.020 (2004), which resulted in the decrease of the average values from 0.154 to 0.076 – 0.098. In the western Mainland, six localities (No. 16 – 21) were surveyed. Three localities out of them showed low average values as well as the Hokkaido and the eastern Mainland populations: 0.019 (No. 18), 0.033 (No. 19), and 0.025 (No. 21). The three other localities showed the higher average values as well as the middle Mainland populations, being 0.092 – 0.165 (No. 16), 0.118 (No. 17), and 0.155 (No. 20). The Ogasawara are the islands which lie south of the middle Mainland, and the samples (No. 22) showed a very different phenomenon from the Mainland populations mentioned above. In spite of the higher average values, 0.225 (2000) and 0.139 (2004), these populations lacked two kinds of the Common Cosmopolitans, In(2R)NS and In(3L)P. Also they lacked two kinds of Rare Cosmopolitans, In(3R)C and In(3R)Mo. These characteristics have not changed since the 1980's surveys in Inoue *et al*. (1982).

In the South-west Islands four localities were surveyed. Although the sample size was small, the Okinoerabu population (No. 23) showed a high average value of 0.338. This value was higher than all the Mainland populations and Ogasawara populations (No. 22). The Okinawa populations (No. 24) showed 0.177 (2003) and 0.231 (2004) on the average, which were in the same level with the Ogasawara populations (No. 22). The different sampling sites may cause lack of uniformity for each inversion frequency between the two samples. On the neighboring small island, Kumeshima (No. 25), the surveys were carried out in the recent three years. These data were similarly high in frequency as the Okinawa (No. 24) and Ogasawara (No. 22) populations, being 0.181- 0.233 on average. The highest average values in the present surveys were observed in the Iriomote populations (No. 26), being 0.493 (2001), 0.511 (2003), and 0.466 (2004). All four Common Cosmopolitans were observed with stable high frequencies in any sample, which were the same results as 1979, 1982, and 1998's surveys in Inoue *et al*. (2002).

On the Unique inversions: These are non-polymorphic inversions. They are all individually different from one another and usually each is recorded only once. Most probably they are selected out soon after they were born and dismissed by a stochastic process. In the present survey a total of 27 paracentric inversions were observed: 7 inversions on the 2L chromosome arm, 6 on the 2R, 7 on

Table 2. The list of the *Unique* inversions found in the present study with their locality numbers and years

Chromosome arm 2L	Chromosome arm 2R
2LW in No. 9, 1981	45B;52F in No. 12, 2002
2LA in No. 9, 1981	51C;59D in No. 21, 2001
29B;36F in No. 9, 1981	45E;55D in No. 12, 2004
30B;31F in No. 9 , 1981	42F;55B in No. 6, 2000
22A;26C in No. 12, 2001	45B;51A in No. 13, 2000
24F;27E-28C in No. 12, 2001 & 2002	43B;55A in No. 9, 1999
26F;35F in No. 13, 2000	
2LR Pericentric inversion 30F;49B in No. 26, 2001	

Chromosome arm 3L	Chromosome arm 3R
3LY in No. 9, 1981	86C;93B in No. 1, 2000
67C;75C in No. 9, 1981	89E;93D in No. 9, 1981
61C;67B in No. 12, 2002	84C:88C in No. 9, 1981
3LM in No. 13, 1994 & 2000	96A;98D in No. 9, 1981
64C;71E in No. 13, 2000	85C;89C in No. 12, 2001 & 2002
63F;69F in No. 25, 2004	86D;93C-D in No. 26 & 28, 2004
66A;69B in No. 28, 2001	86E;96B in No. 23, 1983
3LR Pericentric inversion 64C;90B in No. 12, 2001	

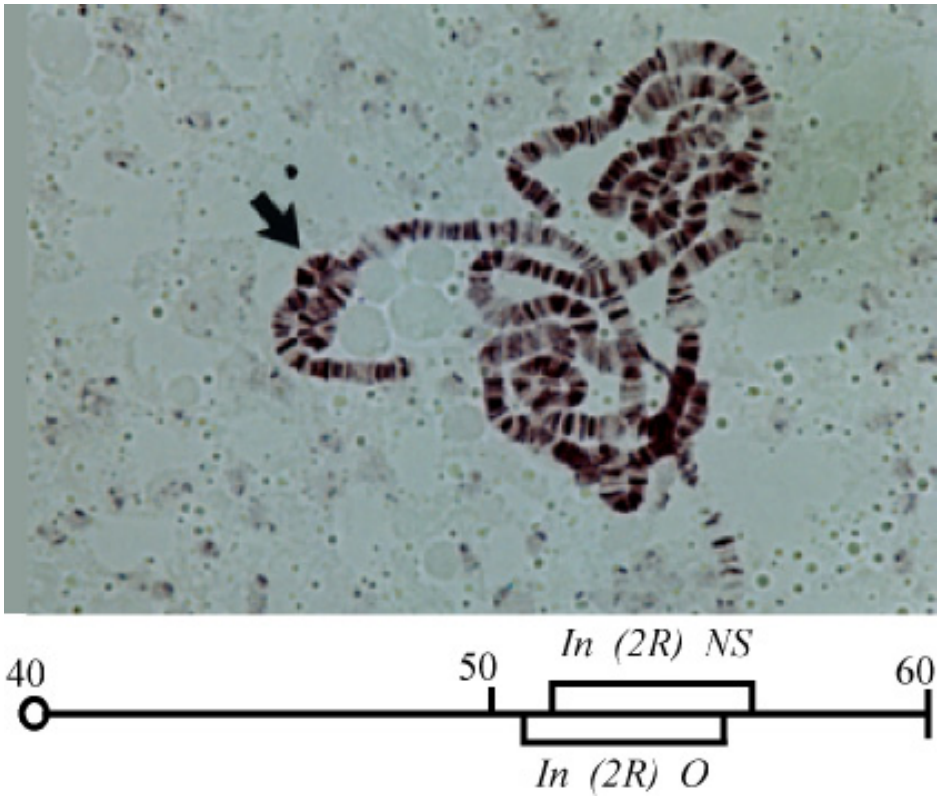

Figure 1. The right arm of the second chromosome with the overlapping complex of In(2R)NS and In(2R)O, and the breakpoint locations of these inversions.

the 3L, and 7 on the 3R. Additionally, two pericentric inversions were observed on the second and third chromosomes, respectively, as shown in Table 2. In *D. melanogaster* the chromosomal variations observed in natural populations are mostly paracentric

inversions, and other types of aberrations are less frequent or indeed rare. It may be caused by some strong affinity within each chromosome arm in *D. melanogaster*, and the degree of arm affinity may be different among *Drosophila* species. For in the study of chromosomal polymorphisms of *D. ananassae* a total of 70 paracentric and 17 pericentric inversions and 13 translocations have been described (Singh, 1998).

On the In(2R)O: This is the paracentric inversion first reported by Inoue *et al.* (2002), being observed in Irabu, Miyako, and Kurima localities in the 1999's surveys with frequencies of 0.011 – 0.091. They are the small islands located between Kumeshima (No. 25) and Iriomote (No. 26) in the South-west Islands. The first reported breakpoints (51A;55C) were corrected by the present detailed analysis to be from 51B to 55E on the right arm of the second chromosome, and we named this In(2R)O. Both of its breakpoints were very close to the Common Cosmopolitans, In(2R)NS (see Figure 1). In the present surveys, In(2R)O was found to distribute in a total of 11 localities. Osaka (No. 13) showed the highest In(2R)O frequency in the Mainland to be 0.090 in 2000, but this inversion was not found there in 1994. Kumeshima (No. 25) also showed the higher frequencies, 0.113 (2001), 0.117 (2002), and 0.086 (2003). So In(2R)O may originate from around the Kumeshima region in the South-west Islands in the latter half of the 1990's. In the present Japan, this inversion prevails more frequently than In(3R)K, which is sometimes classified in the Rare Cosmopolitans because of its world-wide distribution. In(3R)K was observed in only localities No. 12, 13, 14, and 27 with low frequencies of 0.002 – 0.008 in the present surveys (Table 1). Out of Japan, we recently found In(2R)O in one line of 25 isofemale lines sampled from Hawaii, 2005.

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Availability of the University of Texas Publications Dealing with *Drosophila*

Marshall R. Wheeler

From 1940 to 1972 many research articles were published by the University Press in the series, "Studies in the Genetics of *Drosophila*" with J.T. Patterson as editor and later (from 1957-1972) with M.R. Wheeler as editor. In 1960 the series title was changed to "Studies in Genetics." There were also a few special issues. Many of these are now out of print (OOP); all known copies of the remaining issues have been made available by Dr. Wheeler. The copies are available from the office of the Editor, *Drosophila Information Service*; contact Dr. James N. Thompson, jr., (jthompson@ou.edu) for details.

Some issues were given titles and subtitles, but the Publication Number (*e.g.*, UTP 4213) is the best reference. This is the complete list of all the publications:

1940: UTP 4032 (OOP). 1942: UTP 4213 (OOP). 1942: UTP 4228 (OOP). 1943: UTP 4313, "Drosophilidae of the Southwest" (OOP). 1944: UTP 4445, with "Drosophilidae of Mexico" (OOP). 1947: UTP 4720, "Isolating Mechanisms" (OOP). 1949: UTP 4920 (OOP). 1952: UTP 5204 (25 copies). 1954: UTP 5422 (OOP). 1957: UTP 5721 (45 copies). 1959: UTP 5914, "Biological Contributions." Dr. Patterson's 80th birthday issue (59 copies). 1960: UTP 6014 (16 copies). 1962: UTP 6205 (63 copies). 1966: UTP 6615, Morgan Centennial Issue (28 copies). 1968: UTP 6818 (24 copies). 1969: UTP 6918, W.S. Stone Memorial Issue (12 copies). 1971: UTP 7103 (22 copies). Final volume, 1972: UTP 7213 (29 copies).

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