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Scientific Correspondence

Bioinformatics and Cytogenetics of Unusual *Drosophila melanogaster* X-chromosome Morphology

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Sir

The morphology of *Drosophila melanogaster* polytene chromosomes is well described (Bridges, 1935; 1938), yet the underlying causes of gross chromosome structures are largely unknown. In particular, Bridges (1935) was able to identify a number of unusual morphologies strewn along the polytene chromosomes, and he gave them visually descriptive names such as, "Chinese lantern", "chains" and "ballet skirt". One general hypothesis is that primary DNA sequence data is a large determinant of these "higher" chromosome morphologies.

Benos *et al.* (2000) used the sequence data from the *D. melanogaster* genome project (Adams *et al.*, 2000) to investigate at the DNA level one unusual morphological structure in region 2B on the X chromosome, which has been alternately termed a "puff" (Bridges, 1935; 1938) or a "bulb" (Offerman, 1936). Specifically, the "bulb" is an unusual banding pattern in chromosome region 2B3–8 of the X chromosome, thought to be caused by ectopic pairing among chromosome bands in the region, resulting in the appearance of some polytene chromosome bands lying parallel to the chromosome rather than perpendicular to it (Offerman, 1936; Bridges, 1938; Benos *et al.*, 2000). Both Offerman (1936) and Bridges (1938) hypothesized that the morphology in region 2B was due to an unknown sequence repeat organized in reverse order, hence, a "reverse repeat". The techniques for determining the nature of the repeat were, of course, unavailable at that time.

Benos *et al.* (2000) used bioinformatical methods to analyze the primary DNA sequence of the 2B3–8 chromosome region, and reported a pair of 1.2-kb DNA sequence repeats, each made up of 3.5 repeats of a ~350 bp satellite element related to the *1.688* family of satellite elements (Waring and Pollack, 1987; DiBartolomeis *et al.*, 1992). These are referred to as satellite-related (SR) arrays (DiBartolomeis *et al.*, 1992), and the SR arrays were found to be in reverse repeat orientation, as predicted by Offerman (1936) and Bridges (1938). Benos *et al.* (2000) thus suggest that these inverse repeats are the cause of the 2B3–8 "bulb" structure.

Are the SR array sequences identified by Benos *et al.* (2000) necessary for the reverse repeat banding pattern? Although their bioinformatic data is consistent with the original hypothesis, no experimental data was provided. However, we found in the literature experimental cytogenetic data that supports the involvement of the SR arrays. Semeshin *et al.* (2001) recently showed (their Figs. 5a and b) that on an X chromosome carrying inversion $In(1)br^{103}$ (Fig. 1), placing the puff region and the proximal (2B8) repeat, but *not* the distal (2B3) repeat, near cytological region 3C2-3, there is no ectopic pairing. This result suggests that a single repeat is not sufficient for the ectopic pairing in this region. This may mean that DNA homology between these two regions, 2B3 and 2B8, is indeed causing ectopic pairing in the 2B region, as predicted by Benos *et al.* (2000).

Potentially complicating the above interpretation, that the SR arrays in 2B cause ectopic pairing, it has

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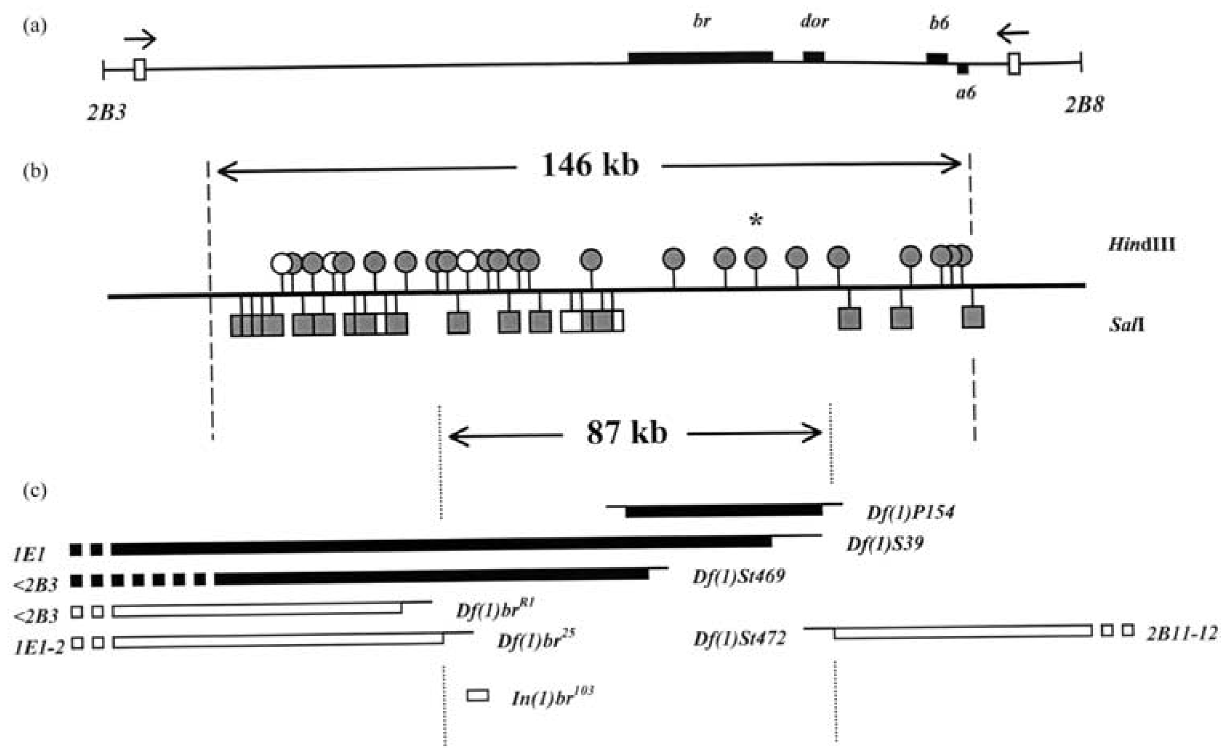

FIGURE 1 Genetic map of *D. melanogaster X* chromosome cytological region 2B3–2B8. (a) Genetic map of the 2B3–2B8 region. Open boxes are the inverted repeats of Benos *et al.* (2000). The arrows indicate the directions of the repeats. Black boxes represent "landmark" known genes in this region [also shown in Fig. 1 of Benos *et al.* (2000)]. These genes are *br* (*broad* (2B4–6), a transcription factor which encodes a family of zinc-finger proteins), *dor* (*deep orange* (2B6–7), which encodes a transporter protein), *b6* (*b6* (2B7), which encodes a receptor-like protein), and *a6* (*a6* (2B7–8), which encodes an oderant/ligand binding protein). (b) Restriction map of the 2B3–2B8 region. *Bam* HI, *Eco* RI, *Hin*dIII, and *Sal* I were used, as in the Belyaeva *et al.* (1987) study. To align the genomic map of Benos *et al.* (2000) with the chromosome aberration map of Belyaeva *et al.* (1987), we artificially cut the sequence of Celera Genomics scaffold AE003421 with *Bam* HI, *Eco* RI, *Hin*dIII, and *Sal* I using Lasergene's MapDraw program. AE003421 spans the 2B1–2B10 region of the *D. melanogaster X* chromosome. For simplicity, results are shown for only two of the four enzymes, *Hind* III (circles) and *Sal* I (squares). Grey-filled shapes denote cut sites that were common to both sequences (i.e. AE003421, and the sequence used in the Belyaeva *et al.* (1987) study), and white-colored shapes denote non-common cut sites. The "*" indicates 8 closely placed *Hin*dIII cut sites. Vertical dashed lines indicate the limits of the Belyaeva *et al.* (1987) genetic map. 146 kb is an approximation. The proximal limit to the Belyaeva *et al.* (1987) map is a *Sal* I cut site within the *a6*locus; the distal limit is a *Bam* HI cut site approximately 7 kb distal to the most distal *Sal* I site shown. Restriction enzyme cut sites are pictured in the correct order relative to each other and to the genes and chromosome aberrations, but their exact positions are not precise at the scale used here. (c) Cytological map showing chromosome aberrations in the 2B3–2B8 region, enabling deletion mapping of chromosome regions necessary for puff formation. Black lines indicate aberrations that, when present, prevent formation of the 2B puff. A white line indicates no effect of the aberration on 2B3–5 puff formation. All results are from Belyaeva *et al.* (1987) and Semeshin *et al.* (2001). Black lines extending from the tips of the thick black or white lines indicate regions of breakpoint uncertainty (Lindsley and Grell, 1968; Belyaeva *et al.*, 1982; 1987; FlyBase 1999). Some deficiencies in the 2B3–2B8 region extend past the 2B3–2B8 region pictured both here and in Belyaeva *et al.* (1987) (see Lindsley and Grell, 1968; Belyaeva *et al.*, 1982; 1987; FlyBase, 1999). These extensions are indicated by a dashed line and the best estimate from the literature of the cytological location of the other breakpoint. Two of the deletions have synonyms (Lindsley and Grell, 1968; Belyaeva *et al.*, 1982; 1987; FlyBase, 1999): $Df(1)br^{R1}(= Df(1)br - rv^1)$; $Df(1)br^{25}(= Df(1)pn7b)$. Vertical dotted lines are a conservative estimate of the region necessary for puff formation. 87 kb is an approximation.

long been known that *D. melanogaster* polytene X chromosomes display a "transcriptional puff" in the 2B3–5 region, caused by a high level of transcription (e.g. Bridges, 1935, p.62 and his Fig. 5; Bridges, 1938; Belyaeva *et al.*, 1987). Therefore, there is a potential confoundment of two unusual chromosomal structures in the same region, since the 2B3–5 puff itself could contribute to ectopic pairing.

Does the region necessary for 2B transcriptional puff formation overlap the SR arrays hypothesized by Benos *et al.* (2000) to cause ectopic pairing? To answer this, we combined chromosome-level deletion-mapping data (Belyaeva *et al.*, 1987) with the DNA sequence data of Benos *et al.* (2000), and demonstrate that the region necessary for transcriptional puff formation does not overlap either SR array (Fig. 1). Belyaeva *et al.* (1987) utilized numerous chromosome rearrangements (see Lindsley and Grell, 1968; Belyaeva *et al.*, 1982; 1987; FlyBase, 1999) to genetically map the region necessary for puff formation to approximately 87 kb, which lies between the breakpoints of deletions $Df(1)br^{25}$ and *Df(1)St472*. This region does not contain the SR arrays identified by Benos *et al.* (2000).

Furthermore, these data conversely show that the SR array sequences are not necessary for transcriptional puff formation. Deletion *Df(1)St472* uncovers (deletes) the proximal repeat of Benos *et al.* (2000), yet puff formation is not disrupted in a *Df(1)St472*

mutant fly. Similarly, deletions $Df(1)br^{R1}$ and $Df(1)br^{25}$ each uncover the distal repeat, and similarly puff formation in $Df(1)br^{R1}$ and $Df(1)br^{25}$ flies is not disrupted.

We conclude that the available experimental genetic evidence supports the hypothesis that the reverse repeat SR array sequences in chromosome region 2B cause ectopic pairing. Additionally, the causes of the 2B transcriptional puff and ectopic pairing appear to be distinct in *D. melanogaster*. Since ectopic pairing in 2B is conserved in the sibling species *D. simulans* (Offerman, 1936), we predict that the same is true in that species.

Cytogenetic analysis of chromosomes carrying tiny deletions encompassing each of the inverted repeats, to our knowledge not currently available (Flybase, 1999; I. F. Zhimulev, personal communication), would more definitively test whether the DNA repeats identified by Benos *et al*. (2000) functionally cause ectopic pairing in 2B. Finally, other cytological regions, which have similar-appearing ectopic pairing, such as regions 3C and 12D-E (Bridges, 1938) would be predicted to contain reverse tandem duplications.

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References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., George, R.A., Lewis, S.E., Richards, S., Ashburner, M., Henderson, S.N., Sutton, G.G., Wortman, J.R., Yandell, M.D., Zhang, Q., Chen, L.X., Brandon, R.C., Rogers, Y.H., Blazej, R.G., Champe, M., Pfeiffer, B.D., Wan, K.H., Doyle, C., Baxter, E.G., Helt, G., Nelson, C.R., Gabor Miklos, GL, Abril, J.F., Agbayani, A., An, H.J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R.M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E.M., Beeson, K.Y., Benos, P.V., Berman, B.P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M.R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K.C., Busam, D.A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J.M., Cawley, S., Dahlke, C., Davenport, L.B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A.D., Dew, I., Dietz, S.M., Dodson, K., Doup, L.E., Downes, M., Dugan-Rocha, S., Dunkov, B.C., Dunn, P., Durbin, K.J., Evangelista, C.C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A.E., Garg, N.S., Gelbart, W.M., Glasser, K., Glodek, A., Gong, F., Gorrell, J.H., Gu, Z., Guan, P., Harris, M., Harris, N.L., Harvey, D., Heiman, T.J., Hernandez, J.R., Houck, J., Hostin, D., Houston, K.A., Howland, T.J., Wei, M.H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G.H., Ke, Z., Kennison, J.A., Ketchum, K.A., Kimmel, B.E., Kodira, C.D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A.A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T.C., McLeod, M.P., McPherson, D., Merkulov, G., Milshina, N.V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S.M., Moy, M., Murphy, B., Murphy, L., Muzny, D.M., Nelson, D.L., Nelson, D.R., Nelson, K.A., Nixon, K., Nusskern, D.R., Pacleb, J.M., Palazzolo, M., Pittman, G.S., Pan, S., Pollard, J., Puri, V., Reese, M.G., Reinert, K., Remington, K., Saunders, R.D., Scheeler, F., Shen, H., Shue, B.C., Siden-Kiamos, I., Simpson, M., Skupski, M.P., Smith, T., Spier, E., Spardling, A.C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A.H., Wang, X., Wang, Z.Y., Wassarman, D.A., Weinstock, G.M., Weissenbach, J., Williams, S.M., Woodage, T., Worley, K.C., Wu, D., Yang, S., Yao, Q.A., Ye, J., Yeh, R.F., Zaveri, J.S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X.H., Zhong, F.N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H.O., Gibbs, R.A., Myers, E.W., Rubin, G.M. and Venter, J.C. (2000) "The genome sequence of *Drosophila melanogaster*", *Science* **287**, 2185–2195.

Belyaeva, E.S., Aizenzon, M.G., Kiss, I., Gorelove, T.D., Pak, S.D., Umbefova, G.K., Kramers, P.G.N. and Zhimulev, I.F. (1982) "Mutations and chromosome rearrangements in the 1E3-4-2B10 region of *Drosophila melanogaster* X-chromosome", *Drosophila Information Service* **58**, 184–190.

Belyaeva, E.S., Protopopov, M.O., Baricheva, E.M., Semeshin, V.F., Izquierdo, M.L. and Zhimulev, I.F. (1987) "Cytogenetic analysis of region 2B3–4–2B11 of the X-chromosome of *Drosophila melanogaster* VI. Molecular and cytological mapping of the ecs locus and the 2B puff", *Chromosoma* **95**, 295–310.

Benos, P.V., Gatt, M.K., Ashburner, M., Murphy, L., Harris, D., Barrell, B., Ferraz, C., Vidal, S., Brun, C., Demailles, J., Cadieu, E., Dreano, S., Gloux, S., Lelaure, V., Mottier, S., Galibert, F., Borkova, D., Minana, B., Kafatos, F.C., Louis, C., Siden-Kiamos, I., Bolshakov, S., Papagiannakis, G., Spanos, L., Cox, S., Madueno, E., de Pablos, B., Modolell, J., Peter, A., Schottler, P., Werner, M., Mourkioti, F., Beinert, N., Dowe, G., Schafer, U., Jackle, H., Bucheton, A., Callister, D.M., Campbell, L.A., Darlamitsou, A., Henderson, N.S., McMillan, P.J., Salles, C., Tait, E.A., Valenti, P., Saunder, R.D. and Glover, D.M. (2000) "From sequence to chromosome: The tip of the X chromosome of *D. melanogaster*", *Science* **287**, 2220–2222.

Bridges, C.B. (1935) "Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*", *Journal of Heredity* **26**, 60–64.

Bridges, C.B. (1938) "A revised map of the salivary gland X-chromosome", *Journal of Heredity* **29**, 11–13.

DiBartolomeis, S.M., Tartof, K.D. and Jackson, F.R. (1992) "A superfamily of *Drosophila*-satellite related (SR) DNA repeats restricted to the X-chromosome euchromatin", *Nucleic Acids Research* **20**, 1113–1116.

FlyBase, (1999) "The FlyBase database of the Drosophila genome projects and community literature, Available from http://flybase.bio.indiana.edu", *Nucleic Acids Research* **27**, 85–88.

Lindsley, D.L. and Grell, E.H. (1968). "Genetic variations of *Drosophila melanogaster*", Carnegie Institute of Washington Publication No. 627.

Offerman, C.A. (1936) "Branched chromosomes as symmetrical duplications", *Journal of Genetics* **32**, 103–116.

Semeshin, V.F., Shloma, V.V. and Zhimulev, I.F. (2001) "Formation and morphology of dark puffs in *Drosophila melanogaster* polytene chromosomes", *Hereditas* **134**, 15–22.

Waring, G.L. and Pollack, J.C. (1987) "Cloning and characterization of a dispersed, multicopy, X-chromosome sequence in *Drosophila melanogaster*", *Proceedings of the National Academy of Sciences USA* **84**, 2843–2947.