5. CONCLUSIONS

Les principals conclusions d'aquest treball són:

1. S'han localitzat 55 marcadors procedents de quatre regions cromosòmiques de *D. melanogaster* als cromosomes de *D. repleta* i *D. buzzatii*. La localització d'aquests marcadors és coherent amb les homologies cromosòmiques proposades entre aquestes espècies i la gran reorganització dels elements cromosòmics que s'ha produït, deguda principalment a la fixació d'inversions paracèntriques. Els resultats també són coherents amb la proposta de nou inversions fixades en el cromosoma 2 entre *D. repleta* i *D. buzzatii*.

2. Les regions cromosòmiques analitzades no presenten diferències significatives en el nombre de punts de trencament d'inversions que contenen. Aquest resultat indica que els punts de trencament es distribueixen homogèniament per tot el cromosoma. Per tant el comportament d'una regió cromosòmica pot ser representatiu del de la resta del genoma. Això fa que amb un nombre relativament petit de marcadors es puguin obtenir bones estimes del número de reordenacions fixades entre diferents espècies.

3. Extrapolant el comportament de les regions cromosòmiques analitzades a tot el cromosoma s'obté que el número mínim d'inversions fixades entre *D. melanogaster* i les dues espècies del grup *repleta* (*D. repleta* i *D. buzzatii*) és de 88±14 inversions (0,71 inversions fixades per milió d'anys). Aquest valor no és significativament diferent de l'obtingut amb la comparació de 160 marcadors distribuïts per tot el cromosoma (114±14 inversions i una taxa de 0,92 inversions per milió d'anys). Aquests resultats mostren que la taxa d'evolució cromosòmica a *Drosophila* és superior a la de la majoria d'organismes analitzats.

4. La mida dels segments cromosòmics conservats entre *D. melanogaster* i les dues espècies del grup *repleta* (*D. repleta* i *D. buzzatii*) és compatible amb el repartiment a l'atzar dels punts de trencament de les inversions, i evidencien la gran flexibilitat que té el genoma de *Drosophila*. No s'han trobat evidències a favor de la conservació d'aquests segments degut a l'existència de restriccions funcionals.

5. S'han clonat i seqüenciat els punts de trencament de la inversió $2q^7$ de *D. buzzatii*. Als cromosomes portadors de la inversió els dos punts de trencament contenen

insercions d'elements transposables. La inversió *2q*^{7} de *D. buzzatii* es va originar per recombinació ectòpica entre dues còpies de *Galileo*, un element transposable de tipus *Foldback*. El fet que el mateix element també hagi originat la inversió *2j* de *D. buzzatii* suggereix que aquest element pot jugar un paper important en l'evolució d'aquesta espècie.

6. S'ha analitzat la variabilitat nucleotídica i estructural de les regions dels punts de trencament de la inversió *2q*^{7}. Als cromosomes portadors de la inversió les insercions d'elements transposables mostren una gran variabilitat estructural. Aquests canvis s'han produït en poc temps, ja que entre aquests cromosomes no existeix variabilitat nucleotídica. Per tant, els punts de trencament de la inversió *2q*^{7} de *D. buzzatii* dels cromosomes portadors de la inversió són punts calents d'inserció d'elements transposables i mostren una gran inestabilitat genètica. En la caracterització dels punts de trencament de la inversió *2j* s'havien obtingut resultats similars, el que suggereix que existeix algun factor que provoca aquesta inestabilitat als punts de trencament de les inversions.

7. La inestabilitat genètica descrita als punts de trencament de les inversions *2j* i *2q*^{7} es pot deure a la presència d'elements del tipus *Foldback*. Aquests elements tenen la capacitat de produir estructures secundàries, que s'ha demostrat que poden originar diferents tipus de reordenacions cromosòmiques. Aquestes estructures també augmenten la capacitat recombinogènica dels elements tipus *Foldback*, el que pot explicar la participació de *Galileo* en la generació de les dues inversions.

8. Els elements transposables trobats als punts de trencament de les inversions *2j* i *2q*^{7} no es distribueixen homogèniament entre els diferents cromosomes ni dins de cada cromosoma. El cromosoma 6 presenta un excés d'elements transposables, i a la resta de cromosomes els elements s'acumulen a les regions proximals.

9. El principal factor que determina la distribució cromosòmica dels elements transposables és probablement la taxa de recombinació. Sembla que els elements s'acumulen preferentment a les regions de baixa recombinació. La reducció de la recombinació que es produeix a les regions incloses a les inversions cromosòmiques als heterocariotips fa que en aquestes regions també s'hi acumulin elements transposables.

10. La localització dels elements transposables *BuT5* i *Galileo* a un punt de trencament de les inversions $2z^3$ i *4s* de *D. buzzatii* respectivament (a cromosomes portadors de la inversió) suggereix que aquests elements podrien estar implicats en la generació de les inversions, o que es poden haver generat punts calents per a la inserció d'elements transposables similars als descrits en les inversions *2j* i $2q^7$.

11. Existeix una associació significativa entre la localització d'alguns elements transposables i els punts de trencament d'inversions polimòrfiques de *D. buzzatii* i fixades entre espècies del complexe *buzzatii*. Tot i ser una evidència indirecta, aquestes associacions reforcen la teoria de la participació dels elements transposables en la generació de les inversions naturals.

12. Alguns dels elements transposables estudiats en aquest treball mostren una certa tendència a localitzar-se a les mateixes bandes cromosòmiques. Aquest fet podria explicar la formació de punts calents d'insercions d'elements transposables, i per tant la reducció de la recombinació no seria l'únic factor que provoca la seva acumulació als punts de trencament de les inversions.

6. REFERÈNCIES

Adams, M.D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al*. 2000. The genome sequence of *Drosophila melanogaster.* Science **287:** 2185-2195.

Ajioka, J.W., D.A. Smoller, R.W. Jones, J.P. Carulli, A.E.C. Vellek *et al*. 1991. *Drosophila genome* project: one-hit coverage in yeast artificial chromosomes. Chromosoma **100**: 495-509.

Andolfatto, P., F. Depaulis i A. Navarro. 2001. Inversion polymorphisms and nucleotide variability in *Drosophila*. Genet. Res. Camb. **77**: 1-8.

Andolfatto, P., J. D. Wall i M. Kreitman. 1999. Unusual haplotype structure at the proximal breakpoint of *In(2L)t* in a natural population of *Drosophila melanogaster*. Genetics **153:** 1297-1311.

Andrews, J., M. Smith, J. Merakovsky, M. Coulson, F. Hannan i L. E. Kelly. 1996. The stoned locus of *Drosophila melanogaster* produces a dicistronic transcript and encodes two distinct polypeptides. Genetics **143**: 1699-1711.

Arkhipova, I. R., N. V. Lyubomirskaya i Y. V. Ilyn. 1995. *Drosophila* retrotranposons. Springer-Verlag, Heidelberg.

Ashburner, M. 1989. Drosophila. A laboratory handbook. Cold spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Ashburner, M., H.L. Carson i J.N. Thompson (editors). 1982. The genetics and biology of *Drosophila.* Vol. 3b. Academic press, London.

Ashburner, M., S. Misra, J. Roote, S. Lewis, R. Blazlej *et al.* 1999. An exploration of the sequence of a 2,9 Mb region of the genome of *Drosophila melanogaster*: the *Adh* region. Genetics **153**: 179-219.

Bagnall, R. D., N. Waseem, P. M. Green i F. Giannelli. 2002. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood **99**: 168-174.

Barker, J. S. F., F. M. Sene, P. D. East i M. A. Q. R. Pereira. 1985. Allozyme and chromosomal polymorphism of *Drosophila buzzatii* in Brazil and Argentina. Genetica **67**: 161-170.

Bartolomé, C., X. Maside i B. Charlesworth. 2002. On the abundance and distribution of transposable elements in the genome of *Drosophila melanogaster*. Mol. Biol. Evol. **19**: 926-937.

BDGP. 2002. The Berkeley Drosophila Genome Project database. http://www.fruitfly.org.

Berg, D.E. i M. M. Howe. 1989. Mobile DNA. American Society for Microbiology, Washington, D.C.

Bergman, C. M., B. D. Pfeiffer, D. E. Rincón-Limas, R. A Hoskins, A. Gnirke, *et al*. 2002. Assessing the impact of comparative genomic sequence data on the functional annotation of the *Drosophila* genome. Genome Biology **3**: research0086.1-0086.20.

Besansky, N. J. i J. R. Powell. 1992. Reassociation kinetics of *Anopheles gambiae* (Diptera: Culicidae) DNA. J. Med. Entomol. **29**: 125-128.

Beverly, S. M. i A. C. Wilson. 1984. Molecular evolution in *Drosophila* and higher Dipterans. II. A time scale for fly evolution. J. Mol. Evol. **21**: 1-13.

Bingham, P.M. i Z. Zachar. 1989. Retrotransposons and the *FB* transposon from *Drosophila melanogaster*. Pp. 485-502 in D. E. Berg and M. M. Howe, eds. American Society for Microbiology, Washington, D.C.

Boeke, J. D. i S. E. Devine. 1998. Yeast retrotransposons: finding a nice quiet neighborhood. Cell **93**: 1087-1089.

Boutanaev, A. M., A. I. Kalmykova, Y. Y. Shevelyov i D. I. Nurminsky. 2002. Large clusters of co-expressed genes in the *Drosophila* genome. Nature **420**: 666-669.

Bridges, C.B. 1935. Salivary chromosome maps, with a key to the banding of the chromosomes of *Drosophila melanogaster*. J. Hered. **26**: 60-64.

Brierley, H. L. i S. S. Potter. 1985. Distinct characteristics of loop sequences of two *Drosophila* foldback transposable elements. Nucleic Acids Res. **13**: 485-500.

Brock, H.W. i D. B. Roberts. 1983. Location of the *Lsp-1* genes in *Drosophila* species by *in situ* hybridization. Genetics **103**: 75-92.

Brogna, S. i M. Ashburner. 1997. The Adh-related gene of *Drosophila melanogaster* is expressed as a functional dicistronic messenger RNA: Multigenic transcription in higher organisms. EMBO J. **16**: 2023-2031.

Busseau, I., A. Pelisson i A. Bucheton. 1989. *I* elements of *Drosophila melanogaster* generate specific chromosomal rearrangements during transposition. Mol. Gen. Genet. **218**: 222-228.

Cáceres, M., A. Barbadilla i A. Ruiz. 1997. Inversion length and breakpoint distribution in the *Drosophila buzzatii* species complex: Is inversion length a selected trait?. Evolution **51**: 1149-1155.

Cáceres, M., A. Barbadilla i A. Ruiz. 1999a. Recombination rate predicts inversion size in diptera. Genetics **153**: 251-259.

Cáceres, M. 2000. Inversiones cromosómicas en *Drosophila*: origen molecular y significado evolutivo de su tamaño. Tesi doctoral, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain.

Cáceres, M., M. Puig i A. Ruiz. 2001. Molecular characterization of two natural hotspots in the *Drosophila* genome induced by transposon insertions. Genome Res. **11**: 1353-1364.

Cáceres, M., J. M. Ranz, A. Barbadilla, M. Long i A. Ruiz. 1999b. Generation of a widespread *Drosophila* inversion by a transposable element. Science **285**: 415-418.

Cai, H., P. Kiefel, J. Yee i I. Duncan. 1994. A yeast artificial chromosome clone map of the *Drosophila* genome. Genetics **136**: 1385-1401.

Calvi, B.R., T. J. Hong, S. D. Findley i W. M. Gelbart. 1991. Evidence for a common evolutionary origin of inverted repeat transposons in *Drosophila* and plants: *hobo*, *Activator*, and *Tam3*. Cell **66:** 465-471.

Capy, P., C. Bazin, D. Higuet i T. Langin. 1998. Dynamics and evolution of transposable elements. Springer-Verlag, Heidelberg, Germany.

Carmena, M. i C. González. 1995. Transposable elements map in a conserved pattern of distribution extending from beta-heterocromatin to centromeres in *Drosophila melanogaster*. Chromosoma **103**: 676-684.

Carson, H. L. 1946. The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. Genetics **31**: 95-113.

Celniker, S. E., D. A. Wheeler, B. Kronmiller, J. W. Carlson, A. Halpern, *et al*. Finishing a whole-genome shotgun: Release 3 of the *Drosophila melanogaste*r euchromatic sequence. Genome Biology **3**: research0079.1-0079.14.

Charlesworth, B. 1996. Background selection and patterns of genetic diversity in *Drosophila melanogaster*. Genet. Res. Camb. **68**: 131-149.

Charlesworth, B. i D. Charlesworth. 1973. Selection of new inversions in multilocus genetic systems. Genet. Res.Camb. **21**: 167-183.

Charlesworth, B., A. Lapid i D. Canada. 1992. The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. II. Inferences on the nature of selection agains elements. Genet. Res. Camb. **60**: 115-130.

Charlesworth, B., P. Jarne i S. Assimacopoulos. 1994a. The distribution of transposable elements within and between chromosomes in a population of *Drosophila melanogaster*. III. Element abundances in heterochromatin. Genet. Res. Camb. **64**: 183-197.

Charlesworth, B., P. Sniegowski i W. Stephan. 1994b. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature **371:** 215-220.

Cirera, S., J. M. Martín-Campos, C. Segarra i M. Aguadé. 1995. Molecular characterization of the breakpoints of an inversion fixed between *Drosophila melanogaster* and *D. subobscura*. Genetics **139:** 321-326.

Clayton, F.E. i W.C. Guest. 1986. Overview of chromosomal evolution in the family *Drosophilidae*. A: The genetics and biology of *Drosophila*. Vol. 3e, pp.1-38. (M. Ashburner, H.L. Carson, J.N. Thompson, editors) Academic Press, London.

Coelho, P. A., J. Queroiz-Machado, D. Hartl i C. E. Sunkel. 1998. Pattern of chromosomal localization of the Hoppel transposable element family in the Drosophila melanogaster subgroup. Chromosome Res. **6**: 385-395.

Coghlan, A. i K. H. Wolfe. 2002. Fourfold faster rate of genome rearrangement i nematodes than in *Drosophila*. Genome Res. **12**: 857-867.

Collins, M. i G. M. Rubin. 1983. High-frequency precise excision of the *Drosophila* foldback transposable element. Nature **303**: 259-260.

Collins, M. i G. M. Rubin. 1984. Structure of chromosomal rearrangements induced by the *FB* transposable element in *Drosophila*. Nature **308**: 323-327.

Colombo, P., J. Yon, K. Garson i M. Fried. 1992. Conservation of the organization of five tightly clustered genes over 600 million years of divergent evolution. Proc. Natl. Acad. Sci. USA **89**: 6358-6362.

Coyne, J. A., W. Meyers, A. P. Crittenden i P. Sniegowski. 1993. The fertility effects of pericentric inversions in *Drosophila melanogaster*. Genetics **134**: 487-496.

Craig, N. L. 1997. Target site selection in transposition. Annu. Rev. Biochem. **66**: 437-474.

Daveran-Mingot, M.-L., N. Campo, P. Ritzenthaler i P. le Bourgeois. 1998. A natural large chromosomal inversion in *Lactococcus lactis* is mediated by homologous recombination between two insertion sequences. J. Bacteriol. **180:** 4834-4842.

Dimitri, P. 1997. Constitutive heterochromatin and transposable elements in *Drosophila melanogaster*. Genetica **100**: 85-93.

Dimitri, P., B. Arca, L. Berghella i E. Mei. 1997. High genetic instability of heterochromatin after transposition of the LINE-like *I* factor in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA. **94**: 8052-8057.

Dowsett, A. P. i M. W. Young. 1982. Differing levels of dispersed repetitive DNA among closely related species of *Drosophila*. Proc. Natl. Acad. Sci. USA **79**: 4570-4574.

Drosopoulou, E. i Z.G. Scouras. 1995. The β-tubulin gene family evolution in the *Drosophila montium* subgroup of the *melanogaster* species group. J. Mol. Evol **41**: 293-298.

Duret, L., G. Marais i C. Biémont. 2000. Transposons but no retrotransposons are located preferentially in regions of high recombination rate in *Caenorhabditis elegans*. Genetics **156**: 1661-1669.

Eanes, W. F., C. Wesley i B. Charlesworth. 1992. Accumulation of P elements in minority inversions in natural populations of *Drosophila melanogaster*. Genet. Res. Camb. **59**: 1-9.

Engels, W. R. 1992. The origin of P elements in *Drosophila melanogaster*. BioEssays **14**: 681-686.

Engels, W. R. i C. R. Preston. 1984. Formation of chromosome rearrangements by *P* factors in *Drosophila*. Genetics **107**:657-678.

Evgen'ev, M. B., H. Zelentsova, H. Poluectova, G. T. Lyozin, V. Veleikodvorskaja *et al*. 2000. Mobile elements and chromosomal evolution in the virilis group of *Drosophila*. Proc. Natl. Acad. Sci. USA **97**: 11337-11342.

Federer, W. T., R. G. D. Steel i B. Wallace. 1967. Mathematical models for chromosomal inversions. Genetics **55**: 783-795.

Finnegan, D.J. 1989. Eukaryotic transposable elements and genome evolution. Trends Genet. **5:** 103-107.

FlyBase. 1999. The Flybase database of the *Drosophila* genome projects and community literature. Nucleic Acids Res. **27**: 85-88. http://flybase.bio.indiana.edu/

Fontdevila, A., A. Ruiz, G. Alonso i J. Ocaña. 1981. The evolutionary history of *Drosophila buzzatii*. I. Natural chromosomal polymorphism in colonized populations of the Old World. Evolution **35**: 148-157.

Fontdevila, A., A. Ruiz, J. Ocaña i G. Alonso. 1982. The evolutionary history of *Drosophila buzzatii*. II. How much has chromosomal polymorphism changed in colonization?. Evolution **36**: 843-851.

Francino, O., O. Cabré i A. Fontdevila. 1993. Distribution of the copia transposable element in the *repleta* group of *Drosophila*. Genet. Sel. Evol. **25**: 501-516.

García Guerreiro, M. P. i A. Fontdevila. 2001. Chromosomal distribution of the transposable elements *Osvaldo* and *blanco* in original and colonizer populations of *Drosophila buzzatii*. Genet. Res. Camb. **77**: 227-238.

Gérard, M., J. Y. Chen, H. Gronemeyer, P. Chambon, D. Duboule i J. Zakany. 1996. In vivo targeted mutagenesis of a regulatory element required for positioning the Hoxd-11 and Hoxd-10 expression boundaries. Genes and Development **10**: 2326-2334.

Gerasimova, T. I., K. Byrd i V. G. Corces. 2000. A chromatin insulator determines the nuclear localization of DNA. Mol. Cell **6**: 1025-1035.

Gibson, T. Y., A. Rosenthal, R.H. Waterston. 1987. Lorist6, a cosmid vector with *Bam*HI, *Not*I, *Sca*I and *Hind*III cloning sites and altered neomycin phosphotransferase gene expression. Gene **53**: 283-286.

Goldberg, M. L., R. Paro i W. J. Gehring. 1982. Molecular cloning of the *white* locus region of *Drosophila melanogaster* using a large transposable element. EMBO J. **1**: 93-98.

Goldberg, M. L., J. Y. Sheen, W. J. Gehring i M. M. Green. 1983. Unequal crossing-over associated with asymmetrical synapsis between nomadic elements in the *Drosophila melanogaster* genome. Proc. Natl. Acad. Sci. USA **80**: 5017-5021.

Goldman, A. S. H. i M. Lichten. 1996. The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. Genetics **144**: 43-55.

Golic, K. G. i M. M. Golic. 1996. Engineering the Drosophila genome: chromosome rearrangements by design. Genetics **144**: 1693-1711.

González, J., E. Betrán, M. Ashburner i A. Ruiz. 2000. Molecular organization of the *Drosophila melanogaster Adh* chromosomal region in *D. repleta* and *D. buzzatii*, two distantly related species of the *Drosophila* subgenus. Chromosome Res. **8**: 375-385.

González, J., J. M. Ranz i A. Ruiz. 2002. Chromosomal elements evolve at different rates in the *Drosophila* genome. Genetics **161**: 1137-1154.

Gordenin, D. A., K. S. Lobachev, N. P. Degtyareva, A. L. Malkova, E. Perkins i M. A. Resnick. 1993. Inverted DNA repeats: a source of eukaryotic genomic instability. Mol. Cell. Biol. **13**:5315-5322.

Gordenin, D. A. i M. A. Resnick. 1998. Yeast ARMs (DNA at-risk motifs) can reveal sources of genome instability. Mutation Research **400**: 45-58.

Gordo, I. i B. Charlesworth. 2001. Genetic linkage and molecular evolution. Curr. Biol. **11**: R684-R686.

Gould, A., A. Morrison, G. Sproat, R. White i R. Krumlauf. 1997. Positive cross-regulation and enhancer sharing: Two mechanisms for specifying overlapping Hox expression patterns. Genes and Development **11**: 900-913.

Gray, Y. H. M. 2000. It takes two transposons to tango. Transposable-element mediated chromosomal rearrangements. Trends Genet. **16**: 461-468.

Gray, Y. H. M., M. Tanaka i J. A. Sved. 1996. *P*-element induced recombination in *Drosophila melanogaster*: hybrid element insertion. Genetics **144**: 1601-1610.

Gubenko, I.S. i M.B. Evgen´ev. 1984. Cytological and linkage maps of *Drosophila virilis* chromosomes. Genetica **65**: 127-139.

Haber, J. E., W. Y. Leung, R. H. Borts i M. Lichten. 1991. The frequency of meiotic recombination in yeast is independent of the number and position of homologous donor sequences: implications for chromosome pairing. Proc. Natl. Acad. Sci. USA **88**: 1120-1124.

Hagstrom, K., M. Muller i P. Schedl. 1996. Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila* bithorax complex. Genes and Development **11**: 900-913.

Harden, N. i M. Ashburner. 1990. Characterization of the *FOB-NOF* transposable element of *Drosophila melanogaster*. Genetics **126**: 387-400.

Hartl, D.L. i E.R. Lozovskaya .1994. Genome evolution: between the nucleosome and the chromosome. EXS. **69**: 579-592.

Hartl, D. L., D. Nurminsky, R. W. Jones i E. R. Lozovskaya. 1994. Genome structure and evolution in *Drosophila*: applications of the framework P1 map. Proc. Natl. Acad. Sci. USA **91**: 6824-6829.

Hartl, D.L. i E.R. Lozovskaya. 1995. The *Drosophila* genome map: a practical guide. Springer-Verlag, New York

Hashimoto, C., K.L. Hudson i K.V. Anderson. 1998. The *Toll* gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell **52**: 269-279.

Hasson, E., H. Naveira i A. Fontdevila. 1992. The breeding sites of Argentinian cactophilic species of the *Drosophila mulleri* complex (subgenus *Drosophila-repleta* group). Rev. Chilena de Historia Natural **65**: 319-326.

Hasson, E., C. Rodríguez, J. J. Fanara, H. Naveira, O. A. Reig i A. Fontdevila. 1995. The evolutionary history of *Drosophila buzzatii*. XXVI. Macrogeographic patterns of inversion polymorphism in New World populations. J. Evol. Biol. **8**: 369-384.

Hasson, E. i W. F. Eanes. 1996. Contrasting histories of three gene regions associated with *In(3L)Payne* of *Drosophila melanogaster*. Genetics **144:** 1565-1575.

Heberlein, U. i G.M. Rubin. 1990. Structural and functional comparisons of the *Drosophila virilis* and *Drosophila melanogaster* rough genes. Proc. Natl. Acad. Sci. USA **87**: 5916-5922.

Heino, T.I., A. O. Saura i V. Sorsa. 1994. Maps of the salivary gland chromosomes of *Drosophila melanogaster*. Dros. Inform. Serv. **73**: 621-738.

Hennig, W. 1999. Heterochromatin. Chromosoma **108**: 1-9.

Hill, W. G. i A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genet. Res. Camb. **8**: 269-294.

Holt, R. A, G. M. Subramanian, A. Halpern, G. G. Sutton, R. Charlab *et al*. 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. Science **298**: 129-149.

Hooper, J.E., M. Pérez-Alonso, J.R. Bermingham, M. Prout, B.A. Rocklein, *et al*. 1992. Comparative studies of Drosophila *antennapedia* genes. Genetics **132**: 453-469.

Hoskins, R. A., C. D. Smith, J. W. Carlson, A. B. Carvalho, A. Halpern *et al*. 2002. Heterochromatic sequences in a *Drosophila* whole-genome shotgun assembly. Genome Biology **3**: research0082.1-0085.16.

Hughes, J. F. i J. M. Coffin. 2001. Evidence for genomic rearrangements mediated by human endogenous retroviruses during primate evolution. Nat. Genet. **29**: 487-489.

Huynen, M. A., B. Snel i P. Bork. 2001. Inversions and the dynamics of eukaryotic gene order. Trends in Genetics **17**: 304-306.

Ji, Y., E. E. Eichler, S. Schwartz i R. Nicholls. 2000. Structure of chromosomal duplicons and their role in mediating human genomic disorders. Genome Res. **10**: 597-610.

John, B. i G. Miklos. 1988. The eukaryote genome in development and evolution. Allen and Unwin, London.

Johnston, J. R, Ed. 1994. Molecular genetics of yeast. A practical approach. The Practical Approach Series, IRL Press.

Junakovic, N., A. Terrinoni, C. Di Franco, C. Vieira i C. Loevenbruck. 1998. Accumulation of transposable elements in the heterochromatin and on the Y chromosome of *Drosophila simulans* and *Drosophila melanogaster*. J Mol Evol **46**: 661-668.

Kafatos, F.C., C. Louis, C. Savakis, D.M. Glover, M. Ashburner *et al*. 1991. Integrated maps of the *Drosophila* genome: progress and prospects. Trends in Genetics **7**: 155-161.

Kaminker, J. S., C. Bergman, B. Kronmiller, J. Carlson, R. Svirskas *et al*. 2002. The transposable elements of the *Drosophila melanogaster* euchromatin: a genomics perspective. Genome Biology **3**: research0084.1-0084.20.

Karch, F., M. Galloni, L. Sipos, J. Gausz, H. Gyurkovics i P. Schedl. 1994. Mcp and Fab-7 map: Molecular analysis of putative boundaries of cis-regulatory domains in the bithorax complex of *Drosophila melanogaster*. Nucleic Acids Res. **22**: 3138-3146.

Karsch-Mizrachi,I. i S.R. Haynes. 1993. The *Rb97D* gene encodes a potential RNA-binding protein required for spermatogenesis in *Drosophila*. Nucleic Acids Research **21**: 2229-2235.

Kaufman, T.C., M.A. Seeger i G. Olsen. 1990. Molecular and genetic organization of the *Antennapedia* gene complex of *Drosophila melanogaster*. Adv. Gen. **27**: 309-362.

Kidwell, M. G. i D. Lisch. 1997. Transposable elements as sources of variation in animals and plants. Proc. Natl. Acad. Sci. USA. **94**: 7704-7711.

Kim, J.M., S. Vanguri, J. D. Boeke, A. Gabriel i D. F. Voytas. 1998. Transposable elements and genome organization: A comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. Genome Res. **8:** 464-478.

Kimmerly, W., K. Stultz, S. Lewis, K. Lewis, V. Lustre *et al*. 1996. A P1-based physical map of the *Drosophila* euchromatic genome. Genome Res. **6**: 414-430.

Koryakov, D. E., A. A. Alekseyenko i I. F. Zhimulev. 1999. Dynamic organization of the beta-heterochromatin in the *Drosophila melanogaster* polytene X chromosome. Mol. Gen. Genet. **260**: 503-509.

Kreitman, M. i R. R. Hudson. 1991. Inferring the evolutionary histories of the *Adh* and *Adh-dup* loci in *Drosophila melanogaster* from patterns of polymorphism and divergence. Genetics **127:** 565-582.

Kress, H. 1993. The salivary gland chromosomes of *Drosophila virilis*: a cytological map, pattern of transcription and aspects of chromosome evolution. Chromosoma **102**: 734-742.

Krimbas, C.B. i J. R. Powell. 1992. *Drosophila* inversion polymorphism. CRC Press, Boca Ratón, FL.

Kuhn, G. C. S., A. Ruiz, M. A. R. Alves i F. M. Sene. 1996. The metaphase and polytene chromosomes of *Drosophila seriema* (*repleta* group; *mulleri* subgroup). Rev. Brasil. Genét. **19**: 209-216.

Kurahashi, H., T. H. Shaik, P. Hu, B. A. Roe, B. S. Emanuel i M. L. Budarf. 2000. Regions of genomic instability on 22q11 and 11q23 as the etiology for the recurrent constitutional t(11;22). Human Molecular Genetics **9**: 1665-1670.

Laayouni, H., M. Santos i A. Fontdevila. 2000. Toward a physical map of *Drosophila buzzatii*: use of randomly amplified polymorphic DNA polymorphisms and sequence-tagged site landmarks. Genetics **156**: 1797-1816.

Labrador, M. i A. Fontdevila. 1994. High transposition rates of *Osvaldo*, a new *Drosophila buzzatii* retrotransposon. Mol. Gen.Genet. **245:** 661-674.

Labrador, M., M. Seleme i A Fontdevila. 1998. The evolutionary history of *Drosophila buzzatii*. XXXIV. The distribution of the retrotranposon *Osvaldo* in original and colonizing populations. Mol. Biol. Evol. **15**: 1532-1547.

Ladeveze, V., S. Aulard, N. Chaminade, G. Periquet, F. Lemeunier. 1998. Hobo transposons causing chromosomal breakpoints. Proc. R. Soc. Lond. B. Biol. Sci **265**: 1157-1159.

Lagerstedt, K., S. L. Karsten, B-M. Carlberg, W. J. Kleijer, T. Tönnesen *et al*. 1997. Double-strand breaks may initiate the inversion mutation causing the Hunter syndrome. Hum. Mol. Genet. **6**: 627-633.

Laird, C. D. 1973. DNA of *Drosophila* chromosomes. Annu. Rev. Genet. **7**: 177-204.

Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody *et al*. 2001. Initial sequencing and analysis of the human genome. Nature **409**: 860-921.

Langley, C. H., E. Montgomery, R. Hudson, N. Kaplan i B. Charlesworth. 1988. On the role of unequal exchange in the containment of transposable element copy number. Genet. Res. **52**: 223-235.

Lathe III, W. C., W. D. Burke, D. G. Eickbush i T. H. Eickbush. 1995. Evolutionary stability of the *R1* retrotransposable element in the genus *Drosophila*. Mol. Biol. Evol. **12**: 1094-1105.

Lefevre, G. Jr. 1976. A photoghraphyc representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. A: *The genetics and biology* of *Drosophila* (ed. M. Ashburner et al.), pp. 31-36. Academic Press, London.

Lemeunier, F. i S. Aulard. 1992. Inversion polymorphism in *Drosophila melanogaster*, pp. 339-405 a Drosophila Inversion Polymorphism. Eds. C. B. Krimbas i J. R. Powell. CRC Press, Boca Ratón, FL.

Levitan, M. 1992. Chromosomal variation in *Drosophila robusta*, pp. 221-338 a Drosophila Inversion Polymorphism. Eds. C. B. Krimbas i J. R. Powell. CRC Press, Boca Ratón, FL.

Levis, R., M. Collins i G. M. Rubin. 1982. FB elements are the common basis for the instability of th w^{DZL} and w^{c} Drosophila mutations. Cell **30**: 551-565.

Lewin, B. 1994. Genes VI. Oxford University Press Inc., New York.

Liao, G-C., E. J. Rehm, and G. Rubin. 2000. Insertion site preferences of the P transposable element in *Drosophila melanogaster.* Proc. Nat. Acad. Sci. USA **97**: 3347-3351.

Lim, J.K. 1988. Intrachromosomal rearrangements mediated by *hobo* transposons in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **85**: 9153-9157.

Lim, J.K. i M. J. Simmons. 1994. Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. Bioessays **16**: 269-275.

Lindsley, D. L. i G. G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press, Inc. San Diego. California.

Lobachev, K. S., B. M. Shor, H. T. Tran, W. Taylor, J. D. Keen *et al*. 1998. Factors affecting inverted repeat stimulation of recombination and deletion in *Saccharomyces cerevisiae*. 1998. Genetics **148**: 1507-1524.

Louis, C., E. Madueño, J. Modolell, M.M. Omar, G. Papagiannakis *et al*. 1997. One-hundred and five new potential *Drosophila melanogaster* genes revealed through STS analysis. Gene **195**: 187-193.

Loukas, M. i F.C. Kafatos. 1986. The actin loci in the genus *Drosophila*: establishment of chromosomal homologies among distantly related species by *in situ* hybridization. Chromosoma **94**: 297-308.

Lozovskaya, E. R., D. A. Petrov i D. L. Hartl. 1993. A combined molecular and cytogenetic approach to genome evolution in *Drosophila* using large-fragment DNA cloning. Chromosoma **102**: 253-266.

Lundin, L. G. 1993. Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse. Genomics **16**: 1-19.

Lyttle, T.W. i D. S. Haymer. 1992. The role of the transposable element *hobo* in the origin of endemic inversions in wild populations of *Drosophila melanogaster*. Genetica **86:** 113-126.

Madueño, E., G. Papagiannakis, G. Rimmington, R.D.C. Saunders, C. Savakis i I. Sidén-Kiamos. 1995. A physical map of the X chromosome of *Drosophila melanogaster*: cosmid contigs and sequence tagged sites. Genetics **139**: 1631-1647.

Maier, D., B.M. Marte, W. Schäfer i Y. Yu, A. Preiss. 1993. *Drosophila* evolution challenges postulated redundancy in the *E(spl)* gene complex. Proc. Natl. Acad. Sci. USA **90**: 5464-5468.

Maier, D., A. Preiss i J.R. Powell. 1990. Regulation of the segmentation gene *fushi tarazu* has been functionally conserved in *Drosophila*. EMBO J. **9**: 3957-3966.

Marín, I. i A. Fontdevila. 1995. Characterization of Gandalf, a new inverted-repeat transposable element of *Drosophila Koepferae*. Mol. Gen. Genet. **248**: 423-433.

Marín, I. i A. Fontdevila. 1996. Evolutionary conservation and molecular characteristics of repetitive sequences of *Drosophila koepferae*. Heredity **76:** 355-366**.**

Martínez-Cruzado, J.C., C. Swimmer, M.C. Fenerjian i F.C. Kafatos. 1998. Evolution of the autosomal chorion locus in *Drosophila*. I. General organization of the locus and sequence comparisons of genes *s15* and *s19* in evolutionary distant species. Genetics **119**: 663-677.

Maside, X., C. Bartolomé, S. Assimacopoulos i B. Charlesworth. 2001. Rates of movement and distribution of transposable elements in *Drosophila melanogaster*: *in situ* hybridization vs Southern blotting data. Genet. Res. Camb. **78**: 121-136.

Mathiopoulos, K.D., A. della Torre, V. Predazzi, V. Petrarca i M.Coluzzi. 1998. Cloning of inversion breakpoints in the *Anopheles gambiae* complex traces a transposable element at the inversion junction. Proc. Natl. Acad. Sci. USA **95:** 12444-12449.

McDonald, J.F. 1993. Evolution and consequences of transposable elements. Curr. Opin. Genet. Dev. **3**: 855-864.

Merriam, J., M. Ashburner, D.L. Hartl i F.C. Kafatos. 1991. Towards cloning and mapping the genome of *Drosophila*. Science: **254**: 221-225.

Mongelard, F. i V. G. Corces. 2001. Two insulators are not better than one. Nature Structural Biology **8**: 192-194.

Montgomery, E., B. Charlesworth i C.H. Langley. 1987. A test for role of natural selection in the stabilization of trasposable element copy number in a population of *Drosophila melanogaster.* Genet. Res. Camb. **49**: 31-41.

Montgomery, E., S.-M. Huang, C. H. Langley i B. H. Judd. 1991. Chromosome rearrangement by ectopic recombination in *Drosophila melanogaster*: Genome structure and evolution. Genetics **129**: 1085-1098.

Moriyama, E.N., D.M. Petrov i D.L. Hartl. 1998. Genome size and intron size in *Drosophila*. Mol. Biol. Evol. **15**: 770-773.

Muller, J. H. 1940. Bearings of the *Drosophila* work on systematics. A: *New Systematics*, pp 185-268. Editat per J. Huxley. Clarendon Press, Oxford.

Nadeau, J. H. i B.A. Taylor. 1984. Lenghts of chromosomal segments conserved since divergence of man and mouse. Proc. Natl. Acad. Sci. USA **81**: 814-818.

Nadeau, J. H. i D. Sankoff. 1998. Counting on comparative maps. Trends Genet. **14**: 495-501.

Nag, D. K. i A. Kurst. 1997. A 140-bp-long palindromic sequence induces double-strand breaks during meiosis in the yeast *Sacharomyces cerevisiae*. Genetics **146**: 835-847.

Navarro, A., E. Betrán, A. Barbadilla i A. Ruiz. 1997. Recombination and gene flux caused by gene conversion and crossing over in inversion heterokaryotypes. Genetics **146**: 695-709.

Naveira, H. i A. Fontdevila. 1985. The evolutionary history of *Drosophila buzzatii*. IX. High frequencies of new chromosome rearrangements induced by introgressive hybridization. Chromosoma **91**: 87-94.

Naylor, J. A., D. Buck, P. Green, H. Williamson, D. Bentley i F. Giannelli. 1995. Investigation of the factor VIII intron 22 repeated region (*int22h*) and the associated inversion junctions. Hum. Mol. Genet. **4**: 1217-1224.

Negre, B., J. M. Ranz, F. Casals, M. Cáceres i A. Ruiz. 2003. A new split of the HOX gene complex in the *repleta* group of *Drosophila*. Enviat.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nurminsky, D. I., E. N. Moriyama, E. R. Lozovskaya i D. L. Hartl. 1996. Molecular phylogeny and genome evolution in the *Drosophila virilis* species group: duplications of the *alcohol dehydrogenase* gene. Mol. Biol. Evol. **13**: 132-149.

O'Hare, K. i G. M. Rubin. 1983. Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34**: 25-35.

Papaceit, M. i E. Juan. 1993. Chromosomal homologies between *Drosophila lebanonensis* and *D. melanogaster* determined by *in situ* hybridization. Chromosoma **102**: 361-368.

Pardue, M. L., S. A. Gerbi, R. A. Eckhardt i J. G. Gall. 1970. Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera. Chromosoma **29**: 268-290.

Patterson, J.T. i W.S. Stone. 1952. Evolution in the genus *Drosophila*. Macmillan, New York.

Petes, T.D. i C. W. Hill. 1988. Recombination between repeated genes in microorganisms. Annu. Rev. Genet. **22**: 147-168.

Pimpinelli, S., M. Berloco, L. Fanti, P. Dimitri, S. Bonaccorsi *et al*. 1995. Transposable elements are stable structural components of *Drosophila melanogaster* heterochromatin. Proc. Natl. Acad. Sci. USA. **92**: 3804-3808.

Piñol, J., O. Francino, A. Fontdevila i O. Cabré. 1988. Rapid isolation of *Drosophila* high molecular weight DNA to obtain genomic libraries. Nucleic Acids Res. **16**: 2736.

Plasterk, R. H. A. 1995. Mechanisms of DNA transposition. A Mobile Genetic Elements. Ed. D. J. Sherratt. Oxford University Press, Oxford.

Plata-Rengijo, P. 1995. Clonación y secuenciación de un mutante white de *Drosophila buzzatii* que contiene un nuevo elemento móvil. Treball de recerca. Universitat Autònoma de Barcelona.

Powell, J. R. 1992. Inversion polymorphism in *Drosophila pseudoobscura* and *Drosophila persimilis*, pp. 73-126 a *Drosophila* Inversion Polymorphism. Editors: B. Krimbas i J. R. Powell. CRC Press, Boca Ratón, FL.

Powell, J.R. i R. DeSalle. 1995. *Drosophila* molecular phylogenies and their uses. Evol. Biol. **28**: 87-138.

Powell, J. R. 1997. Progress and prospects in evolutionary biology: the *Drosophila* model. Oxford University Press, New York.

Randazzo, F. M., M. A. Seeger, C. A. Huss., M. A. Sweeney, J. K. Cecil i T. C. Kaufman. 1993. Structural changes in the *Antennapedia* complex of *Drosophila pseudoobscura*. Genetics **133**: 319-330.

Ranz, J.M., C. Segarra i A. Ruiz. 1997. Chromosomal homology and molecular organization of muller´s elements *D* and *E* in the *Drosophila repleta* species group. Genetics **145**: 281-295.

Ranz, J.M. 1998. Cartografía física comparada de los elementos cromosómicos *E* y *D* en el género *Drosophila*. Tesi doctoral. Universitat Autònoma de Barcelona.

Ranz, J.M., M. Cáceres i A. Ruiz. 1999. Comparative mapping of cosmids and gene clones from a 1.6 Mb chromosomal region of *Drosophila melanogaster* in three species of the distantly related subgenus *Drosophila*. Chromosoma **108:** 32-45.

Ranz. J. M., F. Casals i A. Ruiz. 2001. How malleable is the eukaryotic genome? Extreme rate of chromosomal rearrangement in the genus *Drosophila*. Genome Res. **11:** 230-239.

Ranz, J. M., J. González, F. Casals i A. Ruiz. 2003. Low occurrence of gene transposition events during the evolution of the genus *Drosophila*. Evolution, en premsa.

Regner, L. P., M. S. O. Pereira, C. E. V. Alonso, E. Abdelhay. i V. L. S. Valente. 1996. Genomic distribution of *P* elements in *Drosophila willistoni* and a search for their relationship with chromosomal inversions. J. Hered. **87:** 191-198.

Rizzon, C., G. Marais, M. Gouy i C. Biemont. 2002. Recombination rate and the distribution of transposable elements in the *Drosophila melanogaster* genome. Genome Res. **12**: 400-407.

Roberts, D.B. i S. Evans-Roberts. 1979. The genetic and cytogenetic localization of the three structural genes coding for the major protein of *Drosophila* larval serum. Genetics **93**: 663-679.

Robin, G. C., C. Claudianos, R. J. Russell i J. G. Oakeshott. 2000. Reconstructing the diversification of alpha-esterases: comparing the gene clusters of *Drosophila buzzatii* and *D. melanogaster*. J Mol Evol **51**: 149-160.

Rodríguez-Trelles, F., G. Álvarez i C. Zapata. 1996. Time-series analysis of seasonal changes of the *O* inversion polymorphism of *Drosophila subobscura*. Genetics **142**: 179-187.

Rodríguez-Trelles, F., L. Alarcón i A. Fontdevila. 2000. Molecular evolution of the *buzzatii* complex (*Drosophila repleta* group): a maximum-likelihood approach. Mol. Biol. Evol. **17**: 1112-1122.

Rozas, J., C. Segarra, G. Ribó i M. Aguadé. 1999. Molecular population genetics of the *rp49* gene region in different chromosomal inversions of *Drosophila suboscura*. Genetics **151**: 189-202.

Rozas, J. i R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics **15**: 174-175.

Ruddle, F. H., J. L. Bartels, K. L. Bentley, C. Kappen, M. T. Murtha. 1994. Evolution of HOX genes. Ann. Rev. Genet. **28**: 423-442.

Ruiz, A. i A. Fontdevila. 1981. Ecología y evolución del subgrupo *mulleri* de *Drosophila* en Venezuela y Colombia. Acta Científica Venezolana **32**: 338-345.

Ruiz, A., A. Fontdevila i M. Wasserman. 1982. The evolutionary history of *Drosophila buzzatii*. III. Cytogenetic relationships between two sibling species of the *buzzatii* cluster. Genetics **101**: 503-518.

Ruiz, A., H. Naveira i A. Fontdevila. 1984. La historia evolutiva de *Drosophila buzzatii* IV. Aspectos citogenéticos de su polimorfismo cromosómico. Genética Ibérica **36**: 13-35.

Ruiz, A., M. Santos, A. Barbadilla, J. E. Quezada-Díaz, E. Hasson i A. Fontdevila. 1991. Genetic variance for body size in a natural population of *Drosophila buzzatii*. Genetics **128**: 739-750.

Ruiz, A. i M. Wasserman. 1993. Evolutionary cytogenetics of the *Drosophila buzzatii* species complex. Heredity **70**: 582-596.

Ruiz, A., J. M. Ranz, M. Cáceres, C. Segarra, A. Navarro i A. Barbadilla. 1997. Chromosomal evolution and comparative gene mapping in the *Drosophila repleta* species group. Braz. J. Genet. **20**: 553-565.

Ruiz, A., A. M. Cansian, G. C. S. Kuhn, M. A. R. Alves i F. M. Sene. 2000. The *Drosophila serido* speciation puzzle: putting new pieces together. Genetica **108**: 217-227.

Russo, C.A.M., N. Takezaki i M. Nei. 1995. Molecular phylogeny and divergence times of drosophilid species. Mol. Biol. Evol. **4**: 406-425.

Sambrook, J., E. F. Fritsch i T. Maniatis. 1989. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SanMiguel, P., B. S. Gaut, A. Tikhonov, Y. Nakajima i J. L. Bennetzen. 1998. The paleontology of intergene retrotransposons of maize. Nat. Genet. **20**: 43-45.

SanMiguel, P., A. Tikhonov, Y. K. Jin, N. Motchoulskaia, D. Zakharov *et al*. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science **274**: 765-768.

Schafer, D. J., D. K Fredline, W. R. Knibb, M. M. Green i J.S.F. Baker. 1993. Genetics and linkage mapping of *Drosophila buzzatii*. J. Hered. **84**: 188-194.

Schaffer, L. G. i J. R. Lupski. 2000. Molecular mechanisms for constitutional chromosomal rearrangements in humans. Annu. Rev. Genet. **34**: 297-329.

Schneuwly, S., A. Kuroiwa i W. J. Gehring. 1987. Molecular analysis of the dominant homeotic *Antennapedia* phenotype. EMBO J. **6**:201-206.

Schulze, D.H. i C.S. Lee. 1986. DNA sequence comparison among closely related *Drosophila* species of the *mulleri* complex. Genetics **113**: 287-303.

Schwartz, A., D. C. Chan, L. G. Brown, R. Alagappan, D. Pettay *et al*. 1998. Reconstructing hominid Y evolution: X-homologous block, created by X-Y transposition, was disrupted by Yp inversion through LINE-LINE recombination. Hum. Mol. Genet. **7:** 1-11.

Segarra, C. i M. Aguadé. 1992. Molecular organization of the *X* chromosome in different species of the *obscura* group of *Drosophila.* Genetics **130**: 513-521.

Segarra, C., E. R. Lozovskaya, G. Ribó, M. Agaudé i D. L. Hartl. 1995. P1 clones from *Drosophila melanogaster* as markers to study the chromosomal evolution of Muller's A element in two species of the obscura group of *Drosophila*. Chromosoma **104**: 129-136.

Seoighe, C., N. Federspiel, T. Jones, N. Hansen, V. Bivolarovic *et al*. 2000. Prevalence of small inversions in yeast gene order evolution. Proc. Natl. Acad. Sci. USA **97**: 14433-14437.

Shapiro, J.A. i B. Cordell. 1982. Eukaryotic Mobile and Repeated Genetic Elements. Biol. Cell. **43**: 31-54.

Sidén-Kiamos, I., R.D.C. Saunders, L. Spanos, T. Majerus, J. Treanear *et al*. 1990. Towards a physical map of the *Drosophila melanogaster* genome: mapping of cosmid clones within defined genomic divisions. Nucleic Acids Research **18**: 6261-6270.

Small, K., J. Iber i T. Warren. 1997. Emerin deletion reveals a common X-chromosome inversion mediated by inverted repeats. Nat. Genet. **16**: 96-99.

Smith, P.A i V.G. Corces. 1991. *Drosophila* transposable elements: Mechanisms of mutagenesis and interactions with the host genome. Adv. Genet. **29**: 229-300.

Smoller, D. A., D. Petrov i D. L. Hartl. 1991. Characterization of bacteriophage P1 library containing inserts of *Drosophila* DNA of 75-100 kilobase pairs. Chromosoma **100**: 487-494.

Sniegowski, P.D. i B. Charlesworth. 1994. Transposable element numbers in cosmopolitan inversions from a natural population of *Drosophila melanogaster*. Genetics **137:** 815-827.

Sorsa, V. 1988. Polytene chromosomes in genetic research. Ellis Horwood Limited, Chichester.

Spellman, P. T. i G. Rubin. 2002. Evidence for large domains of similarly expressed genes in the *Drosophila* genome. J. Biol. **1**: 5.

Sperlich, D. 1963. Chromosomal polymorphism and position effect in *Drosophila subobscura*. Proc. 11th Int. Congr. Genet. **1**: 166.

Sperlich, D. i P. Pfriem. 1986. Chromosomal polymorphism in natural and experimental populations. Pp. 257-309 in M. Ashburner, H. L. Carson i J. N. Thompson, eds. The genetics and biology of *Drosophila*. Vol. 3. Jr. Academic Press, London.

Spicer, G. S. 1988. Molecular evolution among some *Drosophila* species groups as indicated by two-dimensional electrophoresis. J. Mol. Evol. **27**: 250-260.

Spradling, A. C. i G. M. Rubin. 1981. Drosophila genome organization: conserved and dynamic aspects. Annu. Rev. Genet. **15**: 219-264.

Spradling, A.C., D.M. Stern, I. Kiss, J. Roote, T. Laverty i G.M. Rubin. 1995. Gene disruptions using P transposable elements: an integral component of the *Drosophila* genome project. Proc. Natl. Acad. Sci. USA 92: 10824-10830.

Stalker, H.D. 1972. Intergroup phylogenies in *Drosophila* as determined by comparisons of salivary banding patterns. Genetics **70**: 457-474.

Stathakis, D.G., E.S. Pentz, M.E. Freeman, J. Kullman, G.R. Hankins *et al*. 1995. The genetic and molecular organization of the *dopa decarboxylase* gene cluster of *Drosophila melanogaster*. Genetics **141**: 629-655.

Steinemann, U.M. 1982. Analysis of chromosomal homologies between two species of the subgenus *Sophophora*: *D. miranda* and *D. melanogaster* using cloned DNA segments. Chromosoma **87**: 77-87.

Steinemann, U.M., W. Pinsker i D. Sperlich. 1984. Chromosome homologies within the *Drosophila obscura* group probed by *in situ* hybridization. Chromosoma **91**: 46-53

Stone, W.S., W.C. Guest i F.D. Wilson. 1960. The evolutionary implications of the cytological polymorphism and phylogeny of the *virilis* group of *Drosophila*. Proc. Natl. Acad. Sci. USA **46**: 350-361.

Streck, R. D., J. E. MacGaffey i S. K. Beckendorf. 1986. The structure of *hobo* transposable elements and their insertion sites. EMBO J. **5**: 3615-3623.

Strutt, D. I. i R.A. White. 1994. Characterization of *T48*, a target of homeotic gene regulation in *Drosophila* embryogenesis. Mech. Dev. **46**: 27-39.

Stutervant, A.H. 1913. The linear arrangement of sex-linked factors in *Drosophila*, as shown by their mode association. J. Exp. Zool. **14**: 43-59.

Stutervant, A.H. 1917. Genetic factors affecting the strength of linkage in *Drosophila*. Pro. Natl. Acda. Sci. USA **22**: 448.

Sturtevant, A. H. i G. W. Beadle. 1936. The relations of inversions in the *X* chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 544-604.

Stutervant, A. H. i T. Dobzansky. 1936. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of species. Proc. Acad. Natl. Sci. USA **22**: 448-450.

Sturtevant, A. H. i E. Novitski. 1941. The homologies of the chromosome elements in the genus *Drosophila*. Genetics **26**: 517-541.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585-595.

Terol, J., M. Pérez-Alonso i R. de Frutos. 1995. Molecular characterization of the *zerknüllt* region of the *Antennapedia* complex of *D. subobscura*. Chromosoma **103**: 613-624.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408**: 796-813.

Thompson, J. D., D. G. Higgins i T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence

weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**:4673-4680.

Tonzetich, J., T. W. Lyttle i H. L. Carson. 1988. Induced and natural break sites in the chromosomes of Hawaiian *Drosophila*. Proc. Natl. Acad. Sci. USA **85**: 1717-1721.

Tosi, D. i F. M. Sene. 1989. Further studies on chromosomal variability in the complex taxon *Drosophila serido* (Diptera, Drosophilidae). Rev. Brasil. Genet. **12**: 729-745.

True, J. R., J. M. Mercer i C. C. Laurie. 1995. Differences in crossover frequency and distribution among three sibling species of Drosophila. Genetics **142**: 507-523.

Truett, M. A., R. S. Jones i S. S. Potter. 1981. Unusual structure of the *FB* family of transposable elements in *Drosophila*. Cell **24**:753-763.

Vaury, C., A. Bucheton i A. Pelisson. 1989. The β heterochromatic sequences flanking the I elements are themselves defective transposable elements. Chromosoma **98**: 215-224.

Vieira, C. P., J. Vieira i D. L. Hartl. 1997a. The evolution of small gene clusters: evidence for an independent origin of the maltase gene cluster in *Drosophila virilis* and *Drsophila melanogaster*. Mol. Biol. Evol.**14**: 985-993.

Vieira, C. P., J. Vieira, D. L. Hartl i E. R. Lozovskaya. 1997b. A framework physical map of *Drosophila virilis* based on P1 clones: applications in genome evolution. Chromosoma **106**: 99-107.

Vieira, C. P., J. Vieira, D. L. Hartl i E. R. Lozovskaya. 1997c. Discordant rates of chromosome evolution in the *Drosophila virilis* species group. Genetics **147**: 223-230.

Virgin, J. B. i J. P. Bailey. 1998. The *M26* hotspot of *Schizosaccharomyces pombe* stimulates meiotic ectopic recombination and chromosomal rearrangements. Genetics **149**:1191-1204.

von Allmen, G., I. Hogga, A. Spierer, F. Karch, W. Bender, H. Gyurkovics i E. Lewis. 1996. Splits in fruitfly *Hox* gene complexes. Nature **380**: 116.

Voytas, D. F. 1996.Retroelements in genome organization. Science **274**: 737-738.

Wasserman, M. 1963. Cytology and phylogeny of *Drosophila*. Am. Nat. **97**: 333-352.

Wasserman, M. i H. R. Koepfer. 1979. Cytogenetics of South American *Drosophila mulleri* complex: the martensis cluster. More sharing of inversions. Genetics **93**: 935-946.

Wasserman, M. 1982. Evolution of the *repleta* group. A The Genetics and Biology of *Drosophila* vol 3b, pp. 61-139. Editat per M. Ashburner, H.L. Carson, J.N. Thompson. Academic Press, New York.

Wasserman, M. i R. H. Richardson. 1987. Evolution of Brazilian *Drosophila mulleri* complex species. J. Hered. **78**: 282-286.

Wasserman, M. 1992. Cytological evolution of the *Drosophila repleta* species group. A: *Drosophila* inversion polymorphism, pp. 455-552. Editat per C.B. Krimbas i J.R. Powell. CRC Press, Boca Ratón, FL.

Waterston, R. H., K. Lindblad-Toh., E. Birney, J. Rogers i J. F. Abril. 2002. Initial sequencing and comparative analysis of the mouse genome. Nature **420**: 520-562.

Wesley , C. S., M. Ben, M. Kreitman, N. Hagag i W. F. Eanes. 1990. Cloning regins of the *Drosophila* genome by microdissection of polytene chromosome DNA and PCR with nonspecific primer. Nucleic Acids Res. **18**: 599-603.

Wesley, C. S. i W. F. Eanes. 1994. Isolation and analysis of the breakpoint sequences of chromosome inversion *In(3L)Payne* in *Drosophila melanogaster*. Evolution **91**: 3132-3136.

Wharton, L.T. 1942. Analysis of the *repleta* group of *Drosophila*. Univ. Texas. Pub. 4228: 23-59.

Wheeler, M.R. 1981. The Drosophilidae: a taxonomic overview. A: The genetics and biology of *Drosophila*. Vol.3a. (M. Ashburner, H.L. Carson, J.N. Thompson, editors) Academic Press, New York, pp. 1-97.

White, M. J. D. 1973. Animal Cytology and Evolution. Cambridge University Press, Cambridge.

Whiting, J. H., M. D. Pliley, J. L. Farmer i D. E. Jeffery. 1989. *In situ* hybridization analysis of chromosomal homologies in *Drosophila melanogaster* and *Drosophila virilis*. Genetics **122**: 99-109.

Wilder, J. i H. Hollocher. 2001. Mobile elements and the genesis of microsatellites in dipterans. Mol. Biol.Evol. **18**: 384-392.

Wright, T. R. F. 1996. Phenotypic analysis of the *dopa decarboxylase* gene cluster mutants in *Drosophila melanogaster*. J. Hered. **87**: 175-190.

Yamamoto, M. T., A. Mitchelson, M. Tudor, K. O'Hare, J. A. Davies i G. L. Miklos. 1990. Molecular and cytogenetic analysis of the heterochromatin-euchromatin junction region of the *Drosophila melanogaster* X chromosome using cloned DNA sequences. Genetics **125**: 821-832.

Yen, P. H., S. P. Tsai, S. L. Wenger, M. W. Steele, T. K. Mohandas i L. J. Shapiro. 1991. X/Y translocations resulting from recombination between homologous sequences on Xp an Yq. Proc. Natl. Acad. Sci. USA. **88**: 8944-8948.

Yoon, J. S. i R. H. Richardson. 1978. A mechanism of chromosomal rearrangements: The role of heterochromatin and ectopic joining. Genetics **88**: 305-316.

Zelentsova H, H. Poluectova, L. Mnjoian, G. Lyozin, V. Veleikodvorskaja *et al*. 1999. Distribution and evolution of mobile elements in the *virilis* species group of *Drosophila*. Chromosoma **108**: 443-56.

Zhou, Z-H., E. Akgün i M. Jasin. 2001. Repeat expansion by homologous recombination in the mouse germ line at palindromic sequences. Proc. Natl. Acad. Sci USA **98**: 8326-8333.

7. ANNEXOS

Annex 1. Ranz, J. M., F. Casals, i A. Ruiz. 2001. How malleable is the eukaryotic genome? Extreme rate of chromosomal rearrangement in the genus *Drosophila.* Genome Research 11: 230-239.

How Malleable is the Eukaryotic Genome? Extreme Rate of Chromosomal Rearrangement in the Genus *Drosophila*

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During the evolution of the genus *Drosophila*, the molecular organization of the major chromosomal elements has been repeatedly rearranged via the fixation of paracentric inversions. Little detailed information is available, however, on the extent and effect of these changes at the molecular level. In principle, a full description of the rate and pattern of change could reveal the limits, if any, to which the eukaryotic genome can accommodate reorganizations. We have constructed a high-density physical map of the largest chromosomal element in *Drosophila repleta* (chromosome 2) and compared the order and distances between the markers with those on the homologous chromosomal element (3R) in *Drosophila melanogaster*. The two species belong to different subgenera (*Drosophila* and *Sophophora*, respectively), which diverged 40–62 million years (Myr) ago and represent, thus, the farthest lineages within the *Drosophila* genus. The comparison reveals extensive reshuffling of gene order from centromere to telomere. Using a maximum likelihood method, we estimate that 114 ± 14 paracentric inversions have been fixed in this chromosomal element since the divergence of the two species, that is, 0.9–1.4 inversions fixed per Myr. Comparison with available rates of chromosomal evolution, taking into account genome size, indicates that the *Drosophila* genome shows the highest rate found so far in any eukaryote. Twenty-one small segments (23–599 kb) comprising at least two independent (nonoverlapping) markers appear to be conserved between *D. melanogaster* and *D. repleta*. These results are consistent with the random breakage model and do not provide significant evidence of functional constraint of any kind. They support the notion that the *Drosophila* genome is extraordinarily malleable and has a modular organization. The high rate of chromosomal change also suggests a very limited transferability of the positional information from the *Drosophila* genome to other insects.

[The sequence data described in this paper have been submitted to the GenBank data library under accession no, AF319441.]

Comparative genomics allows us to infer the rates and patterns of genome evolution. The comparison of genomes between distantly related species is made possible by the construction of high-density linkage and/or physical maps and will be greatly facilitated and accelerated by the sequencing of entire genomes in a handful of archetypal species. Critical to this approach is that the analysis of linkage (synteny) and order (colinearity) relationships must be based on orthologous coding markers (Type I markers; O'Brien et al. 1997). Comparative mapping has already yielded important insights into how the genomes of plants and mammals have evolved (Paterson et al. 1996; Gale and Devos 1998; O' Brien et al. 1999).

Drosophila melanogaster was the subject of the first genetic map (Sturtevant 1913) and the first interspecific comparative study (Sturtevant 1921), and is currently the genetically best-characterized insect. Its relatively small (180 Mb) genome, whose euchromatic portion (120 Mb) has been recently sequenced and annotated (Adams et al. 2000), is the obligatory reference for comparative genomics in insects. The vast amount of cytogenetic information accumulated over the years on many *Drosophila* species (Krimbas and Powell 1992; Powell 1997) suggests that the six chromosomal elements (A–F) that constituted the *Drosophila* ancient genome (Muller 1940; Sturtevant and Novitski 1941) have maintained their integrity in many lineages but have been internally rearranged, most often by the fixation of paracentric inversions. Recent results using DNA markers and in situ hybridization mapping (Whiting et al. 1989; Segarra and Aguadé 1992; Segarra et al. 1995, 1996; Vieira et al. 1997a, 1997b) are consistent with this conclusion. Nevertheless, comparative studies carried out so far either lack resolution or involve only closely related species. Even in the most representative lineages of the genus we still do not know the real extent of chromosomal reorganization and whether or not all chromosomal regions are equally affected.

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We have investigated how the molecular organization of the largest chromosomal element (Muller's element E), has been modified during the 80–124 Myr of separate evolution of the two main lineages in the genus *Drosophila*, represented by the *Drosophila* and *Sophophora* subgenera (Spicer 1988; Russo et al. 1995). The study seeks first to determine the rate of genome reorganization in *Drosophila* and to compare its dynamics with those of other organisms; second, to help to reconstruct the ancestral *Drosophila* genome and to detect those regions, if any, whose conservation could be the result of selective constraints; third, to throw light on the limits of genome reorganization; and fourth, to assess the feasibility of transferring positional information from the *D. melanogaster* genome sequence to other, more poorly characterized, insects. This transferability has important practical consequences (cross-genome map-based cloning) for insect species of economic and medical interest.

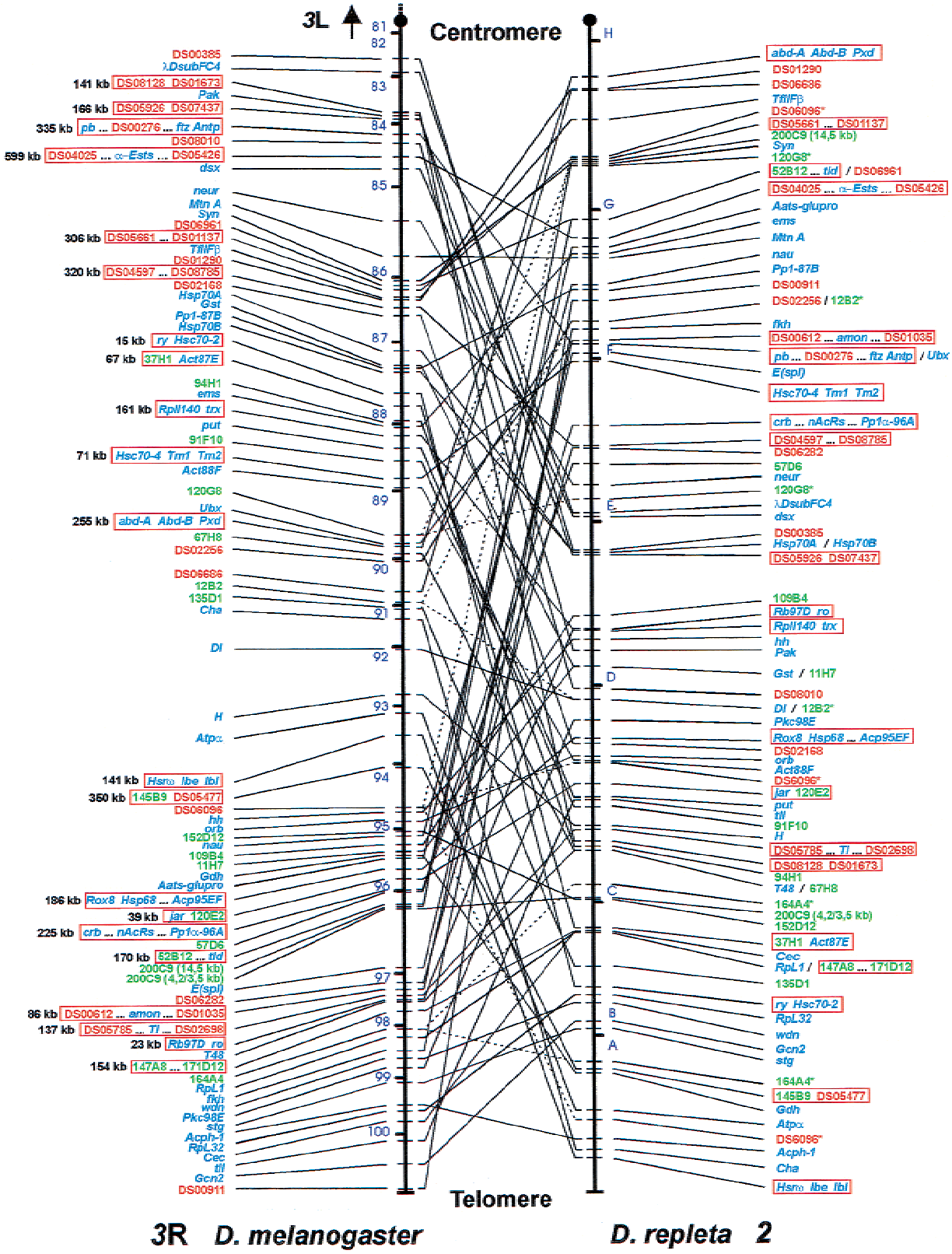
A detailed physical map of *Drosophila repleta* chromosome 2 was assembled and its gene arrangement compared with that of the homologous right arm of the metacentric chromosome 3 (3R) of *D. melanogaster*, whose euchromatic fraction contains 28 Mb of DNA (Adams et al. 2000). *D. repleta* belongs to the *repleta* species group of the *Drosophila* subgenus (Wasserman 1992), whereas *D. melanogaster* pertains to the *Sophophora* subgenus (Powell 1997). The complete map encompasses 160 DNA markers precisely mapped to the salivary gland chromosome *2* of *D. repleta* by in situ hybridization and located accurately on the annotated nucleotide sequence of *D. melanogaster* chromosome 3R (Adams et al. 2000). Markers include clones bearing known protein-coding genes, cosmids, and P1 phages. The study also comprises a thorough comparative analysis of four particular chromosomal regions, ranging from ~0.7 to 1.8 Mb, of chromosomal arm 3R. We have thus been able to produce a general picture of the evolution of the entire chromosomal element and to zoom in on certain regions for a finer-scale analysis at the megabase level. Altogether our work represents the most comprehensive genome comparison performed between two insect species so far and has revealed that the *Drosophila* genome is extraordinarily dynamic and malleable, a finding with important implications.

RESULTS

Chief Map Features

Of the 186 DNA probes assayed by in situ hybridization on the polytene chromosomes of *D. repleta*, 154 (82.8%) gave positive results providing 158 orthologous markers for comparison (supplemental Table 1, available on-line at http://www.genome.org). Representative examples are provided in supplemental Fig. 1 (available on-line at http://www.genome.org). Among the markers mapped interspecifically, there are genes, cosmids, and P1 phages. Some of our results have been reported previously (Ranz et al. 1997, 1999) and are included here for the sake of completeness only. Two additional genes mapped by other authors, *Hsrω* (Peters et al. 1984) and *orb* (H. Naveira, pers. comm.), have been included in the final map. The locations of the 160 markers on chromosome 2 of *D. repleta* and chromosomal arm 3R of *D. melanogaster* are shown in Figure 1. Bridges (1935) partitioned the cytological map of chromosomal arm 3R into 20 sections (81–100). All sections have markers (8.4 per section on average) except section 81. The most proximal and distal markers are P1 phages DS00385 and DS00911, located near the centromere (82E1-2) and close to the telomere (100E1-F5), respectively. All markers, without exception, mapped to chromosome 2 of *D. repleta* (Fig. 1). Thus, this chromosomal element has not been involved in reciprocal translocations or pericentric inversions since the divergence between *D. melanogaster* and *D. repleta*, and its gene content has been largely preserved during a total time span of 80–124 Myr. The euchromatic DNA content of chromosomal arm 3R is ~28,000 kb (Adams et al. 2000). Thus, the average marker density in *D. melanogaster* is 1 per 175 kb. Chromosome 2 represents ~23% of the euchromatic genome in *D. repleta* (Wasserman 1992) and holds ~35 Mb of DNA (Schulze and Lee 1986). It was divided by Wharton (1942) into 38 divisions; our physical map contains up to 14 markers per division (4.2 on average), and the average marker density is 1 per 219 kb. This density is comparable to that

Figure 1 (following page) Large-scale comparison of the gene organization of Muller's element E between *Drosophila melanogaster* and *Drosophila repleta*. Connecting lines match the cytological position of orthologous markers. Dashed lines point out those markers whose location can be assigned to a single chromosomal site in *D. melanogaster* but that produce more than one hybridization signal in *D. repleta*. Mapped markers are indicated in blue (genes), green (cosmids), or red (P1 phages). When two or more markers provide redundant mapping information, only representative markers are shown. For precise mapping location of all the markers in *D. melanogaster* and *D. repleta*, see supplemental Table 1 (available on-line at http://www.genome.org). Red open rectangles indicate the 21 conserved segments comprising at least two independent (nonoverlapping) markers that presumably represent ancient physical associations not disrupted during the evolution of the two compared lineages. Their estimated sizes in *D. melanogaster* are indicated. Two genes localized by other authors, *Hsrω* (Peters et al. 1984) and *orb* (H. Naveira, pers. comm.), are also included. Cosmid 200C9 does not appear as a single marker but as two sets of independent subclones (Ranz et al. 1999). Chromosomal arm 3R of *D. melanogaster* is partitioned into 20 (from 81–100) out of 102 numbered sections in which the *D. melanogaster* genome is subdivided (Bridges 1935). Chromosome *2* of *D. repleta* shows its seven main lettered sections according to Wharton (1942).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120G8
Ubx
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Atpα
141 kb Hsmω lbe lbl
350 kb 145B9 DS05477
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225 kb crb ... nAcRs ... Pp1α-96A
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137 kb DS05785 ... Tl ... DS02698
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D. repleta 2

Table 1. High-Resolution Mapping in *Drosophila repleta* of Markers from Four Regions of Chromosomal Arm 3R of *Drosophila melanogaster*

Chromosomal region	Size (Mb)	Number of markers	Minimum coverage	Number of breakpoints	Density (breakpoints per Mb) ± SD[a]
83E1-84E1	1.814	21	74.89%	6	3.31 ± 1.31
86A4-E2	1.139	13	81.79%	7	6.15 ± 2.27
95A1-96A23	1.217	41	75.46%	12	9.86 ± 2.78
97B1-E6	0.732	25	74.79%	6	8.20 ± 3.30
Total	4.902	100	76.62%	31	6.32 ± 1.03

[a]See González et al. (2000)

obtained in the most refined comparative study performed between man and mouse for the human chromosome 7 (Thomas et al. 2000).

Genome Evolution at the Megabase Level

Four chromosomal regions of *D. melanogaster* chromosomal arm 3R, going from ~0.7 to 1.8 Mb in length, have been investigated in great detail (Table 1). The 100 markers mapped in *D. repleta* that come from these *D. melanogaster* regions yield an average density of one marker per 49 kb and a minimum coverage of 75%–82%. The number of disruptions of the marker order in each region provides a minimum estimate of the number of rearrangement breakpoints fixed since the divergence between *D. melanogaster* and *D. repleta*. The breakpoint density thus estimated does not vary significantly among the four regions (Table 1), pointing to a random distribution of breakpoints along chromosome arm 3R of *D. melanogaster*. Extrapolation of the average density (±SD), 6.32 (±1.03) breakpoints per Mb, to the entire chromosomal element gives a minimum of 177.07 (±28.88) breakpoints or 89 (±14) paracentric inversions fixed in this chromosomal element between *D. melanogaster* and *D. repleta*.

Comparative Mapping of Muller's Element E

The comparison of gene order and distances between *D. melanogaster* chromosomal arm 3R and *D. repleta* chromosome 2 (Fig. 1) indicates that element E has undergone an extensive internal reshuffling that extends throughout its entire length. Both fixation of paracentric inversions and gene transpositions could in principle explain this chromosomal reshuffling. Paracentric inversions are known to be very abundant in *Drosophila*, both as intraspecific polymorphisms and as interspecific fixed differences (Krimbas and Powell 1992; Powell 1997). However, gene transpositions usually involve a particular class of genes only, those that are tandem repeated, like the histone cluster (Steinemann 1982; Steinemann et al. 1984) or the 5S RNA genes (Alonso and Berendes 1975). This kind of gene is absent from our sample of markers. Furthermore, gene transposition seems to have a very low rate of occurrence in *Drosophila*. This was corroborated by comparing the molecular organization of chromosome 2 of *D. repleta* with that of *Drosophila buzzatii*, another species in the *repleta* group (data not shown). All changes of location detected can be explained by inversions that are fixed in this chromosome between both species; therefore, on the basis of our sample of 160 markers, no detectable gene transposition has taken place since the divergence between *D. repleta* and *D. buzzatii*, 22–15 Myr ago (Spicer 1988; Russo et al. 1995). Accordingly, we have considered that paracentric inversions, rather than transposition, are chiefly responsible for the observed pattern of disruption of colinearity.

An unbiased estimate of the number of fixed inversions can be obtained using a maximum likelihood (ML) method (Ranz et al. 1997) that assumes a random distribution of breakpoints along the chromosome in the reference species (*D. melanogaster*). This assumption seems to hold true in our case (see below). Our ML method, unlike the method of Nadeau and Taylor (1984), does not require a random distribution of markers through the genome. Our sample of markers combines those selected to cover four particular regions, with additional markers spread throughout chromosomal arm 3R. Furthermore, our method makes full use of the information about both conserved and nonconserved chromosomal segments. Application of this ML method to our data (Fig. 1) yielded an estimate of 228 (±28) fixed breakpoints, that is, 114 ± 14 fixed inversions. This rate is consistent with the minimum estimate previously calculated from detailed data at the megabase level as indicated by the wide overlapping of their respective 95% confidence interval.

Finally, 21 chromosomal segments comprising at least two independent (nonoverlapping) markers have seemingly been conserved between *D. melanogaster* and *D. repleta* (Fig. 1). These conserved segments are quite small with sizes ranging in *D. melanogaster* from 23–599 kb (188 kb on average).

Colinearity Conservation

By using nonparametric correlation tests, we deter-

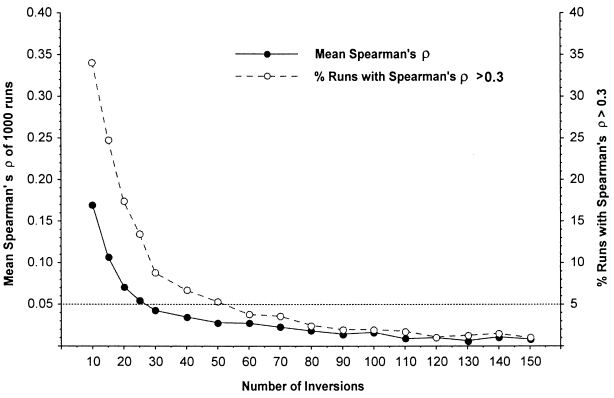

Figure 2. Randomization of chromosomal gene content after fixation of an increasing number of inversions. The conserved segments defined by at least two consecutive and independent markers (nonoverlapping) were considered as a single effective chromosomal site. This yielded a total of 87 positions for the analysis. Solid circles represent the mean values of Spearman's ρ from 1000 runs. Open circles represent the percentages of runs with a Spearman's ρ >0.3, that is, a correlation similar to that found between *Drosophila melanogaster* and *Drosophila repleta*.

mined whether or not the gene organization of chromosomal element E has been randomized between *D. melanogaster* and *D. repleta*. A nonsignificant correlation between the rank order of markers in two species can be taken as evidence of random organization of the gene content of a particular element. In our case, however, a significant correlation of gene order was found between chromosome 2 of *D. repleta* and arm 3R of *D. melanogaster* (four and 10 ties in *D. melanogaster* and *D. repleta*, respectively, Spearman $\rho = 0.336$, $P = 0.001$; Kendall $\tau = 0.217$, $P = 0.003$), considering 87 effective chromosomal sites (see Fig. 2 legend for details). This correlation is unexpected given the estimated number of fixed paracentric inversions if these were generated and fixed at random. Computer simulations showed that after fixation of only 60 inversions, the chromosomal gene content is completely randomized in >95% of runs, and with 110 inversions, a significant correlation >0.3 is only found in 1.8% of cases (Fig. 2).

DISCUSSION

Rates of Chromosomal Evolution

We have estimated that 114 ± 14 paracentric inversions have been fixed in Muller's element E between *D. repleta* and *D. melanogaster*. The low coefficient of variation of this estimate (12%) and its agreement with the lower bound of 89 ± 14 obtained by the in-depth analysis of four particular chromosomal regions support its high reliability. Considering the divergence time between the *Drosophila* and *Sophophora* subgenera (Spicer 1988; Russo et al. 1995), we estimate an evolution rate of 0.9–1.4 chromosomal inversions fixed per million years. Table 2 shows a comparison of this rate with those observed in other eukaryotes. We have used the number of disruptions per Mb per Myr to standardize the data because the genome size and type of chromosomal rearrangements vary among species. Our estimate is similar to that obtained by Segarra et al. (1995), who compared the physical maps of chromosome *X* between *D. melanogaster* and *Drosophila pseudoobscura* with a smaller number of markers. Altogether, the estimates in *Drosophila* show that its genome evolves two orders of magnitude faster than that of mammals and at least fivefold faster than the most dynamic plant genomes, the *Arabidopsis-Brassica* clade. The limited density of orthologous markers in many comparisons can not explain such a huge disparity in rates of evolution.

Table 2. Rates of Chromosome Evolution in Different Taxa

Comparison	Chromosomal disruptions	Method	Divergence time (Myr)	Genome size studied (Mb)	Disruption per Mb per Myr
Drosophila melanogaster/ Drosophila repleta	228	RSR	62[h]	28[m]–35[n]	0.06567-0.05253
Drosophila melanogaster/ Drosophila pseudoobscura	112[a]	NT	30[i]	22[m,o]	0.08485
Homo sapiens/Mus musculus	180[b]	NT	114[j]	3000[p]–2700[p]	0.00026–0.00029
Homo sapiens/Sus scrofa	35[c]	NT	93[j]	3000[p]–2700[p]	0.00006–0.00007
Mus musculus/Sus scrofa	77[c]	NT	114[j]	2700[p]	0.00013
Homo sapiens/Capra hircus	100[d]	NT	93[j]	3000[p,q]	0.00018
Mus musculus/Capra hircus	187[d]	NT	114[j]	2700[p]–3000[q]	0.00030–0.00027
Arabidopsis thaliana/Brassica nigra	90[e]	NT	35[i]	120[r]–360[e]	0.01071–0.00357
Lycopersicon/Capsicum ssp.	22[f]	Direct count	40[k]	950[r]–1900/3800[r]	0.00029–0.00010
Zea mays/Sorghum bicolor	15[g]	Direct count	24[l]	2500[r]–760[r]	0.00013–0.00041

RSR, see Ranz et al. (1997); NT, see Nadeau and Taylor (1984).
[a]Segarra et al. (1995); [b]Ehrlich et al. (1997); [c]Johansson et al. (1998); [d]Schibler et al. (1998a); [e]Lagercrantz (1998); [f]Livingstone et al. (1999); [g]Whitkus et al. (1992); [h]Spicer (1988); [i]Throckmorton (1975); [j]Janke et al. (1994); [k]Paterson et al. (1996); [l]Gaut and Doebley (1997); [m]Adams et al. (2000); [n]Schulze and Lee (1986); [o]Powell (1997); [p]Dear (1997); [q]Schibler et al. (1998b); [r]Arumuganathan et al. (1991).

Three different factors may contribute to this extreme rate of chromosomal rearrangement in *Drosophila*: a shorter generation time, a greater mutation rate, and a less detrimental effect on fertility of inversions, which accordingly would have a higher fixation probability. In *Drosophila*, crossing over is suppressed in males and significantly reduced within the inversion segment in heterokaryotypic females, particularly in case of small inversions, for mechanical reasons (Navarro and Ruiz 1997). Furthermore, single crossovers within the inversion segment produce no inviable zygotes because the resulting unbalanced chromosomes are always set into the polar bodies due to the ordered oogenesis (Sturtevant and Beadle 1936; Carson 1946). Only four-strand double crossovers within the inversion segment, which are likely significant in large inversions only, yield unbalanced gametes (Navarro et al. 1997). However, in mammals and plants, unlike *Drosophila*, most heterozygotes for chromosomal rearrangements have reduced fertility (White 1973; Burnham 1980).

Patterns of Genome Evolution in *Drosophila*

We have detected 21 associations of markers (Fig. 1), which appear to have been conserved since the divergence of *D. melanogaster* and *D. repleta*, that is, they were present in the genome of their common ancestor. Natural selection might be invoked to explain their preservation, implying that because of functional constraints, the gene organization of these segments can not be disrupted without detrimental consequences. Alternatively, these segments could be the by-product of the fixation of rearrangements with randomly distributed breakpoints (Ohno 1973; Nadeau and Taylor 1984). The random breakage (RB) hypothesis can be tested by computing the probability of recovering by chance a chromosomal segment with a relative size equal to or larger than the observed value: $P = e^{-2nl}$, where $2n$ is the number of fixed inversion breakpoints and l is the relative segment size (Nadeau and Taylor 1984; Ranz et al. 1999). Within the sample of 21 conserved segments detected, only one is large enough (599 kb) to give a significant result ($P = 0.0073$). However, with 114 fixed inversions, we would expect to find $228 \times 0.0073 = 1.66$ segments as large as this one in chromosome 2 of *D. repleta* under the RB model. Thus, there is no firm evidence to reject the RB model, that is, evidence for functional constraint. The extraordinary malleability of the *Drosophila* genome is epitomized by the organization of the Hox complex, which is widely conserved in the animal kingdom (Ruddle et al. 1994). In *Drosophila*, by contrast, the presumed single Hox ancestral complex has been disrupted at least twice: one split between *Antp* and *Ubx* took place in the lineage leading to *D. melanogaster* (Lindsley and Zimm 1992) and the other one between *Ubx* and *abd-A* occurred in the lineage leading to *D. repleta* (Fig. 1) and *Drosophila virilis* (von Allmen et al. 1996).

Our results point to a modular organization of the *Drosophila* genome. The proper function of each gene would depend essentially on the physical conservation of its own regulatory sequences located in its immediate vicinity and not on interactions with the surrounding genes. Thus, any module (the gene plus its regulatory sequences) can change its localization within the euchromatin without loss of function. Hox genes appear to be consistent with this view. They are largely autonomous, each with independent regulatory elements apparently insulated from the others (Karch et al. 1994; Hagstrom et al. 1996). Conversely, in vertebrates such genes possess shared regulatory elements, and their regulation seems to require tight colinear clusters (Gérard et al. 1996; Gould et al. 1997). Therefore, there are fewer functional constraints keeping Hox genes together in *Drosophila*. A few exceptions to this modular organization where two close genes are coregulated have been reported in *Drosophila* (Andrews et al. 1996; Brogna and Ashburner 1997; Zhang et al. 1999). In these cases, the interaction in *cis* established between the neighboring genes could prevent chromosomal disruption (Lundin 1993). However, our results suggest that these kinds of interactions are not common, and when they occur must involve genes included in short chromosomal stretches only.

If the *Drosophila* genome has a modular organization, how can we account for the unexpected correlation found for the gene order between *D. melanogaster* and *D. repleta*? This correlation would be consistent with the existence of underlying functional constraints acting on a regional, rather than local, scale. There is, however, another more parsimonious explanation. If large inversions have a low probability of fixation because of their fertility effects (Navarro et al. 1997), which seems to be the case (Cáceres et al. 1997), then the randomization of gene order would proceed at a slower rate than is implied in Figure 2. Computer simulations with 110 fixed inversions of an allowed relative size not >30% of the chromosome yielded correlation coefficients >0.3 in 41.2% of runs (results not shown), which supports this simpler explanation.

Transferability of Positional Information from the *D. melanogaster* Genome

Synteny conservation has been reported among mosquitoes and *D. melanogaster* (Matthews and Munstermann 1994). However, the indispensable condition for a useful transfer of mapping information is the additional conservation of colinearity. The high rate of evolution found in *Drosophila* limits the transfer of such information from the *D. melanogaster* genome to other insects. The crucial parameter is the size of the conserved chromosomal fragment, which under the RB

model is a function of the rate of chromosomal change and the time elapsed since the divergence from the common ancestor. Using the average rate in *Drosophila* of ~1.85 disruptions per million years, we have calculated the likelihood of conservation of a chromosomal segment as a function of its size at three different phylogenetic distances, that is, divergence times. The results, shown in Figure 3, are offered as a first approximation only. Variation in evolution rate among chromosomal elements (Vieira et al. 1997a; González et al. 2000) and among phylogenetic lineages should be considered. For example, inversions and translocations are found in different mosquito genera and *Chironomus*, while only the latter are observed in *Ceratitis* and *Musca* (White 1973; Matthews and Munstermann 1994). Nevertheless, some useful predictions can be made. Only nearby genes in *D. melanogaster* are expected to be still adjacent in different insect orders (Fig. 3). This could be the case with *engrailed* and *invected*, two genes 15 kb apart in *D. melanogaster* (Goldsborough and Kornberg 1994; Adams et al. 2000), which seem to be also together in *Bombyx mori* (Wu et al. 1999). Information transferability within the genus *Drosophila* is much easier within the *Sophophora* subgenus than between different subgenera (Fig. 3). However, even in the latter case, the *D. melanogaster* genome may be useful over short chromosomal distances. For instance, chromosome 2 of *D. repleta* can be envisaged as a collection of 229 fragments homologous to those in chromosomal arm 3R of *D. melanogaster* with a predicted average size of 122 kb. Despite this small size, positional information from *D. melanogaster*

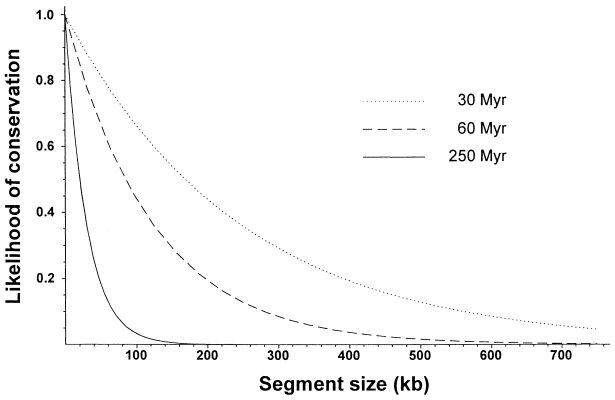

Figure 3. Potential transferability of positional information from *Drosophila melanogaster* to taxa at different phylogenetic distances. The probability that a chromosomal stretch with a relative length *l* has not been disrupted after the fixation of *2n* breakpoints is $P = e^{-2nl}$. *2n* depends on the evolutionary divergence time between lineages compared. Divergence times indicated in the chart $\times 2$ were taken from the comparisons among groups of species within the *Sophophora* subgenus (Throckmorton 1975), among subgenera within the *Drosophila* genus (Spicer 1988), and among some of the main insect orders (Friedrich and Tautz 1997), respectively. A rate of 1.85 disruptions per Myr was assumed in all cases.

was used for cloning purposes in a distantly related species included in the *Drosophila* subgenus (Cáceres et al. 1999).

METHODS

Flies

The following species and stocks were used: *D. melanogaster* (Canton S), *D. repleta* (1611.2 and 1611.6 from The National *Drosophila* Species Resource Center, Bowling Green, Ohio), *D. buzzatii* (st-1), *D. hydei* (HY-8), and *D. virilis* (VIR-Tokyo).

DNA Probes

Eighty-three gene clones, 51 cosmids, and 52 P1 phages were used as probes. Thirty-seven gene clones and 31 cosmids were hybridized previously (Ranz et al. 1997, 1999), and the remaining clones, 118, have been hybridized in this work. Most gene clones come from genomic or cDNA *D. melanogaster* libraries and were kindly provided by different authors (supplemental Table 2, available on-line at http://www.genome.org). The *D. buzzatii double sex* (*dsx*) clone was isolated by PCR (supplemental Table 2), and the PCR product was cloned into a PGEM-T vector (Promega) and sequenced with an ALF express DNA automated sequencer (Pharmacia Biotech). BLASTX (Altschul et al. 1997) gave a probability of matching by chance with *dsx* sequences from *D. melanogaster* and *Bractocera tryoni* lesser than E-16, confirming the identity of the cloned sequence (GenBank accession no. AF319441). Cosmids and P1 phages belong to the *D. melanogaster* libraries of the European (EDGP 2000) and Berkeley (BDGP 2000) *Drosophila* Genome Projects, respectively. DNA preparation was performed essentially as described in Sambrook et al. (1989) for recombinant plasmids, recombinant phages and cosmids, and as in Hartl and Lozovskaya (1995) for P1 phages.

Construction of the Map and in situ Hybridization

All clones were hybridized to the salivary gland chromosomes of the source species (*D. melanogaster* in most cases) as control. When assayed on the chromosomes of *D. repleta*, 63 gene clones (75.9%), 48 cosmids (94.1%), and 43 P1 phages (82.7%) yielded detectable hybridization signals (supplemental Table 1). Most clones gave a single signal (see supplemental Fig. 1), but seven gene clones, nine cosmids, and five P1 phages produced two or more hybridization signals. Five of those genes (*Act87E*, *Hsp70A*, *Hsp70B*, *Pp1-87B*, and λ*DsubFC4*) belong to gene families whose members are conserved and dispersed through the genome. In these cases, as discussed previously (Ranz et al. 1997), the strongest signal invariably corresponds to the chromosomal site of the probed gene. Four markers identified from consistent secondary signals (*Hsc70-2*, *Hsc70-4*, *Hsp68*, and *Act88F*) were included in the final map. In the case of cosmids and P1 phages that produce several hybridization signals in *D. repleta*, we have considered that the clone encompasses a rearrangement breakpoint fixed during the divergence of *D. melanogaster* and *D. repleta* as the most likely explanation. Evidence supporting this interpretation has been provided elsewhere (Ranz et al. 1999).

Salivary gland chromosome preparation, probe labeling by nick translation, hybridization, and detection were carried out for all species as described (Ranz et al. 1997) except that hybridization to *D. repleta* chromosomes was performed at

25°C instead of the usual 37°C for control hybridizations. Micrographs were taken by phase contrast with a Nikon Optiphot-1 microscope and a Nikon H-III photomicrographic system at 600× magnification using EKTAR-25 Kodak film and a blue filter. The localization of probes was determined using the photographic map (Lefevre 1976) and the electron microscopy map (Heino et al. 1994) of *D. melanogaster*. For *D. repleta*, we used the map drawn by Wharton (1942).

Analysis of the Degree of Genome Rearrangement

Given the variable marker density throughout chromosomal arm 3R, the maximum likelihood method described by Ranz et al. (1997), which does not assume any particular marker distribution, was used. To apply this method, markers were anchored in the genome sequence of the reference species, *D. melanogaster*. Then, all the chromosomal segments delimited by neighboring markers were checked for conservation in *D. repleta* and their sizes estimated using precise molecular information (Adams et al. 2000; BDGP 2000). Genes and P1 phages containing STS were easily anchored in the sequence of chromosomal arm 3R and their sizes determined. For some P1 phages and cosmids, molecular information was not available. In these cases, an open reading frame mapped to the same cytological position was used as a reference and assumed to be the midpoint of the clone. When unknown, an average size of 80 and 40 kb was assumed for P1 phages and cosmids, respectively (Siden-Kiamos et al. 1990; Sternberg 1990; Smoller et al. 1991). Southern analysis and comparison of restriction profiles were performed when necessary to test for the inclusion of a gene within a cosmid or P1 phage or for possible overlap between adjacent clones (see Ranz et al. 1999 for methods). For genes, as well as for cosmids and P1 phages that yielded only one hybridization signal on *D. repleta* chromosome 2, it was assumed that no chromosomal disruption occurred during the evolution of these two lineages, that is, they are conserved. A total of 60 genomic stretches fit into this category, while the remaining 83 were considered nonconserved (seven containing exactly one breakpoint, three exactly two, one exactly three, and 72 at least one).

Computer Simulation

The randomization of gene order in a chromosome subject to the sequential fixation of paracentric inversions was studied by computer simulation. Briefly, an ideal chromosome consisting of 3500 positions (genes) with the same marker arrangement as in *D. melanogaster* chromosomal arm 3R was simulated. Increasing numbers of inversions with a random distribution of breakpoints were then generated, and each time Spearman's coefficient of rank correlation between the initial and final marker arrangement was calculated. Each simulation was repeated 1000 times.

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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., et al. 2000. The genome sequence of *Drosophila melanogaster*. *Science* **287:** 2185–2195.

Alonso, C. and Berendes, H.D. 1975. The location of 5S (ribosomal) RNA genes in *Drosophila hydei*. *Chromosoma* **51:** 347–356.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389–3402.

Andrews, J., Smith, M., Merakovsky, J., Coulson, M., Hannan, F., and Kelly, L.E. 1996. The stoned locus of *Drosophila melanogaster* produces a dicistronic transcript and encodes two distinct polypeptides. *Genetics* **143:** 1699–1711.

Arumuganathan, K. and Earle, E.D. 1991. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* **9:** 208–218.

BDGP. 2000. The Berkeley Drosophila Genome Project database. http://www.fruitfly.org

Bridges, C.B. 1935. Salivary chromosome maps, with a key to the banding of the chromosomes of *Drosophila melanogaster*. *J. Hered.* **26:** 60–64.

Brogna, S. and Ashburner, M. 1997. The Adh-related gene of *Drosophila melanogaster* is expressed as a functional dicistronic messenger RNA: Multigenic transcription in higher organisms. *EMBO J.* **16:** 2023–2031.

Burnham, C.R. 1980. *Discussions in cytogenetics*. C.R. Burnham, MN.

Cáceres, M., Barbadilla, A. and Ruiz, A. 1997. Inversion length and breakpoint distribution in the *Drosophila buzzatii* species complex: Is inversion length a selected trait? *Evolution* **51:** 1149–1155.

Cáceres, M., Ranz, J.M., Barbadilla, A., Long, M., and Ruiz, A. 1999. Generation of a widespread *Drosophila* inversion by a transposable element. *Science* **285:** 415–418.

Carson, H.L. 1946. The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. *Genetics* **31:** 95–113.

Dear, P.H. 1997. *Genome mapping*, 1st ed. Oxford University Press, New York.

EDGP. 2000. The European *Drosophila* Genome Project database. http://edgp.ebi.ac.uk

Ehrlich, J., Sankoff, D., and Nadeau, J.H. 1997. Synteny conservation and chromosome rearrangements during mammalian evolution. *Genetics* **147:** 289–296.

FlyBase 1999. The *Drosophila* database. *Nucleic Acid Res.* **24:** 53–56.

Friedrich, M. and Tautz, D. 1997. An episodic change of rDNA nucleotide substitution rate has occurred during the emergence of the insect order Diptera. *Mol. Biol. Evol.* **14:** 644–653.

Gale, M.D. and Devos, K.M. 1998. Plant comparative genetics after 10 years. *Science* **282:** 656–659.

Gaut, B. and Doebley, J. 1997. DNA sequence evidence for the segmental allotetraploid origin of maize. *Proc. Natl. Acad. Sci.* **94:** 6809–6814.

Gérard, M., Chen, J.Y., Gronemeyer, H., Chambon, P., Duboule, D., and Zakany, J. 1996. In vivo targeted mutagenesis of a regulatory element required for positioning the Hoxd-11 and Hoxd-10 expression boundaries. *Genes & Dev.* **10:** 2326–2334.

Goldsborough, A.S. and Kornberg, T.B. 1994. Allele-specific quantification of *Drosophila* engrailed and invected transcripts. *Proc. Natl. Acad. Sci.* **91:** 12696–12700.

González, J., Betrán, E., Ashburner, M., and Ruiz, A. 2000. Molecular organization of the *Drosophila melanogaster* Adh chromosomal region in *D. repleta* and *D. buzzatii*, two distantly related species of the *Drosophila* subgenus. *Chromosome Res.* **8:** 375–385.

Gould, A., Morrison, A., Sproat, G., White, R., and Krumlauf, R. 1997. Positive cross-regulation and enhancer sharing: Two

mechanisms for specifying overlapping Hox expression patterns. *Genes & Dev.* **11:** 900–913.
Hagstrom, K., Muller, M., and Schedl, P. 1996. Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila* bithorax complex. *Genes & Dev.* **10:** 3202–3215.
Hartl, D.L. and Lozovskaya, E.R. 1995. *The* Drosophila *genome map: A practical guide.* Springer, New York.
Heino, T.I., Saura, A.O., and Sorsa, V. 1994. Maps of the salivary gland chromosomes of *Drosophila melanogaster*. *Dros. Inform. Serv.* **73:** 621–738.
Janke, A., Feldmaier-Fuchs, G., Thomas, W.K., von Haeseler, A., and Pääbo, S. 1994. The marsupial mitochondrial genome and the evolution of placental mammals. *Genetics* **137:** 243–256.
Johansson, M., Ellegren, H., and Andersson, L. 1995. Comparative mapping reveals extensive linkage conservation—but with gene order rearrangements—between the pig and the human genomes. *Genomics* **25:** 682–690.
Karch, F., Galloni, M., Sipos, L., Gausz, J., Gyurkovics, H., and Schedl, P. 1994. Mcp and Fab-7: Molecular analysis of putative boundaries of cis-regulatory domains in the bithorax complex of *Drosophila melanogaster*. *Nucleic Acids Res.* **22:** 3138–3146.
Krimbas, C.B. and Powell, J.R. 1992. *Drosophila inversion polymorphism*. CRC, Boca Raton, FL.
Lagercrantz, U. 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. *Genetics* **150:** 1217–1228.
Lefevre Jr., G. 1976. A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In *The genetics and biology of* Drosophila (ed. M. Ashburner, et al.), pp. 31–66. Academic Press, London.
Lindsley, D.L. and Zimm, G.G. 1992. *The genome of* Drosophila melanogaster. Academic Press, San Diego.
Livingstone, K.D., Lackney, V.K., Blauth, J.R., van Wijk, R., and Jahn, M.K. 1999. Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. *Genetics* **152:** 1183–1202.
Lundin, L.G. 1993. Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse. *Genomics* **16:** 1–19.
Matthews, T.C. and Munstermann, L.E. 1994. Chromosomal repatterning and linkage group conservation in mosquito karyotypic evolution. *Evolution* **48:** 146–154.
Muller, J.H. 1940. *Bearings of the* Drosophila *work on systematics*. In *New systematic* (ed. J. Huxley) pp. 185–268. Clarendon Press, Oxford.
Nadeau, J.H. and Taylor, B.A. 1984. Lengths of chromosomal segments conserved since divergence of man and mouse. *Proc. Natl. Acad. Sci.* **81:** 814–818.
Navarro, A. and Ruiz, A. 1997. On the fertility effects of pericentric inversions. *Genetics* **147:** 931–933.
Navarro, A., Betrán, E., Barbadilla, A., and Ruiz, A. 1997. Recombination and gene flux caused by gene conversion and crossing over in inversion heterokaryotypes. *Genetics* **146:** 695–709.
Ohno, S. 1973. Ancient linkage groups and frozen accidents. *Nature* **244:** 259–262.
O'Brien, S.J., Wienberg, J., and Lyons, L.A. 1997. Comparative genomics: Lessons from cats. *Trends Genet.* **13:** 393–399.
O'Brien, S.J., Menotti-Raymond, M., Murphy, W.J., Nash, W.G. Wienberg, J., Stanyon, R., Copeland, N.G., Jenkins, N.A., Womack, J.E., and Marshall Graves, J.A. 1999. The promise of comparative genomics in mammals. *Science* **286:** 458–481.
Paterson, A.H., Lan, T.-H., Reischmann, K.P., Chang, C., Lin, Y.-R., Liu, S.-C., Burow, M.D., Kowalski, S.P., Katsar, C.S., DelMonte T.A., Feldmann, K.A., Schertz, K.F., and Wendel, J.F. 1996. Toward a unified genetic map of higher plants, transcending the monocot-dicot divergence. *Nat. Genet.* **14:** 380–382.
Peters, F.P.A.M.N., Lubsen, N.H., Walldorf, U., Moormann, R.J.M., and Hovemann, B. 1984. The unusual structure of heat shock locus 2–48B in *D. hydei*. *Mol. Gen. Genet.* **197:** 392–398.
Powell, J.R. 1997. *Progress and prospects in evolutionary biology: The* Drosophila *model*. Oxford University Press, New York.
Ranz, J.M., Segarra, C. and Ruiz, A. 1997. Chromosomal homology and molecular organization of Muller's elements *D* and *E* in the *Drosophila repleta* species group. *Genetics* **145:** 281–295.
Ranz, J.M., Cáceres, M., and Ruiz, A. 1999. Comparative mapping of cosmids and gene clones from a 1.6 Mb chromosomal region of *Drosophila melanogaster* in three species of the distantly related subgenus *Drosophila*. *Chromosoma* **108:** 32–43.
Ruddle, F.H., Bartels, J.L., Bentley, K.L., Kappen, C., Murtha, M.T., and Pendleton, J.W. 1994. Evolution of HOX-genes. *Ann. Rev. Genet.* **28:** 423–442.
Russo, C.A.M., Takezaki, N., and Nei, M. 1995. Molecular phylogeny and divergence times of Drosophilid species. *Mol. Biol. Evol.* **12:** 391–404.
Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning, A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schibler, L., Vaiman, D., Oustry, A., Giraud-Delville, C., and Cribiu, E.P. 1998a. Comparative gene mapping: A fine-scale survey of chromosome rearrangements between ruminants and humans. *Genome Res.* **8:** 901–915.
Schibler, L., Vaiman, D., Oustry, A., Guinec, N., Dangy-Caye, A.-L., Billault, A., and Cribiu, E.P. 1998b. Construction and extensive characterization of a goat bacterial artificial chromosome library with three fold genome coverage. *Mamm. Genome* **9:** 119–124.
Schulze, D.H. and Lee, C.S. 1986. DNA sequence comparison among closely related *Drosophila* species of the mulleri complex. *Genetics* **113:** 287–303.
Segarra, C., and Aguadé, M. 1992. Molecular organization of the *X* chromosome in different species of the *obscura* group of *Drosophila*. *Genetics* **130:** 513–521.
Segarra, C., Lozovskaya, E.R., Ribó, G., Aguadé, M., and Hartl, D.L. 1995. P1 clones from *Drosophila melanogaster* as markers to study the chromosomal evolution of Muller's A element in two species of the *obscura* group of *Drosophila*. *Chromosoma* **104:** 129–136.
Segarra, C., Ribó, G., and Aguadé, M. 1996. Differentiation of Muller's chromosomal elements D and E in the *obscura* group of *Drosophila*. *Genetics* **144:** 139–146.
Siden-Kiamos, I., Saunders, R.D., Spanos, L., Majerus, T., Treanear, J., Savakis, C., Louis, C., Glover, D.M., Ashburner, M. and Kafatos, F.C. 1990. Towards a physical map of the *Drosophila melanogaster* genome: Mapping of cosmid clones within defined genomic divisions. *Nucleic Acids Res.* **18:** 6261–6270.
Smoller, D.A., Petrov, D., and Hartl, D.L. Characterization of bacteriophage P1 library containing inserts of *Drosophila* DNA of 75–100 kilobase pairs. *Chromosoma* **100:** 487–494.
Spicer, G.S. 1988. Molecular evolution among some *Drosophila* species groups as indicated by two-dimensional electrophoresis. *J. Mol. Evol.* **27:** 250–260.
Steinemann, U.M. 1982. Analysis of chromosomal homologies between two species of the subgenus Sophophora: *D. miranda* and *D. melanogaster* using cloned DNA segments. *Chromosoma* **87:** 77–88.
Steinemann, U.M., Pinsker, W., and Sperlich, D. 1984. Chromosome homologies within the *Drosophila obscura* group probed by in situ hybridization. *Chromosoma* **91:** 46–53.
Sternberg, N. 1990. Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. *Proc. Natl. Acad. Sci.* **87:** 103–107.
Sturtevant, A.H. 1913. The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *J. Exp. Zool.* **14:** 43–59.
———. 1921. A case of rearrangement of genes in *Drosophila*. *Proc. Nat. Acad. Sci.* **7:** 235–237.
Sturtevant, A.H. and Beadle, G.W. 1936. The relations of inversions in the *X* chromosome of *D. melanogaster* to crossing over and disjunction. *Genetics* **21:** 544–604.
Sturtevant, A.H. and Novitski, E. 1941. The homologies of the chromosome elements in the genus *Drosophila*. *Genetics* **26:** 517–541.
Thomas, J.W., Summers, T.J., Lee-Lin, S.-Q., Braden Maduro, V.V., Idol, J.R., Mastrian, S.D., Ryan, J.F., Jamison, D.C., and Green,

E.D. 2000. Comparative genome mapping in the sequence-based era: Early experience with human chromosome 7. *Genome Res*. **10:** 624–633.

Throckmorton, L.H. 1975. The phylogeny, ecology, and geography of *Drosophila*. In *Handbook of genetics* (ed. R.C. King), Vol. 3, pp. 421–469. Plenum, New York.

Vieira, J., Vieira, C.P., Hartl, D.L., and Lozovskaya, E.R. 1997a. Discordant rates of chromosome evolution in the *Drosophila virilis* species group. *Genetics* **147:** 223–230.

———. 1997b. A framework physical map of *Drosophila virilis* based on P1 clones: Applications in genome evolution. *Chromosoma* **106:** 99–107.

von Allmen, G., Hogga, I., Spierer, A., Karch, F., Bender, W., Gyurkovics, H. and Lewis, E. 1996. Splits in fruitfly Hox gene complexes. *Nature* **380:** 116.

Wasserman, M. 1992. Cytological evolution of the *Drosophila repleta* species group. In *Drosophila inversion polymorphism* (ed. C.B. Krimbas et al.), pp. 455–552. CRC, Boca Raton, FL.

Wharton, L.T. 1942. Analysis of the *repleta* group of *Drosophila*. University of Texas Publication. **4228:** 23–52.

White, M.J.D. 1973. *Animal cytology and evolution*. Cambridge University Press, London.

Whiting Jr., J.H., Plliley, M.D., Farmer, J.L., and Jeffery, D.E. 1989. In situ hybridization analysis of chromosomal homologies in *Drosophila melanogaster* and *Drosophila virilis*. *Genetics* **122:** 99–109.

Whitkus, R., Doebley, J., and Lee, M. 1992. Comparative genome mapping of shorgum and maize. *Genetics* **132:** 1119–1130.

Wu, C., Asakawa, S., Shimizu, N., and Kawasaki, S. 1999. Construction and characterization of bacterial artificial chromosome libraries from the silkworm, *Bombyx mori*. *Mol. Gen. Genet*. **261:** 698–706.

Zhang, Y., Roote, J., Brogna, S., Davis, A.W., Barbash, D.A., Nash, D., and Ashburner, M. 1999. Stress sensitive B encodes an adenine nucleotide translocase in *Drosophila melanogaster*. *Genetics* **153:** 891–903.

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Annex 2. Inversions cromosòmiques del complexe *buzzatii*.

Inversió[a]	Espècie	Punts de trencament	Referència
Xj	-	E2d-G1a	(1)
Xr	-	D3a-F3a	(1)
Xs	*D. starmeri*	F1e-F3d	(1)
Xq	*D. starmeri*	D3d-G1a	(1)
Xy	*D. starmeri*	F1a-E2d	(2)
2m	-	D3d-F2a	(3)
2n	-	F2a-G1g	(3)
2l	-	C7e-D5a	(3)
$2e^2$	-	F6a-F3a	(3)
$2u^6$	-	D1g-F2a	(3)
$2w^7$	-	D1g-G1a	(3)
$2x^7$	-	D5c-F2a	(3)
$2y^7$	-	G1a-D1g	(3)
$2z^7$	-	F2a-F6a	(3)
$2e^8$	-	B2a-C6a	(3)
$2j^9$	-	D4a-F4d	(3)
$2f^8$	*D. borborema*	C7d-E6a	(4)
$2g^8$	*D. borborema*	C6c-E2h	(4)
$2h^8$	*D. borborema*	C6a-C1h	(4)
2j	*D. buzzatii*	C6b-E5a	(5)
$2y^3$	*D. buzzatii*	D1a-E3a	(5)
$2z^3$	*D. buzzatii*	E4b/c-F1f	(5) (6) (7)
$2q^7$	*D. buzzatii*	D3c-G2f	(5) (7)
$2c^9$	*D. buzzatii*	C1a-D1a	(5)
$2d^9$	*D. buzzatii*	D5b-G3d	(5)
$2e^9$	*D. buzzatii*	C3c-C6h	(5)
$2f^9$	*D. buzzatii*	C6h-G2f	(5)
$2g^9$	*D. buzzatii*	B3e-C3a	(5)
$2h^9$	*D. buzzatii*	E1a-E2e	(5)
$2i^9$	*D. buzzatii*	F3c-G2f	(5)
$2r^9$	*D. buzzatii*	C5f-D2a	(5)

2s[9]	*D. buzzatii*	E1a-G2a	(5)
2k[9]	*D. koepferae*	B1d-D4a	(8)
2l[9]	*D. koepferae*	A1a-B3e	(8)
2m[9]	*D. koepferae*	C3b-E1e	(8)
2n[9]	*D. koepferae*	E5c-F4a	(8)
2u[9]	*D. koepferae*	F6a-C3b	(3)
2v[9]	*D. koepferae*	F1c-F4g	(3)
2w[9]	*D. koepferae*	A4d-E5a	(3)
2x[9]	*D. koepferae*	E5a-F6a	(3)
2g[2]	*D. martensis*	B4c-D5a	(1)
2o[9]	*D. martensis*	B4c-C4e	(2)
2p[9]	*D. martensis*	C6a-D3d	(2)
2p[8]	*D. richardsoni*	F2a-G2b	(3)
2q[8]	*D. richardsoni*	E5e-D3e	(3)
2a[8]	*D. serido*	D1c-F3a	(4)
2b[8]	*D. serido*	B4a-F2a	(4)
2c[8]	*D. serido*	C1a-A4a	(4)
2d[8]	*D. serido*	F2a-E4a	(4)
2w[8]	*D. serido*	E4g-D3d	(9)
2x[8]	*D. serido*	B2a-C6e	(9)
2y[8] (*2"e"*)	*D. serido*	E1g-F6a	(9) (10)
2z[8] (*2"d"*)	*D. serido*	D3d-G1g	(9) (10)
2y[9]	*D. serido*	C4f-E1d	(9)
2 "a"	*D. serido (IV)*	C2c-E1d	(10)
2f[2]	*D. starmeri*	B3a-C7e	(3)
2t[6]	*D. starmeri*	D1g-D5a	(3)
2w[6]	*D. starmeri*	C7e-C6a	(1)
2x[6]	*D. starmeri*	F4a-E2e	(1)
2y[6]	*D. starmeri*	B1b-B3a	(1)
2z[6]	*D. starmeri*	F1c-D5a	(1)
2a[7]	*D. starmeri*	E6a-E2e	(1)
2e[7]	*D. starmeri*	B4e-C3f	(3)
2b[7]	*D. starmeri*	F4a-E6g	(1)

2c^{7}	*D. starmeri*	E2e-E6g	(1)
2r^{7}	*D. starmeri*	B1b-C4a	(1)
2q^{9}	*D. starmeri*	F6b-G4b	(2)
2w^{6}	*D. uniseta*	B3f-D2e	(1)
2t^{9}	*D. venezolana*	D2b-E4a	(3)
3k	-	D5a-G1h	(3)
3v	-	D4b-E4a	(3)
3w	-	E4a-F4c	(3)
3r^{2}	-	B1c-C5d	(3)
3u	*D. borborema*	D4b-F4g	(11)
3j^{2}	*D. buzzatii*	A2c-F2b	(5)
3k^{2}	*D. koepferae*	D2b-F3g	(8)
3y	*D. starmeri*	C5d-B5a	(1)
3z	*D. starmeri*	D1h-E5d	(1)
3a^{2}	*D. starmeri*	C1b-C5e	(1)
3e^{2}	*D. starmeri*	F3f-G1a	(1)
4s	*D. buzzatii*	D1d-F1c	(5)
4m	*D. koepferae*	E3d-G2c	(8)
5g	-	D3a-F2d	(8)
5d^{2}	-	E1a-F1a	(3)
5c^{2}	*D. buzzatii*	D4d-E3g	(12)
5w	*D. koepferae*	D4g-F1a	(8)
5e (*5"e"*)	*D. serido*	C3a-F1a	(9) (10)
5d	*D. seriema*	F2a-G2e	(11)
5q	*D. starmeri*	C2c-D4a	(1)

(1) Wasserman i Koepfer 1979; (2) Ruiz i Fontdevila 1981; (3) Ruiz i Wasserman 1993; (4) Wasserman i Richardson 1987; (5) Ruiz *et al*. 1984; (6) Laayouni *et al*. 2000; (7) Aquest treball; (8) Ruiz *et al*. 1982; (9) Ruiz *et al*. 2000; (10) Tosi i Sene 1989; (11) Kuhn *et al*. 1996; (12) Barker *et al*. 1985.

[a] Entre parèntesi s'indica la denominació anterior d'algunes inversions.

Annex 3. Inversions induïdes per introgressió híbrida a *D. buzzzatii* (Naveira i Fontdevila 1985).

Inversió	Punts de trencament
In(2)1	B2e-D4l
In(2)2	B1e-D4b
In(3)1	A5e-C5b
In(3)2	E1c-G2a
In(3)3	B2c-E1a
In(3)4	A1a-A2d
In(4)1	A5b-D1b
In(4)2	B4e-E4a
In(4)2	B4e-G3a
In(4)3	E1h-F4c
In(4)4	D2c-F2d
In(5)1 (*5I*)[a]	F2b-F2e
In(5)2	A3g-B1e
In(5)3	F1d-F2e
In(5)4	E4f-F2e
In(5)5	C2e-G2a
In(5)6	A4e-F3f
In(5)7	D1b-F1d

[a] La inversió *In(5)1* també s'anomena inversió *5I* (Ruiz *et al*. 1991).

moltes gràcies a tots i totes els qui heu fet possible la realització d'aquest treball, hi heu participat, i l'heu fet més agradable

defensa de la alegría

Defender la alegría como una trinchera
defenderla del caos y de las pesadillas
de la ajada miseria y de los miserables
de las ausencias breves y las definitivas

defender la alegría como un atributo
defenderla del pasmo y de las anestesias
de los pocos neutrales y los muchos neutrones
de los graves diagnósticos y de las escopetas

defender la alegría como un estandarte
defenderla del rayo y la melancolía
de los males endémicos y de los académicos
del rufián caballero y del oportunista

defender la alegría como una certidumbre
defenderla a pesar de dios y de la muerte
de los parcos suicidas y de los homicidas
y del dolor de estar absurdamente alegres

defender la alegría como algo inevitable
defenderla del mar y las lágrimas tibias
de las buenas costumbres y de los apellidos
del azar y también
también de la alegría

Mario Benedetti