Differentiation of Muller’s Chromosomal Elements D and E in the Obscura Group of Drosophila

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ABSTRACT

Twenty-two markers located on Muller’s elements D or E have been mapped by in situ hybridization on polytene chromosomes of Drosophila. This technique is sensitive enough to give positive results in cross-hybridizations and thus can be applied in evolutionary studies. Initially, it was used to recognize homologous chromosomal elements among different Drosophila species (Steinemann 1982; Steinemann et al. 1984; Whiting et al. 1989; Papaceit and Juan 1993; among others). Another more detailed approach has been to identify homologous segments within a particular chromosomal element among different species (Segarra and Aguadé 1992; Segarra et al. 1995). These latter studies indicate extensive reshuffling within Muller’s A element (Muller 1940) according to comparisons of, for instance, D. melanogaster with D. pseudoobscura or with D. subobscura, and even from comparisons of the two obscura group species.

In the present study, this latter approach is extended to Muller’s elements D and E. Twenty-two probes that hybridize to these elements in D. melanogaster have been mapped in six species of the obscura group. The obscura group species studied are included in two different clusters. The first cluster is the D. subobscura cluster with the three Palearctic and closely related species: D. subobscura, D. maderensis and D. guanche. The other cluster is the D. pseudoobscura cluster that includes the three Nearctic and closely related species: D. pseudoobscura, D. persimilis and D. miranda. As indicated in Table 1, Muller’s element E is autosomal in the seven species, while element D is autosomal in D. subobscura, D. maderensis and D. guanche and corresponds to the right arm (XR) of the metacentric sex chromosome in D. pseudoobscura, D. persimilis and D. miranda. Muller’s elements D and E are fused to form the 3L and 3R chromosomal arms, respectively, of the metacentric autosome 3 of D. melanogaster (Buzzati-Traverso 1955).

The accurate mapping of these markers in the obscura group species contributes to their genetic characterization. As a second aim, this study attempts to corroborate previously proposed chromosomal homologies among the species in the same obscura cluster. Third, it tries to identify homologous chromosomal segments between the species of different clusters. Fourth, the extent of reorganization within D and E elements during the divergence of the species can be analyzed. In fact, the rate of evolution of the different chromosomal elements may be affected by their autosomal or sex chromosomal character. Charlesworth et al. (1987) propose that the fixation rate of underdominant chromosomal rearrangements is higher with sex linkage than for autosomes and indicate that in the genus Drosophila there are slightly more X-linked than autosomal-fixed paracentric inversions. If sex chromosomes indeed evolve faster, one would expect a priori that the distribution of markers would be more conserved in element E, which is autosomal in the seven species studied.
DNA plasmids or phages including totally or partially known ADE recombinant phage detection conditions were markers; the name of those phages begins with recombinant phage genomic library of recombinant phage. Extra-macrochaetae heat shock protein 83 (D. melanogaster) Esterase-5 (U. pseudo) Est-5 D. mel Accessory male protein 70A (D. mel) Acp 70A Ecdysone-inducible E74 (D. mel) E 74 probes were labeled with 1Gbiotin dUTP (Boehringer Mannheim). D. persimilis D. sub, D. subobscura; D. mel, D. melanogaster; D. pseudo, D. pseudoobscura. 

**MATERIALS AND METHODS**

Two strains of D. subobscura were used: ch cu, which has J, and O, ch chromosomal arrangements, and an isochromosomal line with the O, arrangement. D. madeirensis and D. guanche strains were available in our laboratory. D. pseudoobscura, D. persimilis and D. miranda strains were kindly provided by R. C. Lewontin.

Preparations of polytene chromosomes suitable for in situ hybridization were obtained following Montgomery et al. (1987). The 22 probes used in the present study are summarized in Table 2. Some probes correspond to recombinant DNA plasmids or phages including totally or partially known genes cloned in D. melanogaster, D. subobscura or D. pseudoobscura. Additionally, some anonymous phages from a random genomic library of D. subobscura (AGUADE 1988) that gave a single hybridization signal in this species were also used as markers; the name of those phages begins with "ΔDsub". Probes were labeled with 16-biotin diUTP (Boehringer Mannheim) by nick translation. Prehybridization, hybridization and detection conditions were as described in Segarra and Aguadé (1992).

The following polytene chromosome photomaps were used to identify the location of hybridization sites: D. madeirensis (PAPACEIT and PREVOSI 1991), D. guanche (MOLTÓ et al. 1987), D. pseudoobscura (STOCKER and KAISTRITIS 1972), D. persimilis (MOORE and TAYLOR 1986), D. miranda (DAS et al. 1982) and D. melanogaster (LEFÈVRE 1976). In the case of D. subobscura, the Kunze-Mühl and Müller (1958) cytological map has been used. This map was taken as a reference to establish chromosomal sections in D. madeirensis and D. guanche photomaps. Unfortunately, sections of the photomaps of D. pseudoobscura and D. persimilis are not in complete agreement, and sections of the D. miranda photomap were newly established by DAS et al. (1982) without considering the previously established subdivisions by Dobzhansky and Tan (1936).

**RESULTS**

All probes that map on the 3L chromosomal arm of D. melanogaster hybridize on chromosome J of the three obscura Palearctic species and on the XR chromosomal arm of the obscura Nearctic representatives. Similarly, all probes hybridizing to the 3R chromosomal arm of D. melanogaster are located on chromosome O of the Palearctic species and on chromosome 2 of the Nearctic representatives of the obscura group species. Photographs showing the hybridization signals of the 22 probes in the different species are available from the authors on request.

Figure 1 shows a linear representation of the location of markers along element D in the different species. Only the close linkage between Acp70A and ΔDsubS40 is conserved in all the six obscura species. Comparing

**TABLE 1**

| Homologies between Muller’s elements and the chromosomes or chromosomal arms of D. melanogaster and the obscura group species |
|---|---|---|---|---|
| Muller’s element | D. subobscura | D. pseudoobscura |
| D | J (autosomal) | XR (sexual) |
| E | O (autosomal) | 2 (autosomal) |

**TABLE 2**

<table>
<thead>
<tr>
<th>Probe name (species)</th>
<th>Symbol</th>
<th>Chromosomal element</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant phage Fa (D. sub)</td>
<td>λDsubFa</td>
<td>D</td>
<td>A. MUNTÉ (personal communication)</td>
</tr>
<tr>
<td>Recombinant phage F62 (D. sub)</td>
<td>λDsubF62</td>
<td>D</td>
<td>J. M. MARTIN-CAMPOS (personal communication)</td>
</tr>
<tr>
<td>Ecdysone-inducible E74 (D. mel)</td>
<td>E74</td>
<td>D</td>
<td>BURIS et al. (1990)</td>
</tr>
<tr>
<td>Recombinant phage S30 (D. sub)</td>
<td>λDsubS30</td>
<td>D</td>
<td>L. SÁNCHEZ (personal communication)</td>
</tr>
<tr>
<td>Recombinant phage S40 (D. sub)</td>
<td>λDsubS40</td>
<td>D</td>
<td>S. CIRERA (personal communication)</td>
</tr>
<tr>
<td>Accessory male protein 70A (D. mel)</td>
<td>Acp70A</td>
<td>D</td>
<td>S. CIRERA (personal communication)</td>
</tr>
<tr>
<td>Esterase-5 (D. pseudo)</td>
<td>E5</td>
<td>D</td>
<td>BRADY et al. (1990)</td>
</tr>
<tr>
<td>Recombinant phage Fj (D. sub)</td>
<td>λDsubFj</td>
<td>D</td>
<td>Present study</td>
</tr>
<tr>
<td>Recombinant phage S25 (D. sub)</td>
<td>λDsubS25</td>
<td>D</td>
<td>L. SÁNCHEZ (personal communication)</td>
</tr>
<tr>
<td>Heat shock protein 83 (D. mel)</td>
<td>hsp83</td>
<td>D</td>
<td>HOLMGREN et al. (1979)</td>
</tr>
<tr>
<td>Extra-macrochaetae (D. mel)</td>
<td>emc</td>
<td>D</td>
<td>GARRELL and MODOLLEL (1990)</td>
</tr>
<tr>
<td>Recombinant phage FC4 (D. sub)</td>
<td>λDsubFC4</td>
<td>E</td>
<td>S. RAMOS-ONSINS (personal communication)</td>
</tr>
<tr>
<td>Recombinant phage S14 (D. sub)</td>
<td>λDsubS14</td>
<td>E</td>
<td>Present study</td>
</tr>
<tr>
<td>Heat shock protein 70 (D. mel)</td>
<td>hsp70</td>
<td>E</td>
<td>LIVAK et al. (1978)</td>
</tr>
<tr>
<td>Xanthine dehydrogenase (D. sub)</td>
<td>Xdh</td>
<td>E</td>
<td>J. M. COMERÓN and M. AGUADÉ (unpublished)</td>
</tr>
<tr>
<td>Recombinant phage S9 (D. sub)</td>
<td>λDsubS9</td>
<td>E</td>
<td>Present study</td>
</tr>
<tr>
<td>Recombinant phage G2 (D. sub)</td>
<td>λDsubG2</td>
<td>E</td>
<td>A. NAVARRO-SABATÉ (personal communication)</td>
</tr>
<tr>
<td>Recombinant phage S6 (D. sub)</td>
<td>λDsubS6</td>
<td>E</td>
<td>SEGARRA and AGUADE (1993)</td>
</tr>
<tr>
<td>Acid phosphatase-1 (D. sub)</td>
<td>AcpH-1</td>
<td>E</td>
<td>S. RAMOS-ONSINS (personal communication)</td>
</tr>
<tr>
<td>Ribosomal protein 49 (D. pseudo)</td>
<td>η49</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Cecropin (D. sub)</td>
<td>Cec</td>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

D. sub, D. subobscura; D. mel, D. melanogaster; D. pseudo, D. pseudoobscura.
any of the species of the obscura group with *D. melanogaster*, the segment delimited by *Acp70A* and *lDsubS40* also seems to be conserved, but the two markers are more tightly linked in the obscura group species than in *D. melanogaster*. Although the segment between *lDsubFy*- *hsp82* and *KUNZE-MÖHL D. madeirensis* probes is also maintained among the Palearctic and Nearctic representatives except for *D. persimilis*, the banding pattern of this segment when comparing, for instance, *D. subobscura* and *D. pseudoobscura*, is different enough for it not to be considered a true homologous segment. Moreover, the three Palearctic species and *D. melanogaster* also share the segment *E74-Acp70A*, although the banding pattern of this segment between *D. melanogaster* and the obscura representatives does not show a good correspondence.

*D. subobscura* and *D. madeirensis* share the same linear array of element *D* markers with similar relative distances (Figure 1). *D. subobscura* and *D. guanche* differ by the inversion of the *E74* and *emc* loci. *D. pseudoobscura* and *D. persimilis* also differ by the inversion of two markers (*lDsubFy* and *lDsubS30*). *D. pseudoobscura* and *D. miranda* differ by the inverted orientation of the three more distal markers (emc-*hsp28/23-lDsubS25*) and also of the four more proximal ones (*lDsubFa*- *EstS*- *E74*- *hsp82*).

Figure 2 shows the location of markers along element *E* in the different species. The order of markers in *D. subobscura* is given according to the *O* gene arrangement that corresponds with that in the cytological map (KUNZE-MÖHL and MÖLLER 1958). The figure also includes the location of the *Antennapedia* (*Antl*) gene that had been mapped previously by TEROL et al. (1991). All probes except the *hsp70* hybridize to a single site in the different species. In *D. melanogaster*, *hsp70* genes are organized in two clusters localized at sections 87A and 87C, but in situ hybridization of *hsp70* probes in this species also give weak signals at positions 95D and 87D (HOLMGREN et al. 1979). In *D. subobscura* and *D. guanche*, only two rather strong hybridization signals were detected. However, in *D. madeirensis* and the three obscura Nearctic species, in addition to these two strong signals, some very weak signals were also detected at other locations on the same chromosomal arm (result not shown).

Between the obscura group species and *D. melanogaster*, only the close linkage between the *rp49* and *Acp1*-loci has been maintained. In addition, the six obscura group species share the nearly telomeric position of *Cec*. In *D. melanogaster*, although *Cec* is also the most distal locus of those analyzed, the distance between *Cec* and the telomere is longer and so delimits a different segment. The close linkage between *lDsubS22* and one of the signals from *hsp70* is also maintained in the six obscura species. This result allowed us to differentiate in these species the two signals detected with the *hsp70* probe (as *hsp70/1* and *hsp70/2*). Another segment delimited by *lDsubS9* and *hsp70/1* seems to be conserved in five of the six obscura species (except for *D. miranda*). Although the length of the segment is fairly conserved, the banding pattern differs when comparing, for instance, *D. subobscura* and *D. pseudoobscura*. Finally, the *Antl-lDsubF4*- *lDsubS6* segment is also maintained in the obscura spe-
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D. subobscura

D. madeirensis

D. guanche

D. pseudoobscura

D. persimilis

D. miranda

D. melanogaster

FIGURE 2.—Schematic representation of the location of markers along element E of the studied species. The different elements have been given the same length to show the relative position of markers among them. Centromere is placed on the left and telomere on the right of the elements. See Table 2 for notation of markers.

D. subobscura and D. madeirensis differ by the inverted orientation of Acph-1-rp49-hsp70/2. The order of these loci in D. guanche is in agreement with that of D. madeirensis. However, in D. guanche there is an additional inversion including Xdh-ADsubS9-hsp70/1-ADsubS89. D. pseudoobscura and D. persimilis show an inverted orientation of Xdh-ADsubS9-hsp70/1-ADsubS2ADsubS14. D. pseudoobscura and D. miranda differ by the inverted position of Xdh-ADsubS9.

Tables 3 and 4 show the location of D and E elements markers, respectively, in the different species according to their photographic or cytological maps (see MATERIALS AND METHODS). There is a discrepancy in the location of the E74 gene in D. pseudoobscura with that previously reported by Jones et al. (1991) that can be attributed to a different interpretation of the polytene chromosome map. Otherwise, the locations of hsp28/23 and hsp70 in the three species of the D. subobscura cluster are in agreement with those previously reported (Molto et al. 1993a,b).

DISCUSSION

The established chromosomal homologies (Buzzati-Traverso 1955) of element D (J chromosome in the Palearctic species and XR chromosomal arm in the Nearctic species), and of element E (O chromosome of the Palearctic species and chromosome 2 of the Nearctic species) are completely supported by the present results. Moreover, no instance of exchange of genetic material among different elements has been detected, which indicates that elements D and E have largely con-

TABLE 3

Cytological location of the 12 probes on Muller's element D of the different species studied

<table>
<thead>
<tr>
<th>Species</th>
<th>emc</th>
<th>hsp83</th>
<th>hsp28/23</th>
<th>XDs 25</th>
<th>hsp70</th>
<th>XDs 40</th>
<th>XDs 30</th>
<th>E74</th>
<th>XDs 62</th>
<th>XDs Fa</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. subobscura</td>
<td>31B</td>
<td>18C</td>
<td>35B</td>
<td>27A</td>
<td>22C</td>
<td>19A</td>
<td>20A</td>
<td>20B</td>
<td>35C</td>
<td>34E</td>
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<tr>
<td>D. madeirensis</td>
<td>31B</td>
<td>18C</td>
<td>35B</td>
<td>27A</td>
<td>22C</td>
<td>19A</td>
<td>20A</td>
<td>20B</td>
<td>35C</td>
<td>34E</td>
</tr>
<tr>
<td>D. guanche</td>
<td>31B</td>
<td>18C</td>
<td>35B</td>
<td>27A</td>
<td>22D</td>
<td>19A</td>
<td>20A</td>
<td>20B</td>
<td>35C</td>
<td>34E</td>
</tr>
<tr>
<td>D. pseudoobscura</td>
<td>38</td>
<td>23</td>
<td>40</td>
<td>40</td>
<td>35</td>
<td>21</td>
<td>25</td>
<td>25</td>
<td>35</td>
<td>23</td>
</tr>
<tr>
<td>D. persimilis</td>
<td>39</td>
<td>24</td>
<td>40</td>
<td>40</td>
<td>35</td>
<td>23</td>
<td>26</td>
<td>26</td>
<td>35</td>
<td>24</td>
</tr>
<tr>
<td>D. miranda</td>
<td>9C</td>
<td></td>
<td></td>
<td>10A</td>
<td>9F</td>
<td>13A</td>
<td></td>
<td>18B</td>
<td>18A</td>
<td>13A</td>
</tr>
<tr>
<td>D. melanogaster</td>
<td>61D</td>
<td>63BC</td>
<td>66B</td>
<td>67B</td>
<td>67B</td>
<td>69A</td>
<td>70A</td>
<td>70DE</td>
<td>72F</td>
<td>74F</td>
</tr>
</tbody>
</table>

See Table 2 for notation of markers.
served their genetic content during the divergence of the species studied.

This conclusion is in agreement with previous studies on the location of other single-copy probes in species of the obscura group and in D. melanogaster (Steinemann 1982; Steinemann et al. 1984; Krishnan et al. 1991). However, in contrast with our results, some exchanges between elements have also been described. These cases are generally associated with multiple-copy genes like 5S rRNA genes, the histone genes (Steinemann 1982; Steinemann et al. 1984; Felger and Pinsker 1987) or tRNA genes (Tonzetich et al. 1990). Exchange of multiple-copy genes between different elements has been discussed as a result of the action of transposable elements (Felger and Pinsker 1987; Tonzetich et al. 1990).

An exchange between elements A and D in the D. pseudoobscura species cluster has also been reported (Segarra and Aguade 1992; Segarra et al. 1995). However, these elements are fused in this cluster, and thus this exchange can be easily explained by a pericentric inversion after the centric fusion. This mechanism may also act in other species with chromosomes generated by centric fusions, e.g., chromosome 3 of D. melanogaster resulting from a centric fusion of D and E elements. In the present study, however, no exchanges between element D (3L chromosomal arm) and element E (3R chromosomal arm) of D. melanogaster have been detected. This is consistent with the low probability of fixation of pericentric inversions, due to the production of aneuploid gametes that leads to low fitness when heterozygous (Charlesworth et al. 1987).

The present results allow us to test the homologies proposed previously within elements D and E in these species. Studies on interspecific hybrids between D. subobscura and D. madeirensis (Krimbas and Loukas 1984; Papaceit and Prevosti 1991) indicate that both are homosequential for the J chromosome (D element), which agrees with the identical distribution of markers in those species (Figure 1). Moreover, interspecific hybrids indicate that element E differs between them by the O3 inversion (Krimbas and Loukas 1984; Papaceit and Prevosti 1991) consistent with our observation of an inverted orientation of Acph-1-rp49-hsp70/2 (Figure 2). Additionally, the mapping of Acph-1 at the most proximal band of section 91C indicates that this marker is located very close to the breakpoint 91BC of the O3 inversion.

**TABLE 4**

<table>
<thead>
<tr>
<th>Species</th>
<th>λDsubFC4</th>
<th>λDsubS14</th>
<th>Antp</th>
<th>hsp70</th>
<th>Xdh</th>
<th>λDsubS9</th>
<th>λDsubG2</th>
<th>λDsubS6</th>
<th>AcpH-1</th>
<th>rp49</th>
<th>Cec</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. subobscura</td>
<td>83B</td>
<td>98D</td>
<td>80C</td>
<td>89A</td>
<td>94A</td>
<td>86C</td>
<td>90D</td>
<td>89A</td>
<td>83C</td>
<td>91C</td>
<td>91C</td>
</tr>
<tr>
<td>D. madeirensis</td>
<td>83B</td>
<td>98D</td>
<td>80C</td>
<td>89A</td>
<td>94A</td>
<td>86C</td>
<td>90D</td>
<td>89A</td>
<td>83C</td>
<td>91C</td>
<td>91C</td>
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<td>D. guanche</td>
<td>82D</td>
<td>98D</td>
<td>80C</td>
<td>89A</td>
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<td>86C</td>
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<td>54</td>
<td>53</td>
<td>48</td>
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</tr>
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<td>D. persimilis</td>
<td>48</td>
<td>52</td>
<td>50</td>
<td>58</td>
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<td>55</td>
<td>54</td>
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<td>45-48</td>
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<td>45</td>
<td>50</td>
<td>35</td>
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<td>35</td>
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<tr>
<td>D. melanogaster</td>
<td>82F</td>
<td>83D</td>
<td>84A</td>
<td>87A</td>
<td>87C</td>
<td>87D</td>
<td>95C</td>
<td>95E</td>
<td>96E</td>
<td>99D</td>
<td>99D</td>
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</tbody>
</table>

See Table 2 for notation of markers.
were obtained at localization signals are arrowheaded. Comparison in inversion that differentiates element an arrow. The location corrected scum section species differ by an inversion of sections HANSKY interpretation accounts for the different location of scua probably due to the partial disagreement of section lim-

hDsubF62 ers when comparing both species (Tables 2). MOORE The slightly different locations of some of the mark-

hsp7O/l-~subGZ-hDsubSl4 er segments can be polymorphic in D. miranda. The presence of this inversion makes it difficult to accurately localize on the DAS et al. (1982) photographic map the markers included in it (Table 3), since the stock used by these authors is unknown and its arrangement is difficult to ascertain by simple inspection of the banding pattern in their map. D. pseudoobscura and D. miranda differ with respect to element E only by a small inversion that affects section 54 (DOBZHANSKY and TAN 1996) and that explains the inverted position of Xdh and λDsubS9 when comparing those species (Figure 2).

In addition to checking previously established intrachromosomal homologies, the present results can be used to study the homologies between D. melanogaster and the obscura group species. Markers are distributed rather evenly along element D of D. melanogaster, the smallest segment being that delimited by λDsubβ and hsp28/23. None of the studied element D segments can be considered homologous between D. melanogaster and the obscura group species. However, markers are distributed in a more clustered fashion on element E of D. melanogaster. The cluster formed by the most closely linked loci is Acph-1-rp49-Cec. Of these loci, only the close linkage between rp49 and Acph-1 is maintained in the obscura species. Thus, only one conserved segment has been detected on element E when comparing D. melanogaster and D. pseudoobscura, for example. Only one conserved segment (z-Pgd) was also detected on element A of these two species after mapping a total of 32 markers (SEGARRA et al. 1995). These conserved segments are among the smallest chromosomal segments studied. The presence of conserved segments shared by D. melanogaster and the obscura group species allows inference of the ancestral relative order of some loci before the split of the melanogaster and obscura groups of Drosophila. According to the most parsimonious explanation, the close linkage between rp49 and Acph-1 (element E) must be ancestral to the divergence of the species studied. The same argument can be applied to element A conserved segments (SEGARRA et al. 1995): z-Pgd (disrupted in the D. subobscura lineage) and c-DSO1481 (disrupted in the D. pseudoobscura lineage).

Moreover, the present results also allow inference of chromosomal homologies of elements D and E between, for instance, D. subobscura (as representative of the Palearctic obscura group species cluster) and D. pseudoobscura (as representative of the Nearctic cluster).
Despite conservation of the genic content of elements D and E, considerable reorganization within each element is detected between these species (Figure 4). Inspection of this figure seems to indicate that reshuffling has affected element D more than E. Nine of the E element markers map on the distal half of this element in both species. In addition, in element D there are markers, such as E74, that move more drastically from one part of the chromosome to the other. Apart from this qualitative observation, three conserved segments (Cec-telomere, Acph-1-rp49 and λDsug2-hsp70/1) were detected in element E, but only one (Acp70A-ADsubS40) in element D, all delimited by closely linked markers. Although the detection of a higher number of conserved segments in element E may be partly attributable to our happening to have studied a higher number of tightly linked markers in this element, this cannot be the only explanation. In fact, the segments E74-hsp83 and hsp28/23-λDsug25 on element D are also delimited by closely linked markers in D. pseudoobscura, and these segments have clearly been disrupted in D. subobscura. Thus, element E seems to be more conserved than D. However, a larger number of markers is required to estimate the number of inversions differentiating elements D and E of D. subobscura and D. pseudoobscura. Even if this number is higher for element D, estimates of the evolutionary rates of both chromosomal elements between the obscura species and an outgroup species (D. melanogaster) would be required to detect in which lineage (D. subobscura or D. pseudoobscura) element D has evolved faster. Only this relative rate test would allow us to contrast the hypothesis (Charlesworth et al. 1987) of a higher rate of fixation of chromosomal inversions in the D. pseudoobscura lineage, given the sex chromosomal character of element D in this species.

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