Unusual Haplotype Structure at the Proximal Breakpoint of In(2L)t in a Natural Population of Drosophila melanogaster

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ABSTRACT

The existence of temporally stable frequency clines for In(2L)t in natural populations of Drosophila melanogaster suggests a role for selection in the maintenance of this polymorphism. We have collected nucleotide polymorphism data from the proximal breakpoint junction regions of In(2L)t to infer its evolutionary history. The finding of a novel LINE-like element near the In(2L)t breakpoint junction in sampled inverted chromosomes supports a transposable element-mediated origin for this inversion. An analysis of nucleotide variation in a Costa Rican population sample of standard and inverted chromosomes indicates a unique and relatively recent origin for In(2L)t. Additional In(2L)t alleles from three geographically diverse populations reveal no detectable geographic differentiation. Low levels of In(2L)t nucleotide polymorphism suggest a recent increase in the inversion’s frequency in tropical populations. An unusual feature of our sample of standard alleles is a marked heterogeneity in levels of linkage disequilibrium among polymorphic sites across the breakpoint region. We introduce a test of neutral equilibrium haplotype structure that corrects both for multiple tests and for an arbitrarily chosen window size. It reveals that an ~1.4-kb region immediately spanning the breakpoint has fewer haplotypes than expected under the neutral model, given the expected level of recombination in this genomic region. Certain features of our data suggest that the unusual pattern in standard chromosomes is the product of selection rather than demography.

INVERSION polymorphisms in the genus Drosophila are widely believed to be among the best examples of balanced polymorphisms. They have been used extensively as model systems for the study of the adaptive processes involved in the maintenance of genetic variation (reviewed in Krimbas and Powell 1992). In(2L)t is a common polymorphic inversion with stable frequency clines in natural populations of Drosophila melanogaster. While relatively rare in temperate climates (i.e., <10% at latitudes above 35°), In(2L)t reaches frequencies of 40–60% in tropical populations of Australasia and Africa (Knibb 1982; Bénassi et al. 1993). The existence of parallel latitudinal clines across different continents and hemispheres offers compelling evidence that natural selection is maintaining inversion polymorphisms (Knibb 1982).

Population genetic models suggest that a possible advantage of inversions lies in their ability to suppress recombination in karyotypic heterozygotes and thus maintain favorable epistatic interactions between alleles at linked loci (Wasserman 1968; Dobzhansky 1970). Despite the evidence for selection on inversions based on biogeographical patterns (Knibb 1982) and cage experiments (Van De Iden and Kamping 1991), surveys of molecular variation on standard and inverted chromosomes have found patterns that are generally consistent with historical and/or equilibrium neutral explanations (Ishii and Charlesworth 1977; Mukai and Voelker 1977; Nei and Li 1980; Strobeck 1983; Aquadro et al. 1986, 1991; Aguadé 1988; Bénassi et al. 1993; Rozas and Aguadé 1994).

In Drosophila, the linkage disequilibrium between selected sites and linked neutral variants predicted by epistatic or balancing selection models decays rapidly in the presence of gene conversion (Ishii and Charlesworth 1977; Strobeck 1983; Hudson and Kaplan 1988; Andolfatto and Nordborg 1998). So the effect of selection on linked neutral variation will not be detected if the rate of exchange between standard and inverted arrangements is too high. The rate of double crossover events and gene conversion has been estimated to be on the order of 10^{-4} to 10^{-5} per site per generation in the middle of inversions (reviewed in Ashburner 1989). In addition, gene conversion events between chromosomal arrangements are readily observed in nucleotide polymorphism data (Rozas and Aguadé 1994). Given such high rates of exchange, the pattern at linked neutral sites may not be particularly informative in such regions.

An alternative is to investigate the distribution of molecular variation within and between chromosomal arrangements at loci closely linked to the inversion breakpoints (Strobeck 1983; Andolfatto and Nord-
bong 1998). Exchange between karyotypes is likely to be reduced at inversion breakpoints due to topological constraints on homologous pairing (Novitski and Braver 1954; Grell 1962; S. Hawley, personal communication). Two studies of loci closely linked to inversion breakpoints in Drosophila (Rozas and Aguadé 1994; Wesley and Eanes 1994) lead to different conclusions regarding the level of exchange between arrangements.

We have investigated nucleotide variation in a 5-kb region surrounding the proximal breakpoint of In(2L)\text{t}. Our \textit{a priori} prediction is that the long-term maintenance of the inversion by selection will have led to the accumulation of a large number of fixed differences between karyotypic classes. Wesley and Eanes (1994) found no evidence for deviations from neutral equilibrium expectations at the breakpoints of In(3L)\text{P}, another common polymorphic inversion in D. melanogaster with frequency clines in nature similar to those of In(2L)\text{t}. One explanation is that selection does not often act long enough for the predicted signature of balancing selection to develop (Hudson et al. 1997). If instead In(2L)\text{t} is a young balanced or locally adapted polymorphism that has recently increased in frequency, we might observe a paucity of nucleotide variation among inverted alleles. By investigating sequences directly spanning the inversion breakpoint, our chances of observing the signature of selection, whether old or recent, will be increased due to the expected reduction in levels of genetic exchange between arrangements.

An unexpected feature of our sample of standard alleles is an unusually strong association among segregating sites close to the breakpoint. The presence of selection and recombination in a region can lead to considerable heterogeneity in levels of linkage disequilibrium (Hudson and Kaplan 1988; Hudson et al. 1997). We introduce a test based on Strobeck (1987) to detect subsets of the data that contain fewer haplotypes than expected under a neutral equilibrium model. Previous tests have not corrected for multiple tests nor for an arbitrarily chosen window size (Kirby and Stephan 1996; Bénassi et al. 1999); we show that this can have an important effect on the interpretation of the data.

**MATERIALS AND METHODS**

Localizing the 34A breakpoint: The In(2L)\text{t} proximal breakpoint (34A8-9) was first localized by \textit{in situ} hybridization on polytene chromosomes (modified from Sniegowski and Charlesworth 1994) to two PI clones (DS00576, DS01619). Subcloning of the shared regions of these two clones led to the recovery of an 8.4-kb S\textit{al} fragment containing the 34A breakpoint (see Figure 1). This 8.4-kb region was randomly sheared by aspiration. Sheared fragments were repaired with two polymerase (Boehringer Mannheim, Indianapolis). Size-selected fragments (0.5–1.6 kb) were ligated into a derivative of pZeoO-2.1 (Invitrogen Inc., San Diego) and chemically transformed. Sequencing templates were prepared by PCR directly from colonies with standard M13-based primers, followed by polyethylene glycol (PEG) precipitation. Templates were sequenced with standard primers using a dRhodamine terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA) and run on an ABI377XL automated sequencer. Sequences were analyzed with ABI sequence analysis v. 3.0 software. Contigs were managed with Sequencher v. 3.0 software. Inverted junction regions A/C and B/D (see Figure 1) were recovered by inverse PCR techniques (Ochman et al. 1993) and were sequenced as above. Sequences corresponding to the 8.4-kb proximal breakpoint and In(2L)\text{t} breakpoint junction regions have been deposited in GenBank under accession nos. AF172291–AF172316. An alignment has been deposited in the EMBL database under accession no. D309454 (ftp://ftp.ebi.ac.uk/pub/databases/embldb/align/). The homologous 5-kb region for one line of \textit{D. simulans} was sequenced for divergence estimates (GenBank accession no. AF172317).

Population samples: Field collections were made from a single, large population of \textit{D. melanogaster} in San Jose, Costa Rica. This tropical population was chosen because it was likely to have an appreciable frequency of In(2L)\text{t}. Wild-caught females were used to start isofemale lines. After several days of egg laying, genomic DNA was prepared from each original wild-caught female and PCR genotyped for In(2L)\text{t}. The use of specific primer pairs allowed the recovery of standard or In(2L)\text{t} alleles from inversion heterozygotes identified in the above screen. For the sampling of standard alleles, a 5-kb region spanning the proximal breakpoint (C/D in Figure 1) was PCR amplified from In(2L)\text{t} heterozygotes (primer C, GCCACGCCTCCCACCTACCTTAC; primer D, AATGCTTGGTG GGCCTTGGGAATTGTG). These PCR-generated templates were sequenced directly with a set of forward and reverse primers. Similarly, for In(2L)\text{t} alleles, the two breakpoint junction regions (A/C and B/D in Figure 1) were amplified separately and sequenced from seven individuals (primer InA412, TTTGATCCACCCACATCTCAGAC; primer InB124, GTAC TTTCACTGTTTGTCTGACAGCC). Inversion frequencies were also determined by PCR for three other populations (Florida City, FL; Yeppoon, Australia; and Zimbabwe, Africa) for comparison to previously published estimates. Australian
and African isofemale lines were kindly provided by C.-I Wu. Primers used for karyotyping individual flies for Int(2L)l are as follows: StC545, GACTTATTCTGCTTGCTGATCAAG; StD18, CTTGTCCCCACCGTACAGTGGTTCGTGC; InA151, TATTGGTTGCGCTGTTTCAG. Expected PCR products are ∼500 bp for primer pair StC545/StD18 and ∼250 bp for primer pair StC545/InA151.

Polymorphism analyses: Tests of the neutral equilibrium model that compare polymorphism and divergence (e.g., Hudson et al. 1987) could not be used here because of uncertainties in the alignment between D. melanogaster and D. simulans. The neutral mutation parameter θ = 4Nθ where N is the effective population size of the species and θ is the neutral mutation rate, was estimated from both π, the average pairwise difference per base pair (Tajima 1983), and S, the number of polymorphic sites in the sample (Watterson 1975). We use Tajima’s D statistic (Tajima 1989) to characterize the skew in the frequency distribution of segregating mutations in our sample. Significance levels for Tajima’s D and statistical tests described below were determined using coalescent simulations that condition on intermediate levels of recombination and the observed number of polymorphic sites in the sample (Hudson 1993). Similar simulations were used to test for the uniformity of polymorphic sites. Here, the statistic used was the length of the longest distance (in base pairs) between two consecutive segregating mutations. All simulations assumed a single panmictic Wright-Fisher population, unless otherwise specified. All simulations were run using modifications of a program kindly provided by R. Hudson.

Tree construction. Int(2L)l historical frequency, and age: Bootstrapped parsimony phylogenies were constructed using PAUP3.1.1 (Swofford 1993). To estimate an age for Int(2L)l, we assume that the inversion has a unique origin (from a standard ancestor) and that the inverted region has been genetically isolated from standard chromosomes. Under a model in which balancing selection maintains the inversion, we assume the inversion immediately rose to a frequency f, where it has been maintained until the present. An alternative model assumes that the inversion is a strictly neutral polymorphism that has drifted to its present frequency.

If we assume the former model and that the inversion is at equilibrium, then θ and f can be estimated from the sample sizes of inverted and standard chromosomes (n and n, respectively) and the observed number of segregating sites within the inverted and standard chromosomes (S and S, respectively; Hudson and Kaplan 1986). Define a = Σ−1/2 j=1. Then, E[S] = 4θn and E[S] = θ(1 − f)n. To estimate θ and f, the expectations are replaced by their observed values. This yields

\[ \hat{\theta} = \frac{S}{S + S} \]

and

\[ \hat{f} = \frac{S}{4\theta n} \]

It is then straightforward to estimate the time to the most recent common ancestor of the inverted chromosomes by substituting (1) into

\[ E[T_{nca}] = 4 Nf (1 - f)^{-1}. \]

This yields an estimate of the minimum age of the inversion. An expected age of the inversion is also calculated based on the average net number of differences between arrangements scaled to the expected divergence time between D. melanogaster and D. simulans. It should be noted that these estimates have very large variances.

We investigate by simulation whether levels of Int(2L)l variation are compatible with assumed historical inversion frequencies under a neutral equilibrium model. We run 100,000 coalescent simulations with sample size n, and population mutation parameter θf. Here θ is estimated from (2), and we determine for which values of f the observation of S segregating sites is within the middle 95% of the simulated distribution of S.

Test of neutral equilibrium haplotype structure: We constructed a test to determine whether any subsets of consecutive segregating sites in our data contain fewer distinct haplotypes than would be expected under a neutral equilibrium model. Suppose we have a data set with n chromosomes and S segregating sites. Define S to be the largest number of consecutive segregating sites that contain only k different haplotypes (1 < k < n). An empirical distribution of S is determined from 100,000 simulations using an infinite-sites, panmictic coalescent model conditional on n and S (Hudson 1993). We then calculate the proportion p of simulated data sets containing at least one stretch of S consecutive segregating sites with k or fewer haplotypes. This is equivalent to calculating the proportion of simulated data sets having S greater than or equal to the S observed in the data. Since choosing any value of k is arbitrary, we must correct for the implicit multiple tests involved. This corrected value P is determined from further coalescent simulations that compare the actual smallest p value with simulated smallest p values. Our test is similar to other tests that use the observed number of haplotypes as a test statistic (e.g., Strobeck 1987; Fu 1996), but our test corrects for multiple windows and an arbitrarily chosen window size. Here window size is measured in segregating sites, not in base pairs, so our test shares affinities with commonly used “scan statistics” (e.g., Karlin and Macken 1991).

A concern is the violation of the “infinitely many sites” assumption of our null model. In particular, mutations can sometimes overlap in actual data, especially in the presence of deletions. It is not clear whether “infinite-sites” simulations are conservative when the actual data has overlapping mutations. On one hand, more windows are considered in the simulations than in the actual data, which is conservative. On the other, missing information for those chromosomes with deletions might lead to fewer haplotypes than expected under the infinite-sites assumption. We believe that both effects are rather minor, but a higher degree of caution should be exercised when interpreting the relevant significance levels.

Two different versions of the Int(2L)l data are considered. In-del is likely to be governed by a different mutational process than simple nucleotide polymorphisms (hereafter SNPs); however, they are included in simulations that condition on S since nothing is assumed about the underlying mutation rate, θ (Hudson 1993). Such information has been included in previous studies (e.g., Hudson et al. 1994; Kirby and Stephan 1996; Cirera and Aguadé 1997). We have collapsed suspicious clusters of polymorphisms in our data into single events (complex mutations, denoted M in Figure 2). Version I includes SNPs and biallelic in-del polymorphisms, while Version II also includes overlapping mutational events.

All parameters used in simulations, including n, S, and C are listed in Table 1. In reality, the effective rate of recombination depends on the arrangement class. Our assumptions of panmixia and the same recombination rate for all individuals are unrealistic in this regard. Nevertheless, we consider it to be a reasonable simplifying assumption. In fact, an explicit demographic model of equilibrium-balancing selection based on estimates of the inversion’s age and historic frequency yielded more significant P values (results not shown). Since individuals were not sampled randomly, both versions used a random sample based on the observed frequency (20.8%) of Int(2L)l
TABLE I
Summary of polymorphism data for the In(2L)t proximal breakpoint

<table>
<thead>
<tr>
<th></th>
<th>n×</th>
<th>S</th>
<th>C_m*</th>
<th>θ*</th>
<th>π</th>
<th>Tajima’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Representative sample</td>
<td>14</td>
<td>154</td>
<td>0.0003</td>
<td>0.0104</td>
<td>0.0125</td>
<td>1.00*</td>
</tr>
<tr>
<td>Standard alleles</td>
<td>11</td>
<td>146</td>
<td>0.0005</td>
<td>0.0100</td>
<td>0.0122</td>
<td>1.07</td>
</tr>
<tr>
<td>In(2L)t alleles</td>
<td>7</td>
<td>12</td>
<td>—</td>
<td>0.0011</td>
<td>0.0009</td>
<td>−0.79</td>
</tr>
</tbody>
</table>

*C_m, θ, π are given per site. In-del variation is included in calculations of θ and π.*
*Number of alleles sampled.*
*Watterson’s (1975) estimate.*
*Based on 21% In(2L)t frequency. Assuming other compatible In(2L)t frequencies did not significantly change these estimates.*
*Tajima’s test by simulation (see methods); P > 0.10 when C = 0; P = 0.001 when C = C_m*.

chromosomes in the San Jose population. This sample included all 11 standard chromosomes as well as cr40i, cr44i, and cr46i (see Figure 2). Since In(2L)t varies in frequency among geographic locations, and the inferred historical frequency was estimated to be between 4 and 23% (see results), we also considered representative samples with zero to four inverted alleles.

Recombination: The implications of recombination on the predictions of population genetic models are poorly understood. Perhaps as a result, investigators often conduct statistical tests with the assumption of no recombination. However, since there is evidence for recombination in our (and other) data, it is more realistic to assume C > 0 in simulations. In addition, ignoring the effect of recombination does not always lead to a conservative test (Wai 1999; resulTs).

The use of estimators of the population recombination rate, C = 4Nr, on the basis of nucleotide polymorphism data (e.g., Hudson 1987; Hey and Wakeley 1997) are problematic because they have large variances and are not necessarily conservative for our test. We consider two estimates of the recombination parameter to be informative. The first, C_m, is defined as the recombination rate for which the P value of our haplotype test is maximal. Assuming C = C_m makes our statistical test conservative with respect to our uncertainty over the true value of C. C_m is a second estimate of 4Nr and assumes that r = p, where p is the per base pair, per generation rate of recombination obtained from laboratory measurements of the exchange of flanking markers (cf. Kliman and Hey 1993; Comeron et al. 1999). N is estimated as θ/4μ, where θ is estimated from the average nucleotide diversity at silent sites and noncoding DNA of loci in regions of intermediate recombination (Moriyama and Powell 1996). The neutral mutation rate, μ, is assumed to be 3 × 10^-8 per site per generation, about twice that estimated for silent sites in Drosophila (Sharp and Li 1989; Fleischer et al. 1998). C_m is not conservative in statistical tests. However, C_m has the advantage of being independent of our polymorphism data set (and thus independent of neutral equilibrium assumptions) and it represents our best a priori guess at the true recombination rate in the chromosomal region studied.

The presence of an inversion in our sample makes the parameter C difficult to interpret. We expect that reduced exchange in inversion heterozygotes (i.e., genetic isolation) will affect the overall levels of exchange. When calculating C_m for use in simulations, we account for lack of recombination in D. melanogaster males and assume an absence of recombination in inversion heterozygotes. Thus, C_m = (1 − 2q(1 − q)) 2Np, where q is the assumed inversion frequency. Note that the maximum effect of an inversion on the recombination rate among standard chromosomes is a factor of two (assuming an inversion at 50% frequency and no recombination in inversion heterozygotes).

Analysis of other data sets: To demonstrate the effect that an arbitrarily chosen window size can have on the likelihood of a data set, we assess the significance of nonneutral haplotype structure reported in additional studies of D. melanogaster populations: the white locus data of Kirby and Stephan (1996), the Acp70A locus data of Currier and Aguadedé (1997), and the vermillion locus data of Begun and Aquadro (1995). All biallelic mutations were considered for the analysis of white (Figure 1 of Kirby and Stephan 1996). We excluded site 383, a multiple hit, in the Acp70A data (see discussion). For the vermillion data, each population sample was considered separately. Sites 1031 and 2532 (which are not biallelic) were
Haplotype Structure at In(2L)t

In(2L)t BP

polyA

polyA
excluded. P values of haplotype tests are reported for \(C = C_{in}\) and \(C = C_{ad}\). \(C_{ad}\) is estimated for each locus, as above, using estimates of \(p\) for each locus (Comeran et al. 1999).

RESULTS

Polymorphism summary: Figure 2 summarizes 176 polymorphic sites found in the sampled 5-kb of region C/D (see Figure 1). The data set contains 131 biallelic single nucleotide polymorphisms (SNPs). In addition, there are 27 biallelic insertion-deletion polymorphisms, denoted by i, d, or M. Complex polymorphisms are denoted by M; they are either multiple insertions (or deletions) that are very close to each other, or one or more nucleotide mutations associated with an insertion or deletion. The spatial distribution of insertion-deletion variation does not appear to be different from that for SNPs and does not significantly affect Tajima’s D statistic (results not shown). The In(2L)t breakpoint occurs between positions 2826 and 2919, where all sampled inverted chromosomes \(n = 18\) contain a 94-bp deletion.

Inversion frequencies and uniqueness of origin: Estimates of In(2L)t frequencies assessed by PCR assay in Yeppoon and Florida City samples were 22.9\% \(n = 43\) and 25.0\% \(n = 100\), respectively. These estimates are similar to those based on the cytology of geographically proximate populations (Mettler et al. 1977; Knibb 1982). In(2L)t frequencies for San Jose and Zimbabwe (Harare and Sengwa) were estimated to be 20.8 \(n = 100\) and 58.2\% \(n = 98\), respectively. To confirm that all In(2L)t chromosomes share the same breakpoint, a number of extracted In(2L)t chromosomes (Costa Rica, \(n = 10\); North Carolina, \(n = 7\); Zimbabwe, \(n = 10\)) were assayed by PCR. In all cases there was agreement between the cytology and PCR results consistent with a single mutational origin for In(2L)t. Sequence from 18 In(2L)t chromosomes (San Jose, \(n = 7\); Florida City, \(n = 3\); Yeppoon \(n = 4\); and Zimbabwe, \(n = 4\)) revealed that all have identical breakpoints.

Recombination within and between chromosomal arrangements: For the chromosomal band 34A8-9, the laboratory estimate of the crossover rate is \(2.9 \times 10^{-3}\) per site per generation in females (Comeran et al. 1999). Assuming an In(2L)t frequency of 20.8\%, no recombination in inversion heterozygotes and a species population size of \(10^6\) (see methods), \(C_{ad}\) is 0.019 per site per generation. There is evidence for a considerable amount of recombination in our sample of standard chromosomes. The positions of 10 inferred minimum recombination events (Hudson and Kaplan 1985) in Figure 2 are denoted by arrows. Adding inverted chromosomes to the analysis revealed no additional recombination events.

Despite the large number of informative polymorphisms, there is almost no evidence for genetic exchange between arrangements in our data. A possible exception is site 174 (Figure 2), which may be a multiple hit. This observation is consistent with the finding of suppressed exchange between standard and inverted classes at the breakpoint of the inversion (Novitski and Braver 1954; Grell 1962). We expect that genetic exchange between chromosomal arrangements will tend to decrease the number of fixed differences between arrangements and increase the number of shared polymorphisms. Of the 120 informative polymorphic sites, we observed 12 fixed differences between arrangements and just 1 shared difference. This pattern is in marked contrast to patterns observed at two additional loci linked to In(2L)t (summarized in Table 2). These two loci, Adh and Fbp2, are further from the breakpoints where the rate of exchange between arrangements is likely to be higher.

History of In(2L)t: A bootstrapped parsimony phylogram for 2.3 kb of sequence (positions 1491 to 3822) immediately spanning the inversion breakpoint is shown in Figure 3. All mutations in this segment are consistent with a single genealogical tree (i.e., there are no inferred recombination events in the segment). From the tree, we can infer that the inversion is relatively recently derived from one of two distinct haplotype classes of standard chromosomes. Trees based on three other recombination-free segments (positions 189–492, 639–1020, and 3946–4354) all produce similar topologies: In(2L)t alleles form a distinct cluster that is closely related to one or more standard haplotypes.

In total, 12 biallelic segregating polymorphisms were found in the 7 sampled In(2L)t alleles. Of these, 9 are SNPs (see Figure 2). Estimates of \(\theta\) and \(\pi\) for the In(2L)t alleles are 11 and 7\%, respectively, of those for standard alleles (Table 1). Assuming the population is at equilibrium, this level of diversity is consistent with a low (i.e., \(\sim 10\%\)) average historical frequency.

In(2L)t appears to be recently derived relative to standard lineages and the D. melanogaster-D. simulans divergence time. We estimate the \(E[\text{T}_{\text{MRECA}}]\) of our sample of In(2L)t alleles to be \(\sim 0.3\) \(N_e\) generations. This roughly corresponds to \(\sim 100,000\) years, assuming an \(N_e \sim 3 \times 10^6\) (Kreitman 1983) and 10 generations per year. Our estimate does not change significantly over a wide range of assumed inversion frequencies. However, since In(2L)t is rare relative to the standard alleles, it is more often heterozygous and thus more susceptible to forces such as background selection (Charlesworth et al. 1993) and selective sweeps (Maynard Smith and Haigh 1974). These forces may lead to an underestimate of the historical frequency of In(2L)t (and thus the \(E[\text{T}_{\text{MRECA}}]\)). However, the magnitude of their effect cannot be meaningfully estimated without a priori knowledge of the inversion’s true historical frequency. As an illustration, the resulting reduction in recombination relative to the standard class \((\sim q^2/1-q)^2\) will be greater than 350-fold for an inversion at 5\% frequency, and only 9-fold for an inversion at 25\%.
TABLE 2
Data on genetic exchange between arrangements at loci linked to inversions

<table>
<thead>
<tr>
<th>Region</th>
<th>Cytological position</th>
<th>Standard $\pi$</th>
<th>Inverted $\pi$</th>
<th>Informative poly (a)</th>
<th>Fixed: shared (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In(2L)t breakpoint</td>
<td>34A8-9</td>
<td>0.0122</td>
<td>0.0009</td>
<td>120</td>
<td>12:1</td>
</tr>
<tr>
<td>Adh-5 and Adh-dup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Zimbabwe + World)</td>
<td>35B3</td>
<td>0.0069</td>
<td>0.0029</td>
<td>70</td>
<td>0:20</td>
</tr>
<tr>
<td>(Ivory Coast)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Spain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fbp2 (Ivory Coast)</td>
<td>30B1-12</td>
<td>0.0072</td>
<td>0.0068</td>
<td>15</td>
<td>0:11</td>
</tr>
<tr>
<td>In(3L)P breakpoints</td>
<td>63B8-11 and 72E1-2</td>
<td>0.0060</td>
<td>0.0003</td>
<td>42</td>
<td>8:0</td>
</tr>
<tr>
<td>Hsp83</td>
<td>63C1</td>
<td>0.0065</td>
<td>0.0009</td>
<td>8</td>
<td>2:0</td>
</tr>
<tr>
<td>Est-6</td>
<td>69A1-5</td>
<td>0.0162</td>
<td>0.0200</td>
<td>30</td>
<td>0:17</td>
</tr>
<tr>
<td>Est-5 D. pseudoobscura</td>
<td>23 (near SR BP 22)</td>
<td>0.0123</td>
<td>0.0013</td>
<td>22</td>
<td>7:1</td>
</tr>
<tr>
<td>rp49 D. subobscura</td>
<td>Near BP of O$<em>{12}$/O$</em>{14}$</td>
<td>0.0062</td>
<td>0.0079</td>
<td>53</td>
<td>2:12</td>
</tr>
</tbody>
</table>

(a) The number of polymorphisms in the sample excluding singletons.
(b) The ratio of fixed polymorphisms between arrangements to the number shared between arrangements.
(c) Present study, including insertion-deletion variation, Table 1.
(d) S.-C. Tsuar, unpublished data; the world sample is that of Kreitman (1983).
(e) Data from Bénassi et al. (1993); restriction site data.
(f) Data from Aguadé (1988); restriction site data.
(g) Data from Westey and Eanes (1994).
(h) Data from Hasson and Eanes (1996).
(i) Data from Rozas and Aguadé (1994).
(j) Data from Babcock and Anderson (1996).

A second estimate of the age can be obtained from the number of fixed differences between In(2L)t and standard lineages: the number (9) is small compared to the average pairwise divergence between standard chromosomes (48.5). This again suggests that In(2L)t is recently derived relative to standard lineages. We estimate the expected age of the inversion (~160,000 years) to be ~4% of the net divergence time for D. melanogaster and D. simulans.

In(2L)t nucleotide diversity and frequencies in natural populations: Several features of the data suggest that In(2L)t is not at equilibrium. First, Tajima’s $D$ for the inverted class is negative (though the frequency spectrum is not significantly skewed). This finding is interesting given that Tajima’s $D$ is significantly positive for the random sample. Second, In(2L)t chromosomes show no segregating variation in a region of elevated $\theta$ (positions 2637 to 3020, Figure 2). These observations suggest a recent origin and rapid increase in the inversion’s frequency.

Evidence that In(2L)t has recently increased in frequency can be inferred from the geographic distribution of In(2L)t variation and the relative levels of diversity for standard and inverted chromosomes. We sequenced an ~0.8-kb region spanning the C/A breakpoint junction of 11 additional In(2L)t chromosomes (positions 2001 to 2819, Figure 2). Table 3 summarizes polymorphic variation found in this subregion for 18 In(2L)t alleles from four geographically diverse populations. Permutation tests (Hudson et al. 1992) failed to

Figure 3—Bootstrapped parsimony phylogram for a 2.3-kb region immediately spanning the In(2L)t breakpoint (positions 1491 to 3822 in Figure 2). The tree indicates that In(2L)t is relatively recently derived from one of two distinct standard haplotype classes. Numbers on nodes indicate the percentage of 1000 bootstrap replicates that supported the node.
<table>
<thead>
<tr>
<th>Position*</th>
<th>2</th>
<th>2</th>
<th>2</th>
<th>2</th>
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* The region sequenced corresponds to positions 2001 to 3068 in Figure 2. Labels of alleles correspond to the following populations: cr, San Jose, Costa Rica; fc, Florida City, FL; y, Yeppoon, Australia; zh and zs, Harare and Sengwa Wildlife Reserve, Zimbabwe.

detect geographic differentiation of In(2L)t alleles (P > 0.4). Using coalescent simulations, we estimate that the level of In(2L)t nucleotide diversity is inconsistent with equilibrium inversion frequencies greater than ~23% (assuming C = C_{ib}). Thus, the inversion is not significantly lacking in variation given its sampled frequencies in Costa Rica, Yeppoon, or Florida City. Under the most conservative and unrealistic assumption of no recombination, In(2L)t diversity is inconsistent with frequencies above ~35%. The Zimbabwe sample well exceeds this upper limit with an In(2L)t frequency of 58.2%. This sample is actually composed of two populations that had similar In(2L)t frequency estimates: Sengwa Wildlife Reserve (61.4%, n = 44) and the capital, Harare (55.5%, n = 54).

Heterogeneity in nucleotide polymorphism: Sliding-window profiles of nucleotide diversity (Figure 4) reveal considerable heterogeneity in levels of polymorphism across region C/D. For example, the large mutation-free stretch centered at ~1600 in Figure 4B (found between positions 1491 and 2019 in Figure 2) is highly unusual (P < 0.004 for all C) assuming a uniform mutation rate. Nonuniformity of mutations could arise from heterogeneity in selective constraint across the region. A comparatively large peak of intraspecific polymorphism is observed at the inversion breakpoint (approximately position 2600 in Figure 4A). Interestingly, most of this variation is distributed between two major haplotype classes and not within them (Figure 4B), reflecting the strong linkage disequilibrium observed between sites close to the breakpoint (see Figure 2). The average pairwise divergence between haplotype classes is ~10% at its highest point. This pattern is reminiscent of that observed between Fast and Slow allozyme classes at the Adh locus (Kreitman and Hudson 1991), a polymorphism believed to be maintained by balancing selection. The divergence between Adh-Fast and Adh-Slow allelic classes is between 6 and 7% at its highest point using a comparable window size. The average divergence between D. melanogaster and D. simulans at silent sites is ~9% (Takano 1998).

There seem to be fewer haplotypes at the breakpoint than expected under a neutral equilibrium model (given the sample size, the number of mutations, and the expected recombination rate). Unfortunately, Figures 4A and 4B are tricky to interpret: for one, the division of standard chromosomes into two “haplotype classes,” as done in Figure 4B, is post hoc. In addition, the chosen window size is arbitrary. Given evidence for heterogeneity in constraint in the region, Figure 4 could be misleading since each window may not contain an equivalent number of “neutral” polymorphisms. In general, it is unclear how to test whether an arbitrarily defined window is unusual after having examined the data. To address this difficulty, the test described below corrects for a post hoc choice of window size.

Test of neutral equilibrium haplotype structure: While there is evidence for recombination in our data (i.e., 10 minimum inferred recombination events), there are also strong associations among sites over a considerable distance spanning the In(2L)t breakpoint (see positions 1491-4109 in Figure 2). Although this observation seems unlikely under the null model, closely linked sites do have correlated histories in neutral genealogies with intermediate recombination (Griffiths 1981). For this reason, tests based on the permutation of sites (e.g., Leicht et al. 1993; Kirby and Stephan 1996), which assume sites are exchangeable, are not appropriate in this context. Instead, we tested the unusual features of our data by coalescent simulations with recombination. Table 4 shows p values from simulations run on the two versions of the n = 14 data (see methods). For example, Figure 2 shows 12 consecutive segregating sites (sites 97–108) that define only two haplotypes. To test how unusual this pattern is, 100,000 replicates were run using a panmictic, no recombination coalescent model that conditioned on n = 14 and S = 154. The proportion (p) of simulated runs containing 12 or more consecutive segregating sites that define only two haplotypes was p = 0.048 (see Table 4, version I, C = 0). The p values for each S, corrected for multiple windows, are reported for C = C_{icw} and C = C_{ib}.
most unusual $S_\delta$ for our data is for $S_\delta = 50$ (or $S_\delta = 60$ for Version II, Table 4).

Final $P$ values that correct for a post hoc choice of window size are shown in Table 4. Each of these values reflect median $P$ values for all possible random samples with three $In(2L)t$ alleles (reflecting the sampled $In(2L)t$ frequency of 20.8%). Since $In(2L)t$ nucleotide diversity levels are consistent with frequencies of between ~4 and 23%, we conducted additional tests on random samples corresponding to $In(2L)t$ frequencies of between 0 and 27%. Most of the tests on Version I of the data were not significant when $C = C_{\text{con}}$. Version II of the data, which includes polymorphisms that overlap with the 94-bp deletion of $In(2L)t$ alleles, is significant when $C = C_{\text{con}}$ for samples that included between zero and three $In(2L)t$ alleles.

Figure 5 shows $P$ vs. $C$ for Version I of the data. Note that $C = 0$ is not conservative and that $P$ decreases (almost) monotonically for $C > C_{\text{con}}$. The pattern in Figure 5 (an initial increase in $P$ for low values of $C$ followed by a steady decrease as $C$ increases) is a general feature of our test (results not shown). When running simulations with our a priori estimate of the recombination rate ($C_{\text{lab}}$, corrected for the inversion’s effect), all tests were highly significant ($P < 0.001$) for both versions of the data, including samples with zero to four $In(2L)t$ alleles.

Multiple tests, recombination, and haplotype structure at other loci: Correcting for multiple tests and an arbitrary window size can have an impact on the significance of the data. For example, the most unusual subset of the Acp70A data (Cirera and Aguadé 1997) is a window of 19 segregating sites with three haplotypes ($p_2 = 0.064, C = C_{\text{con}}$). By our test (Table 5), the corrected $P = 0.130$ ($C = C_{\text{con}}$). For the vermilion data of Begun and Aquadro (1995), the lowest $p_2$ is lower than

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Figure 4.—(A) Sliding-window profile of nucleotide diversity ($\pi$) for a representative random sample of alleles ($In(2L)t$ frequency = 21%). A peak of polymorphism is revealed at the inversion breakpoint, indicated by an arrow. The window size is 200 bp; the increment is 10%. Nucleotide positions do not correspond exactly to those in Figure 2 since gaps have been excluded. (B) The distribution of nucleotide diversity within and between haplotype classes. This analysis reveals that most of the diversity at the breakpoint is distributed between (solid line) and not within (dotted lines) the two haplotype classes. Class 1 (defined arbitrarily) includes lines cr08s, cr30s, cr47s, cr52s, and cr69s; class 2 includes the remaining standard and $In(2L)t$ alleles.
0.05 for all non-African populations when \( C = C_{\text{con}} \) (Beijing, California, Ecuador, and Taiwan). Two of these populations (Ecuador and Taiwan) are no longer significant when correcting for the post hoc choice of window size under conservative recombination (\( P = 0.064 \) and \( P = 0.091 \), respectively, Table 5).

The assumed rate of recombination also has a large effect on our interpretation of the data (Table 5). For example, under conservative recombination the white locus (Kirby and Stephan 1996) shows no sign of unusual haplotype structure (\( P = 0.903 \), \( C = C_{\text{con}} \)). However, highly significant nonneutral haplotype structure is detected for the white data set when we assume \( C = C_{\text{lab}} \) (\( P = 0.001 \)). As expected, highly significant haplotype structure is also detected in the Acp70A4 and non-African vermilion samples when \( C = C_{\text{lab}} \) (\( P = 0.004 \)). Interestingly, significant haplotype structure is not detected in the two African samples of vermilion, even when \( C = C_{\text{lab}} \) (\( P = 0.817 \) and 0.270 for Kenya and Zimbabwe, respectively). Thus, these two samples are in accordance with the predictions of a neutral equilibrium model under expected levels of recombination (i.e., \( C = C_{\text{lab}} \)).

Open reading frames: A scan of 8.4 kb of the proximal breakpoint sequence (see Figure 1) with open reading frame (ORF) Finder (http://www.ncbi.nlm.nih.gov) revealed an \( \sim200 \)-amino-acid open reading frame between positions 7701 and 8343. A search of the protein databases revealed that this putative exon encodes a shaggy-like protein kinase. The position and direction of this exon is indicated in Figure 1. This putative exon was not included in our polymorphism study. GRAIL v. 1.3 software (http://compbio.ornl.gov) identified a second putative ORF (Figure 1) between positions 3078 and \( \sim3227 \). This putative ORF is oriented in the same direction as the shaggy-like ORF and contains a helix-loop-helix dimerization motif. It is unknown whether

![Graph showing relationship between P value and C = 4N\( r \) for a representative random sample of alleles based on an In(2L)\( r \) frequency of 21%. Our a priori estimate of the population recombination rate corrected for inversion frequency, \( C_{\text{lab}} = 90 \). The general behavior of our statistic \( P \) with respect to \( C \) is robust to different representative samples of our data and the three additional data sets analyzed.](image-url)
TABLE 5

Haplotype tests on white, Acp70A, and vermilion

<table>
<thead>
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<th>Data set</th>
<th>$C = C_{\text{cont}}$</th>
<th>$C = C_{\text{lab}}$</th>
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<td>white</td>
<td>0.903</td>
<td>0.001</td>
</tr>
<tr>
<td>Acp70A</td>
<td>0.130</td>
<td>0.004</td>
</tr>
<tr>
<td>vermilion</td>
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<td>Beijing</td>
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<td>0.000*</td>
</tr>
<tr>
<td>California</td>
<td>0.016</td>
<td>0.000*</td>
</tr>
<tr>
<td>Ecuador</td>
<td>0.064</td>
<td>0.000*</td>
</tr>
<tr>
<td>Taiwan</td>
<td>0.091</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kenya</td>
<td>0.996</td>
<td>0.817</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>0.927</td>
<td>0.270</td>
</tr>
</tbody>
</table>

Significant tests are indicated in italic type. *$P < 10^{-5}$.

or not these ORFs are contained in a single gene. The proximity of this putative coding region to the In(2L)I breakpoint raises the possibility that the inversion may affect patterns of gene expression. This finding would not be unprecedented in the literature (cf. Wesley and Eanes 1994).

A scan for ORFs in the In(2L)I B/D junction region revealed a 93-amino-acid open reading frame oriented toward the breakpoint that encodes the 3′-end of a novel putative reverse transcriptase of the LINE family of retroelements (Dinocera and Sakaki 1990). It is likely that this element was involved in In(2L)I’s formation since it lies within 0 and 20 bp of the B/D breakpoint junction. The element is present in all sampled inverted chromosomes ($n = 18$) and absent from all sampled standard chromosomes ($n = 11$). The element was also absent in a larger ($n = 24$), geographically diverse (including Miami, Yeppoon, and Zimbabwe) sample of standard chromosomes. Details of this novel transposable element will be described elsewhere.

DISCUSSION

Origin and age of In(2L)I. A LINE-like retrotransposon is found immediately at one of the breakpoint junctions. This element is absent in standard chromosomes but fixed in In(2L)I chromosomes, suggesting a transposable element-mediated origin for the inversion. This finding supports other recent work indicating a role for transposable elements in the formation of naturally occurring chromosomal rearrangements (Lyttle and Haymer 1992; Mathiopoulos et al. 1998).

Patterns of polymorphism and divergence between chromosomal arrangements in the San Jose population sample suggest that In(2L)I has a unique and relatively recent origin. Our estimate of In(2L)I’s age, $\sim 160,000$ years old (or $\sim 1.6$ million generations), is similar to the age reported for In(3L)P (Hasson and Eanes 1996). The inverted sample of alleles has relatively low levels of nucleotide diversity and divergence from standard alleles. A survey of three other geographically diverse populations revealed neither significantly more variation nor evidence for geographic differentiation of In(2L)I alleles. Thus, despite their wide geographic distributions and high frequencies in tropical populations (reviewed in Lemeunier and Aulard 1992), In(2L)I and In(3L)P are not long-lived balanced polymorphisms.

An alternative to the “long-lived balanced polymorphism” scenario is that In(2L)I has very recently increased in frequency in some populations due to selection. If so, the inverted class should have either reduced levels of variation, as observed in one allelic class at the Sod locus of D. melanogaster (Hudson et al. 1994), or a sharply negative skew in the frequency spectrum of segregating mutations (Braverman et al. 1995). The moderate number of fixed differences between karyotypes does suggest that In(2L)I is young relative to standard lineages. However, while Tajima’s $D$ is negative, there are probably too few segregating sites for us to have power to detect selection on In(2L)I based on the frequency spectrum of mutations (also true of In(3L)P; see Wesley and Eanes 1994).

In(2L)I is not at equilibrium in natural populations: A recent increase in the frequency of In(2L)I can be inferred by considering relative levels of nucleotide diversity in standard and inverted arrangement classes (Tables 1 and 2) as well as world-wide inversion frequency estimates (Knibb 1982; Bénasssi et al. 1993; this study). Our simulations indicate that levels of In(2L)I diversity are consistent with historical frequencies of between 4 and 23% when we assume $C = C_{\text{lab}}$ (or 3 and 35% when we assume $C = 0$). This implies that In(2L)I is not at equilibrium in populations with In(2L)I frequencies that exceed 23–35%. Examples of such populations include those in northern Australia, New Guinea, southern Japan (Knibb 1982), West Africa (Bénasssi et al. 1993), and Zimbabwe (this study). Despite extensive biogeographical and experimental evidence for selection on inversions (reviewed in Krimbas and Poweil 1992), our results represent the first evidence from nucleotide polymorphism data that inversions are not at neutral equilibrium in tropical populations. Possible explanations for a recent increase in frequency of In(2L)I include founder and/or hitchhiking events associated with the inversion. However, the lack of geographic differentiation of In(2L)I alleles and the inversion’s high frequency in multiple populations suggests that its high frequency is not an isolated phenomenon. Since the expected time to equilibrium is relatively long ($\sim 4N_{i}$ generations), In(2L)I could be a locally adaptive or balanced polymorphism that is too young to be at equilibrium.

Genetic exchange between arrangements is suppressed at inversion breakpoints: Our data suggest that exchange between arrangements, including gene con-
version, is significantly suppressed at breakpoint regions relative to other regions. This is manifested by the lack of shared polymorphism between arrangements (Table 2) and the low diversity of In(2L)t and In(3L)P alleles (relative to standard alleles) at the junction region. We find no evidence for genetic exchange between karyotypic classes despite the large number of informative sites in the region sequenced (with the possible exception of site 174, Figure 2). In contrast, patterns of variation at Adh and Fabp2 are consistent with considerable levels of between-karyotype exchange at these loci. Patterns of nucleotide polymorphism at multiple loci linked to In(3L)P lead to a similar conclusion (Table 3).

The distribution of polymorphism within and between arrangements at rp49 is significantly different from that observed at both In(2L)t and In(3L)P breakpoints (Table 2, two-tailed \( P < 0.01 \) and \( P < 0.04 \), respectively, by a Fisher’s exact test). Est-5 of D. pseudoobscura (Table 2) shows a pattern more consistent with the patterns observed at In(2L)t and In(3L)P. The rp49 data may be unusual due to the complex nature of the associated inversion system in D. subobscura (Rozas et al. 1999). With the exception of rp49, the data suggest that the rate of exchange between karyotypes at inversion breakpoints is likely to be lower than the reciprocal of the effective population size of the species (Ishii and Charlesworth 1977) and is not constant across the inverted region as is assumed in some recent models (e.g., Navarro et al. 1997).

Unusual haplotype structure at the In(2L)t breakpoint: An unexpected feature of our data is a marked heterogeneity in levels of linkage disequilibrium across the sequence (Figure 2) among standard chromosomes. Two deeply diverged standard arrangement haplotypes exhibit strong associations among sites close to the inversion breakpoint. Yet there is ample evidence for recombination in other regions of the data set.

Our statistical test reveals that an \( \sim 1.4 \)-kb region immediately spanning the In(2L)t breakpoint (Figure 2) has too few haplotypes to be compatible with a neutral equilibrium model under the expected level of recombination (i.e., \( C_{\text{inh}} \), see Table 4). It also suggests that the haplotype structure observed at In(2L)t is more extreme than at two other loci (i.e., white and Acp70A, Table 5) where unusual haplotype structure had previously been reported (Kirby and Stephan 1996; Cirera and Aguadé 1997). An advantage of our test is that it can identify unusual regions that are not apparent when initially viewing the data. The level at which the null hypothesis could be rejected depends on both the amount of information included (i.e., with different versions of the data) and the assumed recombination rate.

Unfortunately, the true recombination rate is unknown. Simulations run with \( C_{\text{inh}} \) do not always show a significant deficiency in the number of haplotypes at the In(2L)t breakpoint. However, on the basis of comparisons of physical and genetic maps (Kimman and Hey 1993; Comeron et al. 1999) and estimates of the effective population size (Kreitman 1983) of D. melanogaster, it seems likely that \( C_{\text{inh}} \) is a severe underestimate of the true population recombination rate. As an illustration, \( C_{\text{inh}} \), our best a priori estimate of \( C \) in this chromosomal region (corrected for the presence of the inversion), is \( >50 \)-fold higher than \( C_{\text{int}} \) and \( \sim 10 \)-fold higher than the \( C \) corresponding to \( P = 0.05 \) (Figure 5). In addition, note that \( C_{\text{inh}} \) (based on \( \rho \)) is likely to be a considerable underestimate of the true rate of exchange because it ignores the added contribution of gene conversion to \( C \). Over small physical distances (i.e., on the order of \( \sim 1 \) kb), gene conversion is expected to contribute as much to the recombination rate as \( \rho \) (Andolfatto and Nordborg 1998).

It is difficult to imagine that recombination rates are different enough in natural populations (relative to lab strains) to account for the unusual haplotype structure observed at the breakpoint. A study of 2nd chromosome recombination rates in the F1 progeny of lab strains and wild-caught D. melanogaster lines suggests that genetic background does not have a large effect (Brooks and Marks 1986). In addition, age and temperature also appear to have relatively minor effects (Ashburner 1989). Even if it is assumed that the true recombination rate is larger than \( C_{\text{inh}} \), it could be argued that local variation in mutation or recombination rates make it easier to observe the strong haplotype structure at the In(2L)t breakpoint. We have no reason to believe that recombination is suppressed to this degree near the breakpoint between standard chromosomes.

Population genetic models: There are several alternatives to the panmictic neutral model that make it more likely to observe fewer-than-expected haplotypes in a sample. These include some forms of selection and population subdivision. Epistatic selection (Lewontin 1974) or balancing selection (Strobeck 1983) can result in a deficiency in the number of haplotypes relative to neutral expectations. Other possibilities include transient selection (Hudson et al. 1994, 1997) or “traffic” models (Kirby and Stephan 1996).

The findings of strong associations among polymorphic sites in standard chromosomes and the extreme divergence between the two major haplotype classes (Figure 4B) raise the possibility that some form of selection on standard alleles predated the appearance of In(2L)t, and that these standard haplotype classes are quite old. Since we do not have a reasonable estimate of the divergence between species across this region, a plausible alternative explanation for the high level of nucleotide polymorphism at the breakpoint is that either the mutation rate or the level of selective constraint varies across the sequenced region. The possibility of a “hotspot” for mutation at the breakpoint seems unlikely given that polymorphisms in the In(2L)t class of chromosomes do not cluster near the location of this elevated window of polymorphism in standard chromosomes.
The finding of at least one putative exon in the sequenced region does suggest that the level of selective constraint is likely to vary. Note, however, that while heterogeneity in selective constraint may be a sufficient explanation for the peak of elevated polymorphism, it does not explain the deficiency in the number of haplotypes.

An alternative to selection models for the pattern at the In(2L)t breakpoint are demographic models (cf. David and Capy 1988). If a single population sample actually consists of individuals from two diverged subpopulations, mutations that contribute to divergence between the populations will appear to be in complete linkage disequilibrium. Strong linkage disequilibrium will result in fewer distinct haplotypes, given the number of mutations observed, than expected in a panmictic population. Recent data on the vermilion locus suggest a deficiency of haplotypes in non-African relative to African populations (Begun and Aquadro 1995). One possibility (as suggested by the authors) is that the African populations are closer to equilibrium while non-African populations have experienced a recent founder event. While consistent with patterns observed at other loci on the X chromosome (Begun and Aquadro 1993), this hypothesis was not supported by a recent multilocus microsatellite study (Irvin et al. 1998).

The main difference between selective and demographic models is that the latter are expected to affect the whole genome with equal strength, while with selection, recombination will tend to uncouple the histories of neutral sites from that of the site under selection. Thus, depending on the rate of recombination, selection models can lead to considerable heterogeneity in linkage disequilibrium across a given region (Hudson and Kaplan 1988; Hudson et al. 1997). The pattern in our data appears to be localized to the breakpoint. Thus, while demographic models cannot be excluded, they seem unlikely given the number of inferred recombination events and rare polymorphisms observed further from the breakpoint (see Figure 2). A similar pattern is observed at the Sod locus (Hudson et al. 1997). However, in the presence of intermediate levels of recombination, closely linked regions may show different patterns of variability even when selection is not operating. So further work is necessary to evaluate the likelihood of these (and other) data under various demographic models. Current work attempts to determine whether or not the unusual haplotype structure we detected at the breakpoint of In(2L)t is a general feature of D. melanogaster populations. Sampling standard alleles from other populations, particularly those in Africa (presumably ancestral, cf. Lachaise et al. 1988), may establish whether epistatic selection or a recent founder event are likely explanations for our data.

Implications for clines and linkage disequilibrium between Adh and In(2L)t: The Fast/Slow allozyme polymorphism of the Adh locus in D. melanogaster is in complete linkage disequilibrium with In(2L)t in many populations (reviewed in LeMeunier and Aulard 1992). Despite this association, Bénassí et al. (1993) demonstrated that among 19 restriction site variants within the Adh locus, only the Fast/Slow site and the Δ1 insertion-deletion were in significant linkage disequilibrium with In(2L)t. Thus, while all In(2L)t chromosomes have the Adh-Slow allele, there is evidence for considerable between-arrangement exchange at Adh (i.e., shared polymorphisms; see Table 2). In fact, laboratory estimates of recombination between Adh and In(2L)t are very high (~10^{-3} to ~10^{-4}, Malpica et al. 1984). Therefore, it is curious that strong linkage disequilibrium is observed between the Adh amino acid polymorphism and In(2L)t. One hypothesis is that some form of epistatic selection maintains linkage disequilibrium (Van DeiIden and Kamping 1989). An alternative is that In(2L)t and Adh-F alleles arose in isolation and have only recently encountered one another (Veuille et al. 1998).

Standard-Adh-Fast and In(2L)t-Adh-Slow alleles coexist at intermediate frequencies in many populations worldwide (Voeiker et al. 1978; Knibb 1986; Bénassí et al. 1993; Veuille et al. 1998; S.-C. Tsaur, unpublished results). Our results suggest that while In(2L)t is relatively young, its age (~1.6 million generations) is at least ~100-fold that of the expected half-life of linkage disequilibrium between Adh and the inversion. Our samples of In(2L)t alleles (including an African population) do not differ significantly at the nucleotide level. There is therefore no evidence that In(2L)t remained isolated for any lengthy period of time. In the absence of selection maintaining linkage disequilibrium, demographic models would have to assume that Standard-Adh-Fast and In(2L)t-Adh-Slow arose in strongly isolated populations and have coexisted for fewer than several thousand generations.

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