Molecular Genetic Variation in the Centromeric Region of the *X* Chromosome in Three *Drosophila ananassae* Populations. I. Contrasts Between the *vermilion* and *forked* Loci

Wolfgang Stephan and Charles H. Langley

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Manuscript received August 1, 1988
Accepted for publication September 30, 1988

ABSTRACT

We have surveyed three natural populations of *Drosophila ananassae* for restriction map variation at the *forked* (*f*) and *vermilion* (*v*) loci, using 6-cutter restriction enzymes. Both loci are located in the centromeric region of the *X* chromosome. Two major conclusions can be drawn from the data. First, we found strong evidence for population subdivision, *i.e.*, significant differences in the frequency distributions of polymorphisms and/or haplotypes between the Burma, India, and Brazil populations. Secondly, the pattern of DNA sequence variation between the two loci is unexpectedly different. The level of nucleotide variation in the *v* locus region is reduced (relative to *f*), especially in the Burma population. Furthermore, in contrast to *v*, we found no insertions/deletions larger than 700 bp and no significant linkage disequilibrium at *f*. The genetic differentiation among subpopulations can readily be attributed to restricted migration as the predominant evolutionary force. According to population genetics theory, the differences in DNA polymorphisms between the two loci are in qualitative agreement with the hypothesis that recombination is reduced in the *v* locus region ("centromere effect") but not at *f*. In order to test this hypothesis directly, we determined the cytogenetic positions of several loci in the centromeric region by *in situ* hybridization and found by comparison with the genetic map that recombination at *v* is indeed very low, much lower than at *f*.

IN the 1980s, developments in DNA technology have allowed population geneticists to measure genetic variation in natural populations in new ways, either by sequencing (KREITMAN 1983) or by restriction mapping techniques (LANGLEY, MONTGOMERY and QUATTLEBAUM 1982; KREITMAN and AGUADÉ 1986a). The initial efforts were primarily devoted to obtaining a rough estimate of the level of DNA polymorphism in *Drosophila melanogaster*, which turned out to be unexpectedly high, both in noncoding and coding regions (LANGLEY, MONTGOMERY and QUATTLEBAUM 1982; KREITMAN 1983; LEIGH BROWN 1983). Those studies led to investigations of more specific topics, such as the difference between restriction site and insertion variation in natural populations (AQUADRO *et al.* 1986), the level of DNA polymorphism in coding *vs.* noncoding regions pertaining to the neutralist-selectionist controversy (KREITMAN and AGUADÉ 1986b; HUDSON, KREITMAN and AGUADÉ 1987) and the existence and range of non-random associations (MIYASHITA and LANGLEY 1988).

In Drosophila, the great majority of the studies of DNA sequence variation focus on *D. melanogaster*. There are only a few exceptions: the recent investigations of AQUADRO, LADO and NOON (1988) comparing the rosy region of *D. melanogaster* with that of its sibling species *Drosophila simulans* and the study by SCHAEFFER, AQUADRO and ANDERSON (1987) on the *Adh* region in *Drosophila pseudoobscura*. Since it is important to determine the pattern of DNA polymorphism in *Drosophila* in general, we initiated investigations of DNA sequence variation in *Drosophila ananassae*. This species provides a potentially interesting comparison, because it is fairly distantly related to *D. melanogaster* [however, less distantly than *D. pseudoobscura* (PATTERSON and STONE 1952)] and because its geographic distribution differs from that of *D. melanogaster*. It is largely tropical (PATTERSON and STONE 1952), but has been found on all continents (MORIWAKI and TOBARI 1975). The zoogeographical center of *D. ananassae* is in Southeast Asia. Of particular interest for this study is the fact that the *X* chromosome of *D. ananassae* is metacentric and that various genes located in the euchromatic middle portion of the *X* chromosome in *D. melanogaster* are in the centromeric region in *D. ananassae*, due to several rearrangements having been fixed in both species since their last common ancestor.

The general question we ask in this paper is, how does recombination affect DNA polymorphism. We report here the results for two loci, *forked* and *vermilion*, located in the centromeric region of the *X* chromosome in *D. ananassae*. Both mutants (*forked* bristles and *vermilion* eye color) were first described

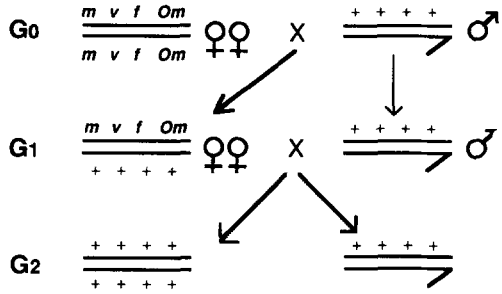

FIGURE 1.—Crossing scheme for extracting the centromere region of the *X* chromosome in *D. ananassae*. The mutant chromosome carries the markers *miniature* (*m*), *vermilion* (*v*), *forked* (*f*), *Om(1D)* (*Om*). In G_0 and G_1, the same male has been used (indicated by an arrow). To confirm the isogenicity of a stock, we crossed a single female of the G_2 generation to her sib brothers and screened the males of the next generation for evidence of recombination.

by KIKKAWA in 1934 [See MORIWAKI and TOBARI (1975)]. We chose these loci, because we expected that both would exhibit the "centromere effect" (BEADLE 1932; MATHER 1939), *i.e.*, reduced levels of recombination. The present study follows the lines of our previous papers in which we explored some of the evolutionary consequences of reduced recombination in the centromere region, such as the accumulation of highly repeated DNA sequences (CHARLESWORTH, LANGLEY and STEPHAN 1986) and transposable elements (LANGLEY *et al.* 1988).

MATERIALS AND METHODS

Strains: Three natural populations of *D. ananassae* were used in this survey: a population from Burma collected by M. TODA in 1982 around Mandalay (19 isofemale lines), an Indian population from collections of F. HIHARA and O. KITEGAWA in 1981 around Hyderabad (20 lines), and a Brazilian population collected in 1986 by B. GOÑI around Sao Paulo (21 lines). These stocks were kindly provided by Y. TOBARI. Since there are no balancers of the *X* chromosome of *D. ananassae* available at present, we used a chromosome carrying a dominant marker (*Om(1D)*; HINTON 1984) to establish lines that are homozygous for the centromeric region of the *X* chromosome. The crossing scheme is depicted in Figure 1.

Cloning of vermilion: A genomic DNA library was made from DNA from a *ca*; *px* stock (HINTON 1984) as follows. 1.5 μg of *ca*; *px* high-molecular weight DNA (see below) was partially digested with various amounts of *Mbo*I (ranging from 0.03 to 0.5 unit) for 15 min. The reactions were terminated by heating the samples up to 65° for 15 min. The digestion which yielded a high proportion of large fragments (>15 kb) was used to prepare the library. After treatment with 1 unit of alkaline phosphatase, the sample was phenol/chloroform extracted and ethanol precipitated, then resuspended in TE (10 mM Tris-HCl (pH 8.0)/1 mM EDTA) and ligated to 3 μg λEMBL4 DNA, digested with *Bam*HI and *Sal*I. The ligated DNA was packaged according to the instructions provided by the supplier (Stratagene).

The library was screened using the 1.9-kb *Xho*I-*Hin*dIII fragment [coordinates 1.1 to 3.0 of SEARLES and VOELKER (1986); see Figure 2] purified from the subclone Spv 8.7 (kindly provided by L. SEARLES). This fragment contains most of the 2.0-kb transcript of *v* from *D. melanogaster*. Plaque hybridization procedures are widely described (*e.g.*, MANIATIS, FRITSCH and SAMBROOK 1982). In our case, hybridization was carried out at low stringency (35°), using 50% formamide. Furthermore, prior to putting the filters into prehybridization solution, they were treated in 0.1× SSC, 1.0% SDS at 65° for 1 hr to reduce background. For the first screen we used nylon membranes (Du Pont), for the second nitrocellulose (Schleicher & Schuell). The final washes were done in 0.1× SSC, 0.5% SDS at 35°.

One of the positive clones (λcpv8) was subcloned into the vector Bluescript KS M13$^+$ (Stratagene) to remove the regions of repetitive DNA identified at both ends of the clone (Figure 2). λcpv8 DNA was completely digested with *Bam*HI and then partially with *Hin*dIII. The fragments were ligated to Bluescript KS M13$^+$, digested with *Bam*HI and *Hin*dIII, as described by the supplier. The lengths of the resulting plasmids were measured by electrophoresis, using a supercoiled DNA plasmid ladder (BRL) as size marker. A subclone obtained in this way was used as a probe in our survey (coordinates −3.2 to +8.8; Figure 3), after comparing its restriction map with that of the original clone.

Restriction map analysis: The restriction map of the *v* locus (Figure 2) was constructed, digesting the clones λcpv4 and λcpv8 with one or two restriction endonucleases and size-fractionating them on 1% agarose gels. We used the same enzymes as SEARLES and VOELKER (1986): *Bam*HI, *Eco*RI, *Hin*dIII, *Sal*I, and *Xho*I. Similarly, the restriction map of the *f* clone, λco6, was obtained (data not shown). λco6 was a gift from S. HORI (Y. HATANO and S. HORI, unpublished data).

Genomic DNA from each of the 60 lines was prepared as described by BINGHAM, LEVIS and RUBIN (1981). The DNA was digested with single restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sac*I, and *Sal*I) and electrophoresed on 1% agarose gels. The DNA fragments were transferred to Zetaprobe-nylon membranes (Bio-Rad) by the usual Southern blotting technique. The filters were hybridized under standard conditions, using 50% formamide, then washed and autoradiographed (MANIATIS, FRITSCH and SAMBROOK 1982). Furthermore, for a standard restriction map, *ca*; *px* genomic DNA was digested with single and pairs of restriction enzymes but otherwise prepared as the DNA from the natural populations.

***In situ* hybridization:** To determine the cytogenetic location of several genes of interest for this study [including *v* and *furrowed* (*fw*)], we prepared probes labeled with biotinylated-UTP (LANGER-SAFER, LEVINE and WARD 1982). Conditions for hybridization and detection are described in MONTGOMERY and LANGLEY (1983). The *fw* clone from *D. melanogaster*, λ320, was a gift from T. GORALSKI and K. CONRAD.

Statistical procedures: The statistical significance of linkage disequilibrium was determined by the Fisher exact test. Furthermore, to test whether or not the observed frequencies of a polymorphism are significantly different between the three populations, we used Monte Carlo simulations on 2 × *N*-contingency tables, as suggested by LEWONTIN and FELSENSTEIN (1965), and implemented in a program kindly provided by W. ENGELS. The levels of nucleotide variation were estimated using the methods of ENGELS (1981), NEI and TAJIMA (1981) and HUDSON (1982).

RESULTS

Characterization of the *v* locus in *D. ananassae*

In this section, we describe briefly the basic cytogenetic and molecular features of the *v* locus and

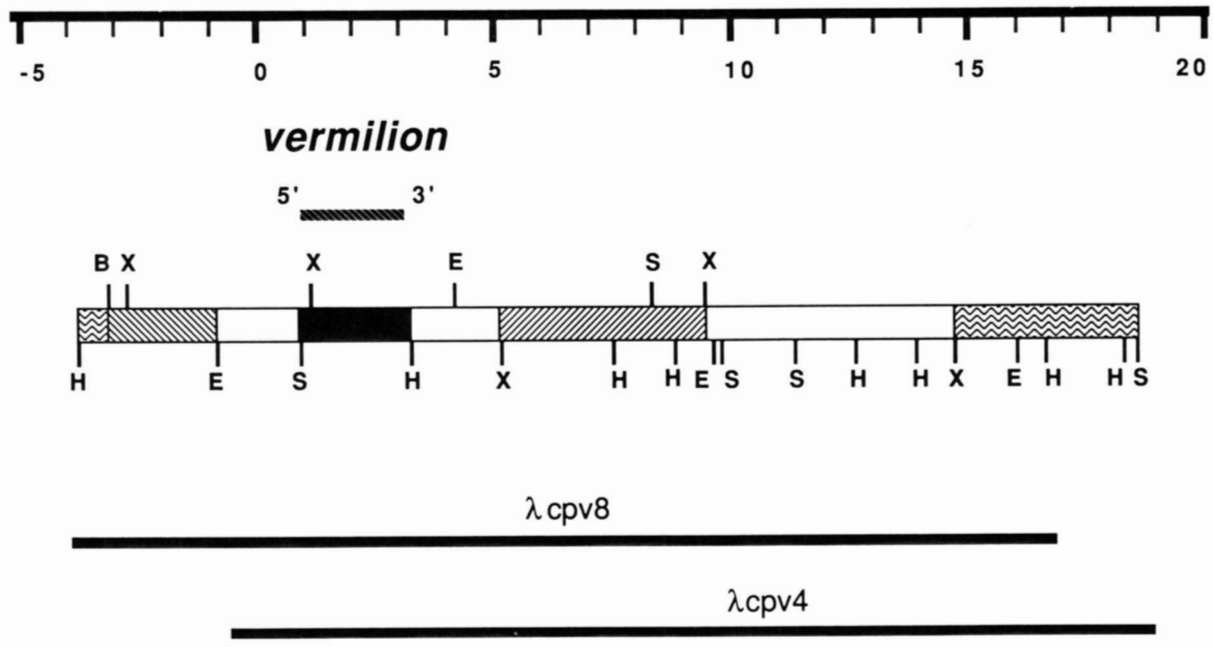

FIGURE 2.—Restriction map of the *v* locus in *D. ananassae*, as obtained by combining restriction data from the clones λcpv4 and λcpv8. The six sites (above the bar representing the *v* region) are shared with *D. melanogaster*. The location of the transcriptional unit is indicated by a solid bar and the two relatively conserved flanking regions by hatched bars. The repetitive DNA identified at both ends of the *v* region is indicated by a wave-like pattern. The coordinates adopted from SEARLES and VOELKER (1986) are the distances in kb. The restriction enzymes are abbreviated as: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

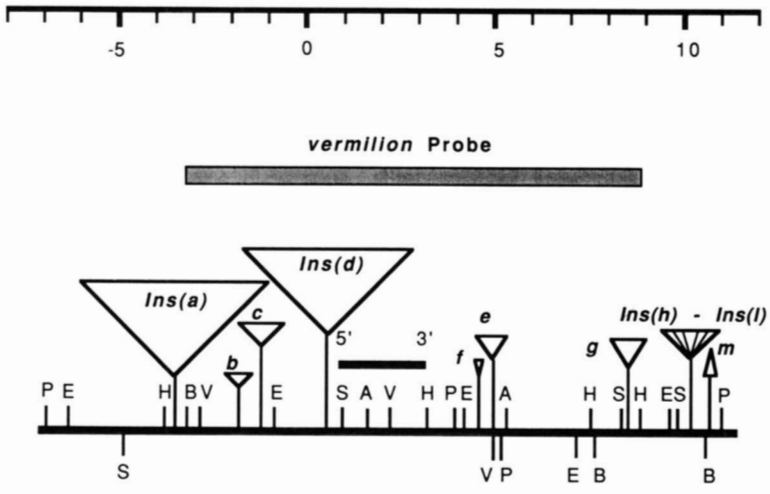

FIGURE 3.—Summary restriction map of DNA sequence variation in the *v* region of *D. ananassae*. The location of the *v* gene is in the approximate center of the region (solid bar). Variant and invariant restriction sites are indicated below and above the line, respectively. In the survey, we used the restriction enzymes *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), *Pvu*II (V), *Sac*I (A), and *Sal*I (S). Insertion and deletion sizes were estimated as follows: *Ins*(*a*) = 5 kb, *Ins*(*b*) = 0.7 kb, *Ins*(*c*) = 1.2 kb, *Ins*(*d*) > 4.5 kb, *Ins*(*e*) = 0.8 kb, *Ins*(*f*) = 0.2 kb, *Ins*(*g*) = 0.9 kb, *Ins*(*h*) = 1.2 kb, *Ins*(*i*) = 0.6 kb, *Ins*(*j*) = 0.1 kb, *Ins*(*k*) = 0.9 kb, *Ins*(*l*) = 1.5 kb, *Del*(*m*) = 0.3 kb. The insertions, *Ins*(*h*) to *Ins*(*l*), found in the interval (9.8, 10.9), are lumped together into one symbol. Positions of insertions are identified only to the indicated restriction fragment. The 5′ and 3′ flanking *Sac*I sites and 3′ flanking *Pvu*II site have not been scored because they are too distant to unambiguously interpret variant restriction fragments.

contrast them with *D. melanogaster*. First, the cytogenetic location of *v* determined by *in situ* hybridization to polytene chromosomes is on the left arm of the *X* chromosome at 13Cp, *i.e.*, very close to the centromere [the coordinates refer to the map of X. TOMIMURA and Y. TOBARI (unpublished results)]. In *D. melanogaster*, the *v* locus is at 10A (SEARLES and VOELKER 1986), that is, further from the centromere. Secondly, the *v* mutant phenotype is apparently not caused by insertions, unlike most of the mutants of *D. melanogaster* analyzed by SEARLES and VOELKER (1986). We found no difference in the restriction fragment length patterns between the DNA isolated from our *ca*; *px* stock and that of *v* mutants carrying the markers *m*, *v*, *f*, and *garnet* (data not shown).

A brief summary of the molecular organization of the *v* locus region is shown in Figure 2, using the coordinate system of SEARLES and VOELKER (1986). The transcriptional unit was localized within the *Sal*I-*Hin*dIII fragment (coordinates 0.9–3.2) and the 3′ and 5′ orientation determined, based on the information available from *D. melanogaster* (L. SEARLES, personal communication). Approximately 13 kb downstream from the transcriptional unit (coordinate >14.6), a major repetitive DNA region was identified that extends to the ends of both λ clones. Evidence for another repetitive region was found at the upstream end of the clone λcpv8 (coordinate <−5.2).

The latter appears to be the edge of a repetitive region which is also found in *D. melanogaster* (L. SEARLES, personal communication). Since we are interested in comparisons between *D. melanogaster* and *D. ananassae*, we made an effort to align the *v* locus restriction maps of these species. Comparing the map obtained by SEARLES and VOELKER (1986) for *D. melanogaster* with our map, six restriction sites appear to be conserved (Figure 2). They are distributed in a region of at least 13 kb around the 2-kb transcriptional unit, suggesting that the *v* region is surprisingly conserved with respect to size. Under the stringency conditions chosen for our cross-hybridization experiments (see MATERIALS AND METHODS), two regions other than the transcriptional unit turned out to be relatively conserved, falling into the intervals (−3.2, −0.9) and +5.1, +9.4) (data not shown). Interestingly, both are separated from the transcriptional unit by less conserved regions of approximately 2-kb lengths.

Restriction map variation of the *v* locus

Restriction site polymorphism: A summary restriction map for the 60 lines of *D. ananassae* from three populations is shown in Figure 3. The *v* transcriptional unit is located in the approximate center of this 18-kb region. Using 7 enzymes, we have scored a total of 25 restriction sites, 6 of which are polymorphic. The distribution of polymorphic sites across the three populations is nonuniform (Table 1). The Burma population (M) shows only two variable sites (out of 22), both variants (*Pvu*II at 5.1 and *Pst*I at 5.2) being *unique*. Similarly, the India population (H) also has two variable sites (out of 22). Both, *Eco*RI at 7.1 and *Bam*HI at 10.6, vary twice within this population. In contrast, in the Brazil population (B), four out of the 23 sites scored are polymorphic, some of them even highly polymorphic. Because of these apparent differences, we estimated the nucleotide variation separately for each population (Table 3). The population differences are reflected by all three measures of average heterozygosity, though not to the same extent. In particular, for the Burma and also for the India populations, HUDSON's method, which is based on the number of segregating sites in the sample leads to values higher than the other two estimators of H, because in these populations all variants are rare. For the Brazil population, all three estimates are nearly identical.

Insertions and deletions: In our sample, all large insertions/deletions (>500 bp) are rare, except the 0.9-kb insertion *Ins*(*g*), which was found in 6 lines of the Burma population and in one line of the India population. The location of this insertion could be identified relatively accurately by the 6-cutter technique, because the insertion site lies in the overlap region of two small fragments, *Hin*dIII-*Hin*dIII and *Sal*I-*Sal*I. Furthermore, since this insert contains three restriction sites (*Hin*dIII, *Pst*I, *Pvu*II), it appears to be a single element that is inserted in the same site in all 7 lines. As the distribution of *Ins*(*g*) indicates, there is also population differentiation with respect to the insertion/deletion dynamics. More extreme examples are the small insertions *Ins*(*f*) and *Ins*(*j*). Both are in high frequency in the Indian and Brazilian populations, but completely absent in the Burmese population.

The distribution of insertions along the *v* locus is non-uniform. Elements are most frequently found in four regions: the 2-kb fragment (coordinates −2.9 to −0.9) is hit in 4 lines by either *Ins*(*b*) or *Ins*(*c*), the 0.9-kb fragment (coordinates 4.2–5.1) in 31 lines by either *Ins*(*f*) or *Ins*(*e*), the 0.5-kb fragment (coordinates 8.3–8.8) in 7 lines by *Ins*(*g*), and the 0.8-kb fragment (coordinates 9.8–10.6) in 26 lines by one of the insertions *Ins*(*h*) to *Ins*(*l*). In three of these regions, the insertions are variable in size, in particular, in the latter where insertions of five different sizes (in the range 0.1 to 1.5 kb) were found. Finally, it is interesting to note that one of these regions coincides with the 2-kb flanking region downstream of the transcriptional unit which, as we mentioned above, is evolutionarily less conserved. Whether that means that the flanking regions of genes are more susceptible to insertions is unclear at present, but speculation is further supported by the fact that the upstream flanking region of the *v* gene is target site of one of the two unique large insertions (*Ins*(*d*) in line H13). A similar behavior was observed at the *v* locus in *D. melanogaster* (C. H. LANGLEY, M. AGUADÉ and N. MIYASHITA, unpublished results).

The *v* haplotype graph: For simplicity, we consider first only restriction site polymorphisms. It is easily seen from Table 1 that there are 7 different haplotypes. The hypothetical phylogenetic relationship of these haplotypes can be represented in a graph, connecting pairs of haplotypes that differ by one site change. In our case, we obtain a *single* graph, *i.e.* there is a one-step transition between all pairs of haplotypes. The resulting graph is a tree, because it contains no closed loops (not shown).

In principle, the same construction may be used to consider restriction site changes and insertion/deletion dynamics simultaneously. To obtain a comprehensive graph we go a step further and lump together those haplotypes which differ from one another only with respect to the variable insertion/deletions at the three regions described above. Thus, we define the following haplotype groups

- α: standard, including insertion/deletion variants in (9.8, 10.9);
- β: α, including insertions in (4.2, 5.3) and +*Bam*HI-site at 10.6;
- β_1: β, including insertions in (−1.9, −3.9);

TABLE 1

Restriction map variants in the *vermilion* region of three *D. ananassae* populations

Lines	Loci								
	*Sal*I −4.9	*Ins* (*c*)	*Ins* (*f*)	*Pst*I 5.2	*Eco*RI 7.1	*Ins* (*g*)	*Ins* (*j*)	*Bam*HI 10.6	Unique variants
M61	+	−	−	−	−	+	−	−	−
M62	+	−	−	−	−	+	−	−	−
M66	+	−	−	−	−	+	−	−	−
M68	+	−	−	−	−	−	−	−	−
M79	+	−	−	−	−	−	−	−	−
M80	+	−	−	−	−	−	−	−	−
M86	+	−	−	−	−	−	−	−	−
M89	+	−	−	−	−	−	−	−	*Ins*(*a*)
M90	+	−	−	−	−	−	−	−	−
M91	+	−	−	−	−	+	−	−	−
M92	+	−	−	−	−	−	−	−	−
M97	+	−	−	+	−	−	−	−	−
M99	+	−	−	−	−	−	−	−	−
M106	+	−	−	−	−	−	−	−	−
M111	+	−	−	−	−	−	−	−	−
M114	+	−	−	−	−	+	−	−	−
M115	+	−	−	−	−	+	−	−	−
M117	+	−	−	−	−	−	−	−	*Ins*(*i*)
M119	+	−	−	−	−	−	−	−	+*Pvu*II(5.1),*Ins*(*h*)
H3	+	−	+	−	−	−	−	+	−
H4	+	−	+	−	−	−	+	+	−
H9	+	−	−	−	−	−	+	+	*Ins*(*e*)
H10	+	+	+	−	−	−	+	+	−
H11	+	−	+	−	−	−	−	+	−
H12	+	+	+	−	+	−	−	+	*Ins*(*k*)
H13	+	−	+	−	−	−	+	+	*Ins*(*d*)
H15	+	−	+	−	−	−	+	+	*Ins*(*b*),*Del*(*m*)
H16	+	−	+	−	−	−	−	+	−
H21	+	−	+	−	−	−	+	+	−
H23	+	−	+	−	−	−	+	+	−
H26	+	−	+	−	−	−	−	+	−
H27	+	−	+	−	−	−	+	+	−
H31	+	−	+	−	−	−	−	+	*Ins*(*l*)
H36	+	+	+	−	+	−	−	+	−
H39	+	−	+	−	−	−	−	+	−
H41	+	−	+	−	−	−	+	+	−
H47	+	−	−	−	−	+	−	−	−
H48	+	−	+	−	−	−	+	+	−
H50	+	−	−	−	−	−	−	−	−
B55	+	−	−	+	−	−	−	−	−
B59	+	−	+	−	−	−	+	+	−
B79	+	−	+	−	−	−	+	+	−
B83	+	−	+	−	−	−	+	+	−
B84	+	−	+	−	−	−	+	+	−
B91	+	−	−	+	−	−	−	−	−
B92	−	−	−	+	−	−	−	−	−
B99	+	−	+	−	−	−	+	+	−
B102	+	−	+	−	−	−	−	+	−
B104	−	−	−	+	−	−	−	−	−
B116	+	−	−	+	−	−	−	−	−
B117	+	−	−	+	−	−	−	−	−
B118	+	−	+	−	−	−	+	+	−
B119	+	−	+	−	−	−	+	+	+*Bam*HI(7.6)
B120	+	−	+	−	−	−	+	+	−
B121	+	−	+	−	−	−	+	+	−
B123	−	−	−	+	−	−	−	−	−
B131	+	−	+	−	−	−	+	+	−
B132	+	−	+	−	−	−	+	+	−
B134	+	−	−	+	−	−	−	−	−
B135	+	−	+	−	−	−	+	+	−

Key: "+", present; "−", absent; M, Burma population; H, India population; B, Brazil population.

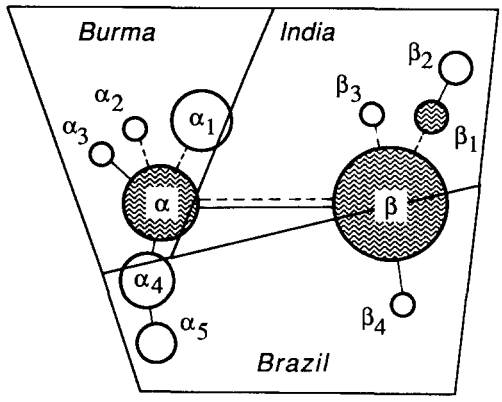

FIGURE 4.—Haplotype graph of the *v* region in *D. ananassae*. This figure represents the proposed phylogenetic relationship and geographical distribution of the *v* haplotypes identified in our sample. The hatched circles indicate haplotype groups, *i.e.* haplotypes *modulo* variable insertions (see text). The area of a circle is proportional to the relative frequency of the corresponding haplotype. The edge between two haplotypes is either due to a restriction site change (solid line) or to an insertion/deletion event (dashed line). The major haplotypes, α and β, are separated from each other by a restriction site change and an insertion. Note that the haplotype graph has no closed loops, indicating that there was no recombination event detectable in our sample. The geometric arrangement of the circles, including intersecting lines, reflects the geographic distribution of the haplotypes, but is, of course, not completely determined by the topology of the graph.

and the haplotypes

α_1: standard, including *Ins(g)*;
α_2: standard, including *Ins(a)*;
α_3: α, including +*Pvu*II-site at 5.1;
α_4: standard, including +*Pst*I-site at 5.2;
α_5: α_4, including −*Sal*I-site at −4.9;
β_2: β_1, including +*Eco*RI-site at 7.1;
β_3: β, including *Ins(d)*;
β_4: β, including +*Bam*HI-site at 7.6.

With these definitions, the phylogenetic relationship and geographic distribution of the haplotypes can be represented in a relatively simple graph (Figure 4).

Two aspects of this graph are immediately obvious. First, all nodes are pairwise connected by a single edge, except for (α,β). α and β differ from each other by an insertion in (4.2, 5.3) and the gain of a *Bam*HI-site at 10.6 (see above definitions). With respect to these differences, the haplotypes can be partitioned into two groups, the α-group $\{\alpha, \alpha_i, i = 1, \ldots, 5\}$ and the β-group $\{\beta; \beta_i, i = 1, \ldots, 4\}$. Their frequencies among all 60 lines are 29 of 60 and 31 of 60, respectively. However, the haplotype distribution between the three populations is drastically different. The Burma population consists only of α-types, whereas the majority (18 out of 20) of the India population is of the β-type. The Brazil population is a 8:13-mixture of α's and β's. Secondly, the haplotype graph is a tree. The absence of closed loops means that, in contrast to the *v* region in *D. melanogaster* (C. H. LANGLEY, M. AGUADÉ and N. MIYASHITA, unpublished results), our data provide no evidence for recombination in the *v* locus region of *D. ananassae*. However, two-step transitions, such as between α and β, may be interpreted as recombinational events.

***In situ* hybridization:** In order to test whether recombination is reduced in the *v* region, we used a more direct method for measuring the recombination frequency, comparing the genetic to the cytogenetic map in the centromeric region of the *X* chromosome. (We are indebted to M. MATSUDA for suggesting this approach.) The genetic map distance between *v* and *fw* is approximately 0.04 map unit (HINTON 1988). By *in situ* hybridization, we localized *fw* on the *right* arm of the chromosome at 14AB, while *v* is located at the left arm at 13Cp (see above). We conclude from these results that the recombination frequency in the *v* locus region is very low, because the distance between *v* and *fw* spans the whole centromere, including extended regions of heterochromatin (KIKKAWA 1938).

Restriction map variation in the *f* locus region

In *D. ananassae*, the *f* locus is located in the centromeric region of the *X* chromosome at 15Cp (M. MATSUDA and S. TANDA, personal communication), *i.e.*, very close to *Om(1D)* which is also at 15Cp (SHRIMPTON, MONTGOMERY and LANGLEY 1986), using the coordinates of Y. TOMIMURA and Y. TOBARI (unpublished results). The genetic map distance between *f* and *Om(1D)*, however, is relatively large: 0.5 map unit. This indicates that the recombination frequency in the *f* locus region is considerably higher than at *v*. Thus, if recombination has an effect on the level of molecular genetic variation, we should expect results that differ from those obtained for *v*.

Restriction site polymorphisms: The results of our survey are summarized in Figure 5 and in Table 2. We have scored a total of 34 restriction sites. Twelve sites are polymorphic, but only two of those are unique, marking a first difference to *v*. The distributions of the polymorphic sites across the three population provides evidence for geographic variation, as for *vermilion*. However, their pattern is different. All 12 polymorphic sites were found in the Burma population, 7 of these 12 sites are polymorphic in the India population as well, whereas only 4 are variable in the Brazil population (which exhibits the highest level of site polymorphism at the *v* locus). These population differences are also expressed by the measures of average heterozygosity (Table 3). For the Burma and India populations, the values of both θ and π are considerably higher than in the *v* region. Population differentiation is shown in more detail by the frequency spectrum (Figure 5), exhibiting that the distribution of 5 polymorphic sites is significantly ($P < 0.01$) different between the populations. Most of the

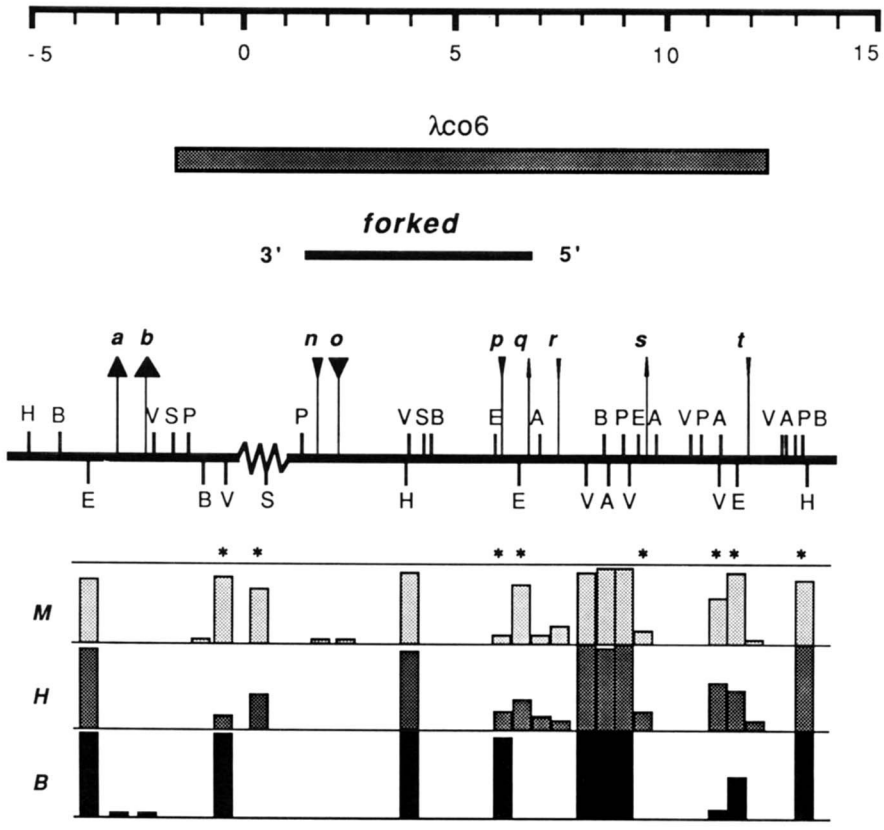

FIGURE 5.—Summary restriction map and frequency spectrum of the *f* region in *D. ananassae*. To localize the transcriptional unit and to determine the 3′ and 5′ orientation, we used information available from *D. melanogaster* (V. CORCÉS, personal communication). Relative to the standard map (*ca*; *px*), the probe, λco6, has a 0.5-kb deletion (*Del*(*h*), see Table 2) (not shown in figure). A restriction fragment length polymorphism, indicated by a zig-zag line, maps into the interval (−0.5, 1.3). It includes *Del*(*c*) to *Ins*(*m*). The sizes of the insertions and deletions were estimated as follows: *Del*(*a*) = 0.4 kb, *Del*(*b*) = 0.5 kb, *Del*(*c*) = 0.1 kb, *Ins*(*d*) = 0.05 kb, *Del*(*e*) = 0.7 kb, *Del*(*f*) = 0.6 kb, *Del*(*g*) = 0.55 kb, *Del*(*h*) = 0.5 kb, *Del*(*i*) = 0.45 kb, *Del*(*j*) = 0.05 kb, *Ins*(*k*) = 0.05 kb, *Ins*(*l*) = 0.4 kb, *Ins*(*m*) = 0.7 kb, *Ins*(*n*) = 0.2 kb, *Ins*(*o*) = 0.4 kb, *Ins*(*p*) = 0.1 kb, *Del*(*q*) = 0.05 kb, *Ins*(*r*) = 0.05 kb, *Del*(*s*) = 0.05 kb, *Ins*(*t*) = 0.05 kb. The frequency distribution of each polymorphism is displayed below the restriction map for the Burma (M), India (H) and Brazil (B) populations. For the restriction fragment length polymorphism in the interval (−0.5, 1.3), the (total) frequency of variants (deviating from the standard *ca*; *px* haplotype) is shown. A star indicates highly significant ($P < 0.01$) population differences for the designated polymorphism.

other variants are rare, such that there is not enough power in the test to show differences.

Insertions and deletions: We found no large insertions (>700 bp) in the *f* locus region, unlike at the *v* locus. However, there is a considerable number of small insertions and deletions (in the range 50 to 100 bp) distributed across most of the 18-kb region under consideration, in particular, at the 5′ end of the transcriptional unit. The most interesting finding is the variable site located in the *Pvu*II-*Hin*dIII fragment at the 3′ flanking region of the gene (coordinates −0.5 to 1.3). The length increments range from −0.7 to +0.7 kb. The frequencies of the variants (those deviating from the standard haplotype *ca*; *px*) are 13 of 19 for the Burma population, 8 of 20 for the India population, and 0 for the Brazil population. These frequency differences are highly significant (Figure 5). The observed length polymorphisms may reflect the expansion/contraction dynamics of tandem-repetitive DNA. The clone, λco6, used as a probe clearly contains some repetitive DNA, which caused a background smear on the autoradiographs. However, the bands could be unambiguously scored, possibly because λco6 has a 0.5-kb deletion (*Del*(*h*)) in this variable region.

Nonrandom associations: We made pairwise tests for linkage disequilibrium for all of the polymorphic restriction sites and insertions/deletions for each population, except for the unique variants. None of them were significant by the criterion of the Fisher exact test. This is consistent with the genetic and cytogenetic results, suggesting that the recombination rate at the *f* locus is not low, though *f* is located in the centromeric region (see below).

DISCUSSION

Comparison of *vermilion* and *forked* in *D. ananassae*

Insertional variation: In the *f* locus region we found no insertions/deletions larger than 700 bp. In contrast, at the *v* locus the majority of insertions found were larger than 500 bp, including two unique insertions of about 5 kb which lie outside the transcriptional unit. The 0.9-kb insertion, *Ins*(*g*), appears to be present in 7 lines at the same position. This is similar to the *yellow-achete-scute* region in *D. melanogaster* for which multiple insertions have been reported (AGUADÉ, MIYASHITA and LANGLEY 1988). The similarity between *v* in *D. ananassae* and *yellow-achete-scute* in *D. melanogaster* can be explained by their location in regions of reduced recombination. This explanation is based on a model developed by LANGLEY *et al.* (1988) that insertions accumulate preferentially in regions of low recombination, while in regions of high recombination asymmetric pairing between homologous elements leads to copy number reduction. The fact that we did not find any large insertions at *f* is consistent with the predictions of this model, since the recombination rate at *f* appears to be much higher than at *v*.

TABLE 2

Restriction map variants in the *forked* region of *D. ananassae*

	Loci																			
Lines	*Eco*RI −3.7	*Pvu*II −0.5	*Ins* (*d*)	*Sal*I 0.4	*Del* (*e*)	*Del* (*h*)	*Del* (*i*)	*Hin*dIII 3.8	*Ins* (*p*)	*Eco*RI 6.5	*Del* (*q*)	*Ins* (*r*)	*Pvu*II 8.1	*Sac*I 8.6	*Del* (*s*)	*Pvu*II 11.2	*Eco*RI 11.6	*Ins* (*t*)	*Hin*dIII 13.2	Unique variants
M61	−	+	−	−	−	−	−	+	−	+	−	−	+	+	−	−	+	−	+	*Ins*(*m*)
M62	+	+	−	ns	+	−	−	+	−	−	−	+	+	+	−	+	+	−	+	*Ins*(*n*)
M66	+	+	−	ns	ns	−	−	+	−	+	+	−	+	+	−	+	+	−	+	*Del*(*g*)
M68	+	+	−	ns	−	−	+	+	−	+	−	+	−	+	−	−	+	−	+	−
M79	+	+	−	ns	−	−	−	+	−	+	+	−	+	+	+	+	−	−	+	*Del*(*f*)
M80	+	+	−	ns	−	+	−	+	−	+	−	−	+	+	−	+	+	−	+	−
M86	−	+	−	ns	−	+	−	+	−	+	−	+	+	+	−	+	+	−	−	−
M89	+	+	−	+	−	−	−	+	−	+	−	−	+	+	−	+	+	−	+	−
M90	+	+	−	ns	+	−	−	+	−	+	−	−	+	+	−	+	+	−	−	*Ins*(*o*)
M91	−	+	−	−	−	−	−	+	−	−	−	−	+	+	−	−	+	−	+	+*Bam*HI(−0.9), −*Pvu*II(9.1)
M92	+	+	−	ns	−	+	−	+	+	+	−	+	−	+	+	+	+	−	+	−
M97	+	+	−	−	−	−	−	+	−	+	−	−	+	+	−	−	+	−	+	−
M99	+	−	−	+	−	−	−	−	+	−	−	−	+	+	−	+	−	−	+	−
M106	−	+	−	−	−	−	−	+	−	+	−	−	+	+	+	−	+	−	−	−
M111	+	+	−	ns	−	+	−	+	−	−	−	−	+	+	−	+	+	−	+	−
M114	+	−	−	ns	−	+	−	−	−	−	−	−	+	+	−	−	+	−	+	−
M115	+	+	−	ns	−	+	−	+	−	+	−	−	+	+	−	−	+	−	−	−
M117	+	−	−	+	−	−	−	+	−	+	−	−	+	+	−	−	+	+	+	−
M119	+	+	−	ns	−	+	−	+	−	+	−	−	+	−	−	+	+	−	+	−
H3	−	−	−	ns	−	+	−	+	+	−	−	−	+	+	−	−	−	−	+	−
H4	+	−	+	+	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	−
H9	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	*Del*(*j*)
H10	+	−	−	+	−	−	−	−	−	−	−	+	+	+	−	+	−	−	+	−
H11	+	−	−	+	−	−	−	+	−	+	+	−	+	+	+	+	−	−	+	−
H12	+	+	−	ns	−	−	+	+	−	+	−	+	+	+	−	+	−	−	+	−
H13	+	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	+	−	+	−
H15	+	−	−	ns	−	−	+	+	−	+	+	−	+	+	+	−	+	−	+	−
H16	+	+	−	ns	−	+	−	+	−	−	−	−	+	+	−	+	−	−	+	−
H21	+	−	+	+	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	−
H23	+	−	−	+	−	−	−	+	+	−	−	−	+	+	−	+	−	−	+	−
H26	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	−
H27	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	−
H31	+	−	−	+	−	−	−	+	+	−	−	−	+	+	−	+	+	−	+	*Ins*(*k*)
H36	+	−	+	+	−	−	−	+	+	−	+	−	+	+	−	+	+	−	+	−
H39	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	−	+	−	+	*Del*(*c*),*Ins*(*l*)
H41	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	+	−	−	+	−
H47	+	−	−	+	−	−	−	+	−	+	−	−	+	+	−	−	−	−	+	−
H48	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
H50	+	−	−	+	−	−	−	−	−	+	−	−	+	+	−	−	+	−	+	−
B55	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B59	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B79	−	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	*Del*(*a*)
B83	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B84	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B91	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	+	−	−	+	−
B93	+	−	−	+	−	−	−	+	−	−	−	−	+	+	−	+	+	−	+	*Del*(*b*)
B99	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B102	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B104	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B116	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B117	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B118	+	+	−	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	−
B119	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B120	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B121	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B123	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B131	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	+	−	+	−
B132	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B134	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−
B135	+	+	−	+	−	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−

The *Sal*I site at 0.4 could not be scored in all lines (indicated by "ns"), probably because it is located within one of several deletions found in that region.

An interesting feature of the *f* locus region is the restriction fragment length polymorphism at the 3′ end of the transcriptional unit. The fact that we could identify a large number of different fragment sizes and that the (total) frequency of the variants is high in the Burma and India populations (but zero in the Brazilian one) seems to suggest that this region is composed of short tandem repeats that can change in copy number very quickly, like the hypervariable minisatellites (JEFFREYS, WILSON and THEIN 1985).

Restriction site variation: For the Burma population, average heterozygosity, H, per nucleotide at v is much lower than at f (Table 3). The same holds true for the India population, but to a lesser extent,

TABLE 3

Estimates of nucleotide variation in the *vermilion* and *forked* locus regions of *D. ananassae*

Variants	*vermilion*			*forked*		
	M	H	B	M	H	B
$\theta \times 10^3$	2.65	2.65	5.21	12.1	6.6	3.65
$\text{Var}(\theta)_1 \times 10^6$	3.63	3.51	6.8	12.3	6.22	3.33
$\text{Var}(\theta)_2 \times 10^6$	6.56	6.28	17.3	71.6	23.4	8.5
$\pi \times 10^3$	0.87	1.52	5.54	8.27	6.22	2.48
$\text{Var}(\pi) \times 10^6$	1.36	2.53	14.2	22.7	14.4	3.63
$\hat{H} \times 10^3$	0.87	1.5	5.35	—	—	2.45

θ and its variances, $\text{Var}(\theta)_1$ for infinite recombination and $\text{Var}(\theta)_2$ for zero recombination, were estimated using HUDSON's (1982) method. π and its sampling variance are calculated according to NEI and TAJIMA (1981). $\hat{H}$ is ENGELS' (1981) estimate of average nucleotide heterozygosity. The value of $\hat{H}$ was not calculated for the Burma and India populations, because the *Sal*I-site in the variable region at position 0.4 could not be scored in all lines (Table 2).

whereas for the Brazil population the opposite was found. The between-population pattern of heterozygosity is different for the two loci:

$$v\text{: } H_M \leq H_H < H_B$$

$$f\text{: } H_M > H_H > H_B,$$

where H_M, H_H and H_B denote the average heterozygosities in the Burma (M), India (H) and Brazil (B) populations, respectively.

At the *v* locus, both the Burma and the India populations show reduced levels of variation, similar to the *su(f)* locus (C. H. LANGLEY, M. AGUADÉ and N. MIYASHITA, unpublished results) or the *yellow-achete-scute* region in *D. melanogaster* (AGUADÉ, MIYASHITA and LANGLEY 1988), while average heterozygosity for the Brazil population is approximately 0.5%, a level common to many loci, including the *f* and *v* loci, in *D. melanogaster* (C. H. LANGLEY, M. AGUADÉ and N. MIYASHITA, unpublished results). The relative order of the levels of heterozygosity in the *v* locus region between the three populations was not expected. An attempt to interpret these results is made below, after discussing the effects of population subdivision.

In the *f* region, average heterozygosity shows the descending order that is to be expected on the basis of the zoogeography of this species: the zoogeographical center of *D. ananassae* is in Southeast Asia (PATTERSON and STONE 1952); from there it spread to Africa and to Central and South America. Thus, the reduction of diversity between the three populations may simply reflect a reduction of the respective population sizes due to restricted migration.

Linkage disequilibria: For both loci we tested all pairs of polymorphisms for linkage disequilibrium. At the *f* locus, we found no significant linkage disequilibrium at all. In contrast, at *v*, all pairs of polymorphic sites were in strong linkage disequilibrium, provided their frequencies were high enough to allow a powerful test. These differences can be explained by the recombination rate differences. The apparent absence of closed loops in the *v* haplotype graph (Figure 4), together with our results from *in situ* hybridization experiments, suggests that recombination in the *v* region is much lower than at *f*. All relevant population genetics models predict that linkage disequilibrium decreases with increasing recombination rate, no matter whether random genetic drift (HILL and ROBERTSON 1966, 1968) or epistasis (KIMURA 1956; LEWONTIN and KOJIMA 1960; FELSENSTEIN 1965) causes the disequilibrium.

Geographic differentiation

Significant differences between the three populations were observed for a large number of polymorphic sites at both loci (for *f*, see the frequency spectrum in Figure 5). The significance tests were carried out by means of Monte Carlo simulations on 2×3-contingency tables (see MATERIALS AND METHODS). For the *v* locus the site differences carry over to haplotypes (Figure 4). The conclusions we can draw from our data are as follows. Because of the descending order of average heterozygosity, $H_M > H_H > H_B$, in the *f* locus region, the Brazil population seems to be a subpopulation of the Burma or India population, while the India population is a subpopulation of the Burma population. In addition, the *v* data suggest that the Brazil population is a 40:60% admixture of the Burma and India populations. Consistent with the notion that the zoogeographical center of *D. ananassae* is in Southeast Asia is our observation that the frequencies of the α haplotypes of the *v* locus, which are characteristic for the Burma population, are more uniformly distributed than the β's (Figure 4).

Molecular genetic variation and recombination in subdivided populations

It remains to provide a qualitative understanding of our two major results, (i) the reduction of the level of nucleotide variation at *v* relative to *f*, and (ii) the reversal of the expected descending order of average heterozygosity at *v*, such that $H_M \leq H_H < H_B$. [Of course, because of the large standard errors (Table 3), the second result has to be considered with some caveats.] The first result is similar to the observations made at *su(f)* and in the *yellow-achete-scute* region in *D. melanogaster* which are completely monomorphic or show very little variation with respect to restriction sites (C. H. LANGLEY, M. AGUADÉ and N. MIYASHITA, unpublished results; AGUADÉ, MIYASHITA and LANGLEY 1988). In both regions, crossing over is infrequent.

We have provided evidence that recombination at the *v* locus is reduced in two ways. First, by determin-

ing the cytogenetic locations of *v* and *fw* by means of *in situ* hybridization and comparing the genetic distances of the intervals (*v*, *fw*) and (*f*, *Om(1D)*) to the corresponding cytogenetic distances, we were able to estimate the relative rates of crossing over at the *v* and *f* regions. Second, indirect evidence for reduced recombination at *v* is detectable in our population genetic data. The haplotype graph of the *v* locus is a tree, containing no closed loops, which is indicative of a complete absence of recombinant haplotypes in our sample. Furthermore, we found large amounts of linkage disequilibrium at *v*, whereas no significant linkage disequilibrium was observed at *f*.

A model recently developed by N. KAPLAN, R. R. HUDSON and C. H. LANGLEY (unpublished results) predicts that the level of nucleotide variation is reduced in regions of low recombination. The model is based on the idea that favorable mutations recurrently occurring in a population and quickly going to fixation can fix a whole region by hitchhiking, if the recombination rate is sufficiently low. The differences in variation we observed in the *v* and *f* regions in the Burma population (which is zoogeographically central) and, to a lesser extent, in the India population are consistent with the qualitative predictions of their model.

The hitchhiking idea may also be used to explain the reverse order of average heterozygosities at *v*, taking into account that, as the *f* data suggest, the India population has a smaller effective population size than the Burma population, and, similarly, that the Brazil population is smaller than the India population. In a smaller population (N, population size), the effect of hitchhiking seems to be reduced, because favorable mutations ($s > 0$, selection coefficient; μ, rate of favorable mutations per gene) occur less often and because the influence of random genetic drift is stronger. Together, this can be expressed by the rate of favorable gene evolution, v, given by $2N\mu u$, with $u = 2s/(1 - e^{-4Ns})$ being the probability of fixation (CROW and KIMURA 1970; p. 426). It is easily seen that v increases with N. However, this discussion of the effect of population size on hitchhiking does not consider recombination. As C. STROBECK pointed out, decreasing population size increases the region over which a favorable mutation sweeping through the population causes homozygosity. Whether this effect is large enough to be critical for the argument presented above can only be seen by analyzing a full model.

Other explanations for reduced nucleotide variation cannot be ruled out at present. For example, it may well be that the mutation rate is different near the centromere, due to the late replication of this region. Another possibility would be that the interaction of mutation-repair, recombination and heterozygosity which seem to be inextricably connected, both genetically and by natural selection, leads to different levels of variation along the chromosome.

We are very grateful to M. MATSUDA and S. TANDA for advice and encouragement at all stages of our experiments. Furthermore, our work profited from interactions with our colleagues M. AGUADÉ, E. GOODE, C. HINTON, R. HUDSON, M. IIZUKA, B. JUDD, N. KAPLAN, B. LANGE, N. MIYASHITA, W. QUATTLEBAUM, G. SIMMONS, R. VOELKER, and M. WATADA. We thank Y. TOBARI, S. HORI, L. SEARLES, V. CORCÉS, T. GORALSKI and K. CONRAD for sharing fly stocks and DNA's. We also thank C. STROBECK, M. TURELLI and an anonymous reviewer for suggestions on improving the manuscript.

LITERATURE CITED

AGUADÉ, M., N. MIYASHITA and C. H. LANGLEY, 1988 Reduced variation in the *yellow-achete-scute* region in natural populations of *Drosophila melanogaster*. Genetics (in press).

AQUADRO, C. F., K. M. LADO and W. A. NOON, 1988 The *rosy* region of *Drosophila melanogaster* and *D. simulans*. I. Contrasting levels of naturally occurring DNA restriction map variation and divergence. Genetics **119:** 875–888.

AQUADRO, C. F., S. F. DEESE, M. M. BLAND, C. H. LANGLEY, C. C. LAURIE-AHLBERG, 1986 Molecular population genetics of the alcohol dehydrogenase gene region of *Drosophila melanogaster*. Genetics **114:** 1165–1190.

BEADLE, G. W., 1932 A possible influence of the spindle fibre on crossing over in *Drosophila*. Proc. Natl. Acad. Sci. USA **18:** 160–165.

BINGHAM, P. M., R. LEVIS and G. M. RUBIN, 1981 Cloning of DNA sequences from the white locus of *D. melanogaster* by a novel and general method. Cell **25:** 693–704.

CHARLESWORTH, B., C. H. LANGLEY and W. STEPHAN, 1986 The evolution of restricted recombination and the accumulation of repeated DNA sequences. Genetics **112:** 947–962.

CROW, J. F., and M. KIMURA, 1970 *An Introduction to Population Genetics Theory*. Burgess, Minneapolis, Minn.

ENGELS, W. R., 1981 Estimating genetic divergence and genetic variability with restriction endonucleases. Proc. Natl. Acad. Sci. USA **78:** 6329–6333.

FELSENSTEIN, J., 1965 The effect of linkage on directional selection. Genetics **52:** 349–363.

HILL, W. G., and A. ROBERTSON, 1966 The effect of linkage on limits to artificial selection. Genet. Res. **8:** 269–294.

HILL, W. G., and A. ROBERTSON, 1968 Linkage disequilibrium in finite populations. Theor. Appl. Genet. **38:** 226–231.

HINTON, C. W., 1984 Morphogenetically specific mutability in *Drosophila ananassae*. Genetics **106:** 631–653.

HINTON, C. W., 1988 Formal relations between *Om* mutants and their suppressors in *Drosophila ananassae*. Genetics (in press).

HUDSON, R. R., 1982 Estimating genetic variability with restriction endonucleases. Genetics **100:** 711–719.

HUDSON, R. R., M. KREITMAN and M. AGUADÉ, 1987 A test of neutral molecular evolution based on nucleotide data. Genetics **116:** 153–159.

JEFFREYS, A. J., V. WILSON and S. L. THEIN, 1985 Hypervariable 'minisatellite' regions in human DNA. Nature **314:** 67–73.

KIKKAWA, H., 1938 Studies on the genetics and cytology of *Drosophila ananassae*. Genetica **20:** 458–516.

KIMURA, M., 1956 A genetic system which leads to closer linkage by natural selection. Evolution **10:** 278–287.

KREITMAN, M., 1983 Nucleotide polymorphism at the alcohol dehydrogenase locus of *Drosophila melanogaster*. Nature **304:** 412–417.

KREITMAN, M., and M. AGUADÉ, 1986a Genetic uniformity in two

populations of *Drosophila melanogaster* as revealed by filter hybridization of four-nucleotide-recognizing enzyme digests. Proc. Natl. Acad. Sci. USA **83:** 3562–3566.

KREITMAN, M. E., and M. AGUADÉ, 1986b Excess polymorphism at the *Adh* locus in *Drosophila melanogaster*. Genetics **114:** 93–110.

LANGER-SAFER, P. R., M. LEVINE and D. C. WARD, 1982 Immunological method for mapping genes on *Drosophila* polytene chromosomes. Proc. Natl. Acad. Sci. USA **79:** 4381–4385.

LANGLEY, C. H., E. MONTGOMERY, R. HUDSON, N. KAPLAN and B. CHARLESWORTH, 1988 On the role of unequal exchange in the containment of transposable element copy number. Genet. Res. (in press).

LANGLEY, C. H., E. A. MONTGOMERY and W. F. QUATTLEBAUM, 1982 Restriction map variation in the *Adh* region of *Drosophila*. Proc. Natl. Acad. Sci. USA **79:** 5631–5635.

LEIGH BROWN, A. J., 1983 Variation at the 87A heat-shock locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 5350–5354.

LEWONTIN, R. C., and J. FELSENSTEIN, 1965 The robustness of homogeneity tests in 2×*N* tables. Biometrics **21:** 19–33.

LEWONTIN, R. C., and K. KOJIMA, 1960 The evolutionary dynamics of complex polymorphisms. Evolution **14:** 458–472.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MATHER, K., 1939 Crossing over and heterochromatin in chromosomes of *Drosophila melanogaster*. Genetics **24:** 413–435.

MIYASHITA, N., and C. H. LANGLEY, 1988 Molecular and phenotypic variation of the *white* locus region in *Drosophila melanogaster*. Genetics **120:** 199–212.

MONTGOMERY, E. A., and C. H. LANGLEY, 1983 Transposable elements in Mendelian populations. II. Distribution of three copia-like elements in a natural population of *Drosophila melanogaster*. Genetics **104:** 473–483.

MORIWAKI, D., and Y. N. TOBARI, 1975 *Drosophila ananassae*. pp. 513–535. In: *Handbook of Genetics*, Vol. 3, Edited by R. C. KING. Plenum, New York.

NEI, M., and F. TAJIMA, 1981 DNA polymorphism detectable by restriction endonucleases. Genetics **97:** 145–163.

PATTERSON, J. T., and W. S. STONE, 1952 *Evolution in the Genus Drosophila*. Macmillan, New York.

SCHAEFFER, S. W., C. F. AQUADRO and W. W. ANDERSON, 1987 Restriction map variation in the alcohol dehydrogenase region of *Drosophila pseudoobscura*. Mol. Biol. Evol. **4:** 252–263.

SEARLES, L. L., and R. A. VOELKER, 1986 Molecular characterization of the *Drosophila* vermilion locus and its suppressible alleles. Proc. Natl. Acad. Sci. USA **83:** 404–408.

SHRIMPTON, A. E., E. A. MONTGOMERY and C. H. LANGLEY, 1986 *Om* mutations in *Drosophila ananassae* are linked to insertions of a transposable element. Genetics **114:** 125–135.

Communicating editor: M. TURELLI