

Chromosomal Patterns of Microsatellite Variability Contrast Sharply in African and Non-African Populations of *Drosophila melanogaster*

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ABSTRACT

Levels of neutral variation are influenced by background selection and hitchhiking. The relative contribution of these evolutionary forces to the distribution of neutral variation is still the subject of ongoing debates. Using 133 microsatellites, we determined levels of variability on X chromosomes and autosomes in African and non-African *D. melanogaster* populations. In the ancestral African populations microsatellite variability was higher on X chromosomes than on autosomes. In non-African populations X-linked polymorphism is significantly more reduced than autosomal variation. In non-African populations we observed a significant positive correlation between X chromosomal polymorphism and recombination rate. These results are consistent with the interpretation that background selection shapes levels of neutral variability in the ancestral populations, while the pattern in derived populations is determined by multiple selective sweeps during the colonization process. Further research, however, is required to investigate the influence of inversion polymorphisms and unequal sex ratios.

IT is well established that neutral variation is affected by selection at linked sites. Two contrasting modes of selection, background selection and hitchhiking, have primarily been investigated. The hitchhiking model assumes that recurrent beneficial mutations are spreading through the population and that linked neutral variants become fixed in association with the beneficial alleles (MAYNARD SMITH and HAIGH 1974; KAPLAN *et al.* 1989). The alternative model of background selection is based on the continuous removal of deleterious mutations from a population (CHARLESWORTH *et al.* 1993). Interestingly, both models make similar predictions for levels of neutral variability in regions of different recombination rates. Neutral variation in regions of low recombination is more suppressed than in regions of high recombination, leading to a positive correlation between recombination rate and variability (MAYNARD SMITH and HAIGH 1974; KAPLAN *et al.* 1989; CHARLESWORTH *et al.* 1993). Experimental data for a range of different species demonstrated that this predicted pattern is a widespread phenomenon (STEPHAN and LANGLEY 1989, 1998; NACHMAN 1997; NACHMAN *et al.* 1998). The qualitative similarity of both models has made it difficult to determine the relative importance of background selection and hitchhiking in shaping the genomic distribution of neutral variation. Recently, it has been shown for a set of reasonable parameters for recombination and deleterious mutation rate that the background selection model provides a good fit to the levels of variation observed in

Drosophila melanogaster (CHARLESWORTH *et al.* 1993; HUDSON 1994). A good fit to the data, however, was also observed for a simple hitchhiking model (WIEHE and STEPHAN 1993; STEPHAN 1995). KIM and STEPHAN (2000) analyzed joint effects of hitchhiking and background selection on neutral variation and found that stationary levels of nucleotide variability in low recombining regions are influenced mainly by hitchhiking, whereas in regions with higher recombination rate background selection has more impact (KIM and STEPHAN 2000).

Approaches to distinguish between background selection and hitchhiking: Three approaches have been proposed to discriminate between the relative roles of background selection and hitchhiking in shaping genome-wide variability. A summary of the expectations under the different approaches is given in Table 1.

First, at mutation-selection equilibrium, the two models differ in their predictions for the single nucleotide polymorphism (SNP)-frequency spectrum in genomic regions of low recombination. The hitchhiking model predicts a surplus of rare alleles compared to neutral expectations for equilibrium populations (BRAVERMAN *et al.* 1995), while under the background selection model in large populations only a slight skew in the allele frequency distribution is expected (HUDSON and KAPLAN 1994). Empirical data, however, did not always detect a statistically significant skew toward rare alleles in the allele frequency distribution (AGUADÉ *et al.* 1989; MARTIN-CAMPOS *et al.* 1992; BEGUN and AQUADRO 1995a). Finally, in a recent study, FAY and WU (2000) found that an excess of derived high frequency alleles is also a unique characteristic of selective sweeps.

A second approach utilizes the comparison of mark-

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TABLE 1

Expectations under different approaches to distinguish hitchhiking and background selection

Expectation	Hitchhiking	Background selection
Excess of rare alleles	+	-
Excess of derived high frequency alleles	+	-
Correlation of variability with recombination rate		
Markers with very high mutation rate	-	+
Markers with low mutation rate	+	+
Higher relative X chromosomal variability	-	+
Lower relative X chromosomal variability	+	-

ers with different mutation rates such as sequence polymorphism and microsatellites (SCHUG *et al.* 1998a). The expectation is that variability, as measured with markers with high mutation rates, should show a correlation with recombination rate under background selection but not under hitchhiking. Markers with low mutation rates should show a correlation in both cases. Recently, levels of microsatellite variability were correlated with recombination rates to discriminate between background selection and hitchhiking for *D. melanogaster* (MICHALAKIS and VEUILLE 1996; SCHLÖTTERER *et al.* 1997; SCHUG *et al.* 1998a). The obtained results were not consistent, due largely to the relatively small number of loci analyzed (SCHLÖTTERER 2000). Furthermore, the relatively low mutation rate of *D. melanogaster* microsatellites (SCHLÖTTERER *et al.* 1998; SCHUG *et al.* 1998b) limits their power to discriminate between background selection and selective sweeps. Human microsatellites, however, have a significantly higher mutation rate than *D. melanogaster* microsatellites, but no correlation between microsatellite variability and recombination rate was detected (PAYSEUR and NACHMAN 2000).

The third approach to distinguish between the two selection models was proposed by AQUADRO *et al.* (1994) and concentrates on different variability levels of X chromosomes and autosomes. Under certain assumptions the models differ in their predictions about the levels of sequence variation on X chromosomes and autosomes (CHARLESWORTH *et al.* 1993). In most species males carry a single X chromosome and two autosomes, while females have two copies of both. In such a heterogametic system recessive deleterious mutations are purged more efficiently from the population when located on the X chromosome (CROW and KIMURA 1970; LANGLEY *et al.* 1981). Because deleterious alleles are removed more rapidly from the X chromosome there is less chance for recombination to combine different neutral

alleles with the deleterious mutation. Given that deleterious alleles and their linked neutral variation are purged from the population, the background selection model predicts more neutral variation on the X chromosome than on the autosomes after correction for different population sizes of the chromosomes and assuming a 1:1 sex ratio. It should be noted, however, that this does not necessarily imply that X chromosomes have a higher absolute level of polymorphism.

Similarly, due to the male heterogamy, beneficial mutations have a longer sojourn time on autosomes than on the X chromosome. In contrast to the background selection model, under which deleterious mutations are removed from the population, the hitchhiking model assumes the fixation of beneficial mutations. It has been shown that beneficial mutations on the X chromosome have a higher fixation rate than autosomes if selection operates on new mutations (CHARLESWORTH *et al.* 1987). Under these assumptions the hitchhiking model, therefore, predicts more neutral variation on the autosomes than on the X chromosome (after correction for the different population sizes of the chromosomes and assuming a 1:1 sex ratio). Recently, BEGUN and WHITLEY (2000) followed this approach and found X chromosomes to harbor less sequence variation than autosomes in *D. simulans*.

In this report we focus mainly on the third approach. We used microsatellites, which are neutral polymorphic markers (SCHLÖTTERER 2000), to determine levels of neutral variability on the X chromosome and autosomes in populations from Africa and non-African populations. The comparison between the two groups of populations was motivated by the colonization history of *D. melanogaster*. It is well established that the species originated in Africa and expanded its habitat only recently, ~10,000 years ago (DAVID and CAPY 1988). Therefore it seemed very likely that different evolutionary forces were operative in these groups and more detailed information can be gleaned if these groups are analyzed separately. African populations are presumably closer to an equilibrium state than non-African populations, which in turn could be influenced by founder events, bottlenecks, and fixation of beneficial mutations associated with the colonization process.

We show that X chromosomes harbor significantly more variation than autosomes in African populations but not in non-African populations. The reduction in variability of non-African compared to African X chromosomes was found to be more pronounced in regions of low recombination rate. We discuss the two selection models and other evolutionary forces such as inversion polymorphism and unequal sex ratios in the light of our data.

MATERIALS AND METHODS

Microsatellites: We analyzed 133 microsatellite loci of which 40 loci are located on the X chromosome and 93 on the

autosomes. The selected microsatellite loci are located in chromosomal regions covering a wide range of recombination rates. All loci were typed in African and non-African populations. The data for 28 loci were taken from the literature (BACHTROG *et al.* 2000). Primer sequences, annealing temperatures, repeat motifs, and cytological positions of all loci are available from the authors' homepage (<http://i122server.vu-wien.ac.at/>). Microsatellite analysis followed standard protocols (SCHLÖTTERER and ZANGERL 1999).

Fly stocks: African isofemale lines were from Zimbabwe or Kenya or from both populations. Depending on the locus, 25–160 flies were typed from these populations. To account for inbreeding effects during propagation of these lines we randomly selected one allele in heterozygous individuals.

Non-African samples were F₁ flies from Austria, France, Germany, Italy, the Netherlands, Russia, and the United States. Twenty-five to 30 individuals per population were typed.

Not all loci were typed for the complete set of populations. The minimum population sample at a locus consisted of 25 African and 60 non-African individuals. If more than a single population was typed for one group (Africa, non-Africa), estimates of variability (see below) were calculated for each population separately and subsequently averaged. This treatment was chosen to avoid a biased variability estimate due to population substructure (Wahlund effect). Furthermore, this strategy accounted for the fact that an unequal number of populations were typed for different loci.

Variability measures, recombination rate, and corrections for effective population sizes of X chromosomes and autosomes: Two measures of microsatellite variability were used in this study: variance in repeat number (GOLDSTEIN *et al.* 1995) and heterozygosity. Both measures were corrected for small sample sizes by multiplying by $n/(n-1)$, where n is the number of typed chromosomes. To account for the dramatic influence of the repeat number on mutation rates (GOLDSTEIN and CLARK 1995; HARR and SCHLÖTTERER 2000; SCHLÖTTERER 2000), we normalized the variance in repeat number for each locus by dividing by the average number of repeats (V_{LC}). This correction accounts for a substantial part of the heterogeneity in microsatellite variability among loci (SCHLÖTTERER 2000). Throughout this text we use V_{LC} as a synonym for variance in repeat number. Furthermore, only microsatellites with <15 repeats were used in this study because longer microsatellites tend to be exponentially more variable (BRINKMANN *et al.* 1998). Variance in repeat number, heterozygosity, and recombination rate (adjusted for no recombination in males) for all loci are listed as a supplemental table at the GENETICS website at <http://www.genetics.org/supplemental>.

To account for different effective population sizes of X chromosomes and autosomes we introduced a correction factor for the X chromosomal variability measures. The correction factor was equal to the ratio of the effective population sizes of autosomes to X chromosomes. This ratio is

$$k = \frac{N_A}{N_X} = \frac{8(N_{ef} + 2N_{em})}{9(N_{ef} + N_{em})}, \quad (1)$$

where N_{ef} and N_{em} are the effective population sizes of females and males, respectively (CABALLERO 1994). Assuming a balanced sex ratio, the correction factor k was 1.33. The adjusted estimates of variability were calculated as

$$H_{corr} = 1 - \frac{1}{\sqrt{1 + k(1/(1 - H_{obs})^2 - 1)}} \quad (2)$$

and

$$V_{corr} = kV_{obs}. \quad (3)$$

It should be noted that Equations 2 and 3 assume a stepwise mutation model (OHTA and KIMURA 1973).

In mammals males and females have different mutation rates (BOHOSSIAN *et al.* 2000), which would require an adjustment for the observed level of variability because then X chromosomes and autosomes would have different mutation rates. A recent study in *D. melanogaster*, however, failed to demonstrate a significant effect for base substitutions (BAUER and AQUADRO 1997); therefore, we also assumed no systematic bias in microsatellite mutation rates among loci located on X chromosomes and autosomes.

Recombination rates for all loci were calculated as outlined in COMERON *et al.* (1999). Units of recombination given in this article are recombination events/(base pair × generation). The lack of recombination in *Drosophila* males was accounted for by a correction factor of $\frac{2}{3}$ for X chromosomes and $\frac{1}{2}$ for autosomal loci. Furthermore we controlled whether, for X chromosomes and autosomes, an equal fraction of loci are located in regions of similar recombination rate (after adjustment for no recombination in males). X chromosomal and autosomal loci, included in this study, therefore experienced on average a similar rate of crossing over ($P > 0.05$, Mann Whitney U -test).

RESULTS

Levels of variability in African and non-African populations: We analyzed 133 microsatellite loci, of which 40 loci were located on the X chromosome and 93 on the autosomes. A complete list of loci and their variability in African and non-African populations is available as a supplement at <http://www.genetics.org/supplemental>.

The joint analysis of all microsatellite loci indicated that both measurements of variability, heterozygosity, and variance in repeat number (corrected for the repeat number – V_{LC}) were significantly higher in African populations than in non-African ones. This difference between African and non-African populations was still significant if autosomal and X chromosomal microsatellite loci were analyzed separately ($P < 0.001$, Mann Whitney U -test, Table 2). This pattern is consistent with recent comparisons of African and non-African populations, which mostly detected higher levels of variability in African populations (BEGUN and AQUADRO 1993, 1995b; AGUADÉ 1998, 1999).

X chromosomal variability is more reduced than autosomal in non-African populations: While both X chromosomal and autosomal loci had a significant reduction in variability in non-African populations, a closer inspection of the data indicated that the relative reduction in variability differed between X chromosomes and autosomes (Figure 1, Table 2). X chromosomal microsatellites are more variable than autosomes in African populations, but in non-African populations X chromosomes are slightly less polymorphic than autosomes. Despite the opposite trend in the two populations, heterozygosities are significantly different between X chromosomes and autosomes in both African and non-African populations ($P < 0.005$, Mann Whitney U -test). V_{LC} showed, especially in African populations, a similar trend, but the

TABLE 2

Differences of autosomal and X chromosomal microsatellite variability in African and non-African populations

Population	Mean heterozygosity			Mean V_{LC}^a		
	Autosome	X chromosome	Difference A:X	Autosome	X chromosome	Difference A:X
Africa	0.62 (0.23)	0.75 (0.12)	**	0.67 (0.86)	0.84 (0.84)	$P = 0.07$
Non-Africa	0.52 (0.22)	0.4 (0.2)	**	0.32 (0.45)	0.24 (0.46)	NS
Difference Africa-non-Africa	**	**		**	**	

Variabilities are not corrected for effective population sizes of the chromosomes. Values in parentheses are standard deviations of the mean. ** $P < 0.01$ (Mann Whitney U -test). A:X, autosomes:X chromosome; NS, not significant.

^a V_{LC} , length corrected variance in repeat number.

difference was not statistically significant. An implication from these results is that variability of microsatellites located on the X chromosome is more strongly reduced in non-African populations. To test for statistical significance we performed an ANOVA and found significant interaction between the factors chromosome (X – autosomes) and population (Africa – non-Africa) on variability (H , $P < 0.0001$; V_{LC} , $P < 0.05$; Table 3).

Up to now, variability measures were not corrected for the different effective population sizes of X chromosomes and autosomes. Assuming a balanced sex ratio (an equal number of males and females), the effective population size of autosomes is 1.33 times larger than the effective population size of X chromosomes. After accounting for these differences in effective population size (see MATERIALS AND METHODS), X chromosomal and autosomal variability was similar in non-African populations (Table 4). The difference between the chromosomes in the African populations, however, became more significant.

Recombination rate and variability: An important aspect of our experimental design was to include loci from a wide range of recombination rates, but also to select chromosomal regions with comparable recombination rates (after correction for absence of recombination in males) on both chromosomes. Since under both selection models a correlation between variability and recom-

bination rate is predicted we tested for such a correlation on autosomes and X chromosomes in African and non-African populations. In African populations we observed an almost significant correlation only between one measure of variability (V_{LC}) and recombination rate for X chromosomal loci ($r = 0.31$, $P = 0.056$; Spearman rank correlation). Interestingly, this correlation was more pronounced for the non-African X chromosomal microsatellites (H , $r = 0.49$, $P < 0.05$; V_{LC} , $r = 0.43$, $P < 0.05$; Spearman rank correlation). No significant correlation for variability (H and V_{LC}) and recombination could be detected for either African or non-African autosomes (non-Africa $r = -0.04$, $P = 0.69$; Africa $r = -0.01$, $P = 0.95$; Spearman rank correlation).

Different effective population sizes of chromosomes:

The relative level of variability on X chromosomes and autosomes is strongly affected by the sex ratio, which in turn depends on the reproductive success of each sex. Therefore, we tested whether different effective population sizes of males and females could explain our results. Our tests are based on a correction factor for the different effective population sizes of the sex chromosomes under various sex ratios.

In the simple case of a balanced sex ratio, the excess of variability on African X chromosomes is statistically significant (Table 4). In non-African populations we noted a reduced heterozygosity of X-linked microsatel-

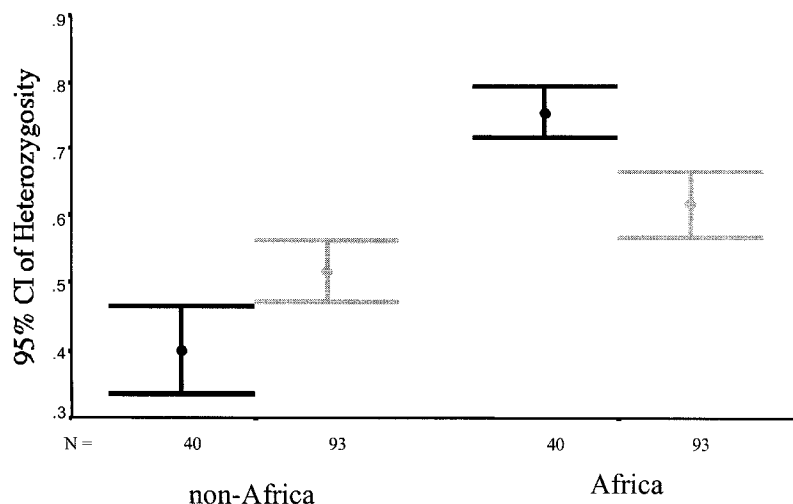


FIGURE 1.—Microsatellite variability on the X chromosome (black bars) and autosomes (gray bars) in African and non-African populations. The error bars indicate the 95% confidence interval of the mean heterozygosity (not corrected for different effective population sizes of X chromosomes and autosomes). The difference between the X chromosome and autosomes in both African and non-African populations is statistically significant ($P < 0.005$, Mann Whitney U -test).

TABLE 3
ANOVA for the impact of chromosome and population on variability

Factor	Heterozygosity ^a				V _{LC} ^b			
	d.f.	Mean square	F	P	d.f.	Mean square	F	P
Chromosome	1	0.02	0.25	0.62	1	0.69	0.39	0.55
Population	1	3.39	52.45	0.0001	1	93.7	48.52	0.0001
Chromosome × population	1	1385	21.4	0.0001	1	7.89	4.08	0.04

^a Heterozygosity was arcsine transformed.

^b V_{LC} (length corrected variance in repeat number) was log transformed.

lites. This difference, however, was not statistically significant.

Given that the ratio of the X chromosome-autosome variability is converse in African and non-African populations we must assume inverted sex ratios in these populations. African populations should have an excess of females while in non-African populations the effective population size of males should be larger.

Because we did not detect a significant difference between autosomal and X chromosomal variability in non-African populations, we did not further pursue the possibility of a larger effective population size of males in non-African populations. To explore the possibility of a nonbalanced sex ratio in African populations, we assumed a larger effective population size in females. Both variability estimators, heterozygosity and V_{LC}, were still significantly higher on African X chromosomes when we assumed a five times larger effective population size of females (Table 4). However, even an assumed 50-fold higher female population size could not account for the observed heterozygosities.

DISCUSSION

Our analysis of a large number of microsatellite loci in African and non-African *D. melanogaster* populations indicated that the level of variability differed between X chromosomes and autosomes. While X chromosomes were significantly more variable in African populations, non-African populations had higher heterozygosities for autosomal microsatellite loci. Similar results were re-

cently obtained from a survey of published *D. melanogaster* sequences (ANDOLFATTO 2001). The sequence analysis of 40 genes in non-African populations of *D. simulans*, a close relative of *D. melanogaster*, which also recently spread from Africa, also revealed lower levels of variability on the X chromosome (BEGUN and WHITLEY 2000). This consistency of various data sets strongly suggests a common underlying biological process. In the following we discuss processes that could, in principle, explain the observed distribution of variability.

Neutral explanations (demographic effects): *Sex ratio:* Male *D. melanogaster* have one X chromosome, while females carry two X chromosomes. Thus, the effective population size of X chromosomes is dependent on the variance of reproductive success of males and females. For African (WU *et al.* 1995; CAPY *et al.* 2000) and non-African (KOROL *et al.* 2000) populations, mate selection has been reported. Furthermore, it has been proposed that due to mate selection X chromosomes can harbor ~90% (rather than 75%) of the autosomal variability (NUNNEY 1993; CHARLESWORTH 2001). At least in European *D. melanogaster* populations, the effect could be countered by large variation in reproductive success among females (BOULETREAU 1978). In summary, there is substantial evidence for variance in reproductive success in *D. melanogaster*, but the extent to which differential reproductive success skews the ratio of X chromosomal to autosomal variability is simply not known.

Our data indicate that, in non-African populations, levels of variability were no longer significantly different among chromosomes when a balanced sex ratio is as-

TABLE 4
Microsatellite variability in African and non-African populations after correction for different effective population sizes of autosomes and X chromosomes

Population	Sex ratio (<i>f:m</i>)	Mean heterozygosity			Mean V _{LC} ^a		
		Autosome	X chromosome	Difference A:X	Autosome	X chromosome	Difference A:X
Africa	1:1	0.62 (0.23)	0.78 (0.11)	**	0.67 (0.86)	1.12 (1.1)	**
Non-Africa	1:1	0.52 (0.22)	0.44 (0.20)	<i>P</i> = 0.058	0.32 (0.45)	0.32 (0.62)	NS
Africa	5:1	0.62 (0.23)	0.75 (0.12)	**	0.67 (0.86)	0.85 (0.85)	*

** *P* < 0.01, * *P* < 0.05 (Mann Whitney *U*-test). Values in parentheses are standard deviations of the mean. *f:m*, females:males; A:X, autosomes:X chromosomes; NS, not significant.

^a V_{LC}, length corrected variance in repeat number.

sumed. For African X chromosomes we found that neither a balanced sex ratio nor a fivefold excess of females could explain the higher variability on African X chromosomes. On the basis of the available data, it is not possible to decide whether the sex ratio in African *D. melanogaster* populations is even more biased than we assumed. Nevertheless, a fivefold excess of females is more conservative than in previous reports, which suggested that X chromosomal variability should be 90% of the autosomal variability (NUNNEY 1993; CHARLESWORTH 2001), which corresponds to a three fold reduction in male effective population size.

Founder effect: It is assumed that *D. melanogaster* started from Africa to colonize the rest of the world $\sim 10,000$ years ago (DAVID and CAPY 1988). Such founder effects are often associated with a loss of genetic variability. Because our data also indicated a loss of variability in the non-African populations, we were interested whether a bottleneck could explain the loss of variability. We pursued two different approaches to test for a bottleneck in the non-African populations.

Our first test takes advantage of the well-investigated relationship between the number of alleles and heterozygosity. After a bottleneck, heterozygosity excess is expected at neutral loci (CORNUET and LUIKART 1996). We used the three different test statistics, sign test, standardized difference test, and Wilcoxon test, all implemented in the Bottleneck 1.2 software (CORNUET and LUIKART 1996). Irrespective of the mutation model used (strict stepwise or two-phase model with 30% nonstepwise mutations) we did not detect a significant heterozygosity excess in the non-African populations ($P > 0.05$).

The second test was based on the property of bottlenecks that they are a genome-wide phenomenon. Hence, if a founder event caused the reduction in variability, then all microsatellite loci located on the same chromosome should be affected to the same extent. To test this, we plotted the observed heterozygosity in non-African and African populations against recombination rate. If the reduction in variability were entirely due to a bottleneck, no correlation between variability and recombination rate should be detected. Interestingly, we observed a significant correlation between recombination rate and heterozygosity on the X chromosome only in non-African populations (non-Africa $r = 0.49$, $P = 0.001$; Africa $r = 0.08$, $P = 0.64$; Spearman rank correlation; Figure 2a). The same effect can also be seen for V_{LC} in non-African populations ($r = 0.43$, $P = 0.005$; Spearman rank correlation), where, in contrast, a marginally significant correlation is also observed in African populations ($r = 0.31$, $P = 0.056$; Spearman rank correlation). On the autosomes, no correlation could be detected (non-Africa $r = -0.04$, $P = 0.69$; Africa $r = -0.01$, $P = 0.95$; Spearman rank correlation; Figure 2b). This test shows that simple changes of population size cannot explain our data. Furthermore, even a reduction in the effective female population size would not have produced

a correlation between variability and recombination rate. The correlation between recombination and levels of variability has been explained by selective sweeps of beneficial mutations (BEGUN and AQUADRO 1992) or background selection (CHARLESWORTH *et al.* 1993). Our observation that a significant correlation exists on the X chromosome suggests that selection has a greater effect on the non-African X chromosome than on autosomes, but provides no further insight whether the fixation of beneficial or the removal of deleterious mutations is determining the pattern of variability.

Differential selective forces are operating in African and non-African populations: Any study attempting to explain the impact of selection on autosomal and X chromosomal genes has to consider the effect of dominance. While the dynamics of dominant mutations will not differ substantially among X chromosomes and autosomes, recessive mutations will be selected more efficiently when located on the X chromosome. Recently, BEGUN and WHITLEY (2000) reviewed the consequences of dominance effects for levels of variability under various selection regimes. For the remainder of the text, however, we assume that mutations are recessive.

Evidence for multiple beneficial mutations on non-African X chromosomes: The genetic consequences of exposure to a novel habitat are well investigated in simple organisms, such as *Escherichia coli* and yeast. Habitat shifts were shown to increase the fixation rate of beneficial mutations, leading to a higher fitness in the new environment (NOVICK and SZILARD 1950; LENSKI and TRAVISANO 1994; IMHOF and SCHLÖTTERER 2001). Similarly, the colonization of more temperate environments outside of Africa has undoubtedly resulted in an increased potential for the fixation of beneficial mutations in *D. melanogaster*. In principle, two different scenarios have to be distinguished. First, the beneficial mutations are novel and occurred during the colonization event. Second, the beneficial mutations were already present in the founder populations, but only with the habitat shift did these mutations become advantageous. While, for the first scenario, lower X-linked variability could be assumed (CHARLESWORTH *et al.* 1987), the predictions are less clear for the second model. ORR and BETANCOURT (2001) considered a sudden environmental change, which rendered previously deleterious alleles (in mutation-selection equilibrium) beneficial (ORR and BETANCOURT 2001). Under this model, the authors showed that a stronger reduction in variability on the X chromosome may or may not be expected if adaptive mutations involve previously deleterious alleles.

Until now, literally nothing has been known about mutations that are beneficial in a novel environment and the characterization of those mutations is among the most exciting challenges to evolutionary biologists. Despite lacking a clear prediction on the nature of beneficial mutations, we tested whether the pattern of variability is consistent with beneficial mutations reducing

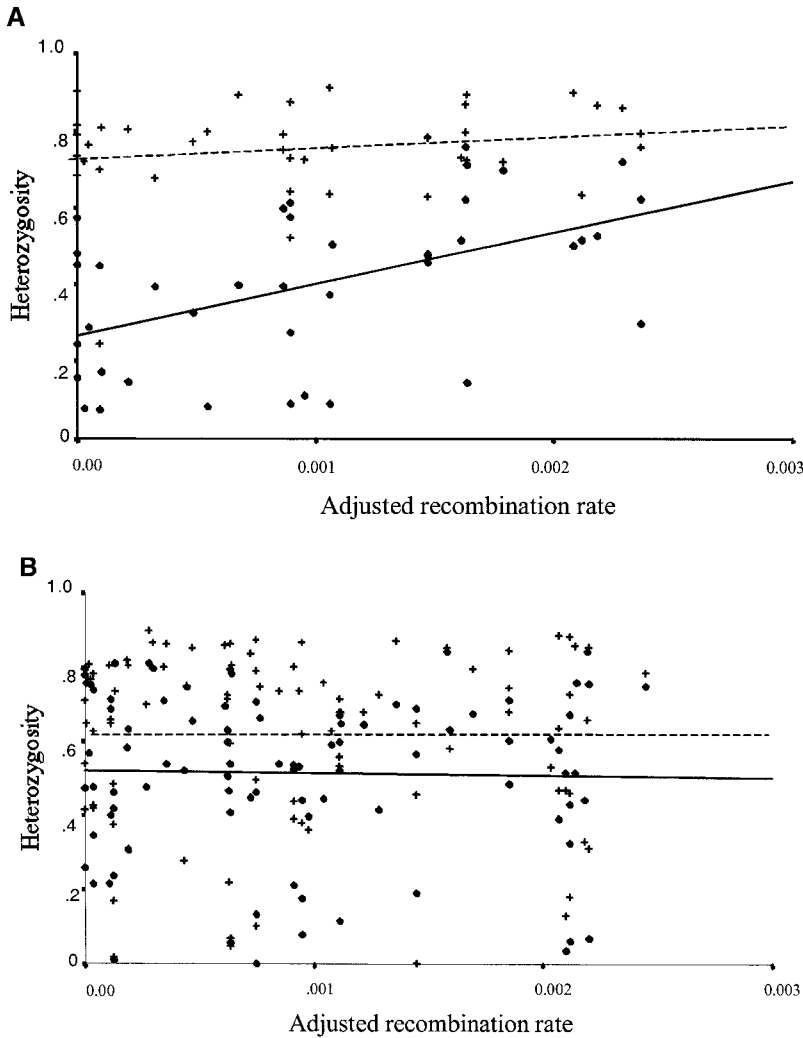


FIGURE 2.—Heterozygosity of non-African and African populations plotted against recombination rate [recombination events/(kilobase × generation)]. Crosses and the dashed line represent African, and dots and the solid line non-African, populations. To account for the absence of recombination in males, we corrected the recombination rates of autosomes and X chromosomes, assuming a 1:1 sex ratio. (A) A significant correlation between heterozygosity and recombination rate was observed for non-African X chromosomal loci (non-Africa $r = 0.49$, $P = 0.001$; Africa $r = 0.08$, $P = 0.64$; Spearman rank correlation). (B) On the autosomes no correlation could be detected (non-Africa $r = -0.04$, $P = 0.69$; Africa $r = -0.01$, $P = 0.95$; Spearman rank correlation).

X chromosomal variation in non-African populations. Because microsatellite loci are neutral markers, reduced microsatellite variability at a given locus is most likely caused by linkage to a selected site. Thus, the prediction is that some microsatellites on the X chromosome will be linked to a beneficial mutation, while others are not linked. Given that many beneficial mutations are required to reduce the variability on the X chromosome, we expect a reduced variability at several microsatellite loci. Consequently, microsatellite variability should show large variation among loci. Because autosomes did not experience a strong reduction in variability, fewer microsatellite loci are expected to be associated with a beneficial mutation. Hence, the variation in variability among loci is expected to be less pronounced among autosomal loci than among X chromosomal loci. To test this we calculated the variance of RV_{LC} ($RV_{LC} = V_{nA}/V_{Af}$) values for X chromosomal and autosomal loci. To obtain a confidence interval for this variance we generated 100 pseudoreplicas by bootstrapping the RV values for each group of loci. Figure 3 shows the mean of the variance of RV_{LC} and its 95% confidence interval. This value re-

flects the variation of RV_{LC} values on X chromosomes and autosomes. Consistent with a higher impact of selective sweeps on the X chromosome, we found a larger variance of RV_{LC} values on the X chromosome. Nevertheless, this result does not indicate whether the beneficial mutations occurring on the X chromosome were already segregating in the African population or are of recent origin. However, it should be noted that our data do not suggest that a larger number of beneficial mutations occurred on the X chromosome than on autosomes. Due only to the larger hitchhiking effect of beneficial mutations on the X chromosome is their presence more obvious. We assume that a similar number of beneficial mutations (per gene) also occurred on the autosomes, but a much smaller genomic region has hitchhiked with them.

The pattern of variability in African populations: One further important result of our survey is that in African populations X chromosomal microsatellites have a significantly higher variability than autosomes. Recently, ANDOLFATTO (2001), who obtained a similar pattern in a survey of published sequence data, proposed that autosomal variability in African populations could be

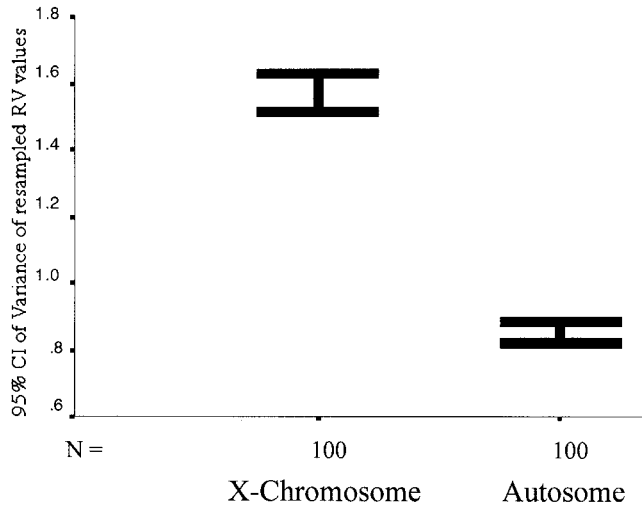


FIGURE 3.—Mean variance of 100 resamples of RV_{LC} ($RV_{LC} = V_{LCnA}/V_{LCnFr}$) values for X chromosomes and autosomes. The error bars indicate the 95% confidence interval of the mean RV_{LC} .

depressed by a recent increase in frequency of chromosomal inversions in these populations. In fact several inversions have been found to form clines with higher frequencies at low latitudes near the equator (LEMEUNIER and AULARD 1992). NAVARRO *et al.* (2000) found that variability levels within inversions could be reduced as long as the inversion has not reached equilibrium (NAVARRO *et al.* 2000). Experimental data, however, indicate that a reduction of variability is confined to the proximity of the inversion breakpoints. No reduced variability was detected in the middle of inversions (HASSON and EANES 1996; AGUADÉ 1998, 1999; ANDOLFATTO *et al.* 1999; BÉNASSI *et al.* 1999; DEPAULIS *et al.* 1999). Furthermore, an interesting finding of ANDOLFATTO (2001) was that *D. simulans*, a sibling species of *D. melanogaster*, which shows very few inversion polymorphisms, showed no elevated X-linked variability in African populations.

Given the possible implications of the data, we tested for an influence of common cosmopolitan inversions,

for which clines with high frequencies in African populations were described. Because most of the lines used were not kept alive, we did not determine the inversion status of each line. Thus, the inversion frequencies in our African samples are not known. However, if these inversions have affected our variability estimates, this should be recognizable even without the knowledge of the inversion status of each of the lines analyzed. We split the autosomal microsatellites into loci, which are located outside and within genomic regions harboring these inversions. No loci were found to map to the inversion breakpoints. Our microsatellite set contained only three of the five inversion locations known to reach high frequencies in Africa. The chromosomal locations of the inversions, as well as the microsatellite variabilities within and outside these regions, are given in Table 5. In contrast to the expectations for recently swept inversions, we did not detect any evidence for a reduction in variability of microsatellites located within the chromosomal regions to be possibly an inversion. This observation holds irrespective of whether we analyzed microsatellites within each inversion individually or all three inversions jointly and compared them to all microsatellites located outside of the inversion regions (Table 5).

While the separate analysis of microsatellite loci within inversions and outside inversions seems not to support the hypothesis of ANDOLFATTO (2001), we note that our test is rather crude and restricted to the impact of common cosmopolitan inversions. However, an alternative explanation of the data may be that the pattern of variability in African populations is consistent with background selection. According to the background selection model, X chromosomes should harbor more neutral variation than autosomes (CHARLESWORTH *et al.* 1987). Recessive deleterious alleles are purged more efficiently from X chromosomes and, thus, fewer neutral alleles are removed together with the deleterious alleles.

CONCLUSION

Our results are consistent with the interpretation that, in the African populations, which are presumably close

TABLE 5

Microsatellite variabilities within and outside of known inversion locations in African populations

Subsample	Cytological location	No. of microsatellites	Mean H	Mean V_{LC}^a
In(2L)t	22D–34A	20	0.61 (0.24)	0.98 (1.18)
In(2R)NS	52A–56F	12	0.58 (0.28)	0.33 (0.28)
In(3L)P	63C–72E	7	0.76 (0.06)	0.61 (0.39)
All inversions		39	0.63 (0.24)	0.71 (0.91)
No inversions		54	0.61 (0.23)	0.64 (0.82)
All loci		93	0.62 (0.23)	0.67 (0.86)

Pairwise differences of mean H and V_{LC} between all subsamples were not significant (Mann Whitney U -test, $P > 0.1$). Values in parentheses are standard deviations of the mean.

^a V_{LC} , length corrected variance in repeat number.

to an equilibrium situation, background selection is the evolutionary force shaping the partitioning of variability among X chromosomes and autosomes. Non-African populations, in contrast, seem to be more affected by the spread of beneficial mutations that were most likely associated with the habitat expansion of *D. melanogaster*. However, the influence of evolutionary forces, such as variation in reproductive success, chromosomal inversions, and founder effects on the observed pattern of variability, is not clear. More theoretical analyses are required to model the expectations for X chromosomal and autosomal variation in equilibrium and nonequilibrium populations.

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