A Microsatellite Variability Screen for Positive Selection Associated With the "Out of Africa" Habitat Expansion of *Drosophila melanogaster*

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ABSTRACT

We report a "hitchhiking mapping" study in *D. melanogaster*, which searches for genomic regions with reduced variability. The study's aim was to identify selective sweeps associated with the "out of Africa" habitat expansion. We scanned 103 microsatellites on chromosome 3 and 102 microsatellites on the *X* chromosome for reduced variability in non-African populations. When the chromosomes were analyzed separately, the number of loci with a significant reduction in variability only slightly exceeded the expectation under neutrality—six loci on the third chromosome and four loci on the *X* chromosome. However, non-African populations also have a more pronounced average loss in variability on the *X* chromosomes as compared to the third chromosome, which suggests the action of selection. Therefore, comparing the *X* chromosome to the autosome yields a higher number of significantly reduced loci. However, a more pronounced loss of variability on the *X* chromosome may be caused by demographic events rather than by natural selection. We therefore explored a range of demographic scenarios and found that some of these captured most, but not all aspects of our data. More theoretical work is needed to evaluate how demographic events might differentially affect *X* chromosomes and autosomes and to estimate the most likely scenario associated with the out of Africa expansion of *D. melanogaster*.

THE "neutral theory of evolution" (Kimura 1983) has dominated the study of molecular evolution for many years, but recent evidence suggests that beneficial mutations may be more abundant than previously assumed. For example, >40% of the amino acid replacements in Drosophila have been estimated to be driven by positive natural selection (Fay and Wu 2001; Fay *et al.* 2002; Smith and Eyre-Walker 2002). Similarly it was suggested that amino acid replacements in *Drosophila melanogaster* are on average beneficial (Bustamante *et al.* 2002). High values of putatively positively selected amino acid replacements have also been estimated for humans (Fay *et al.* 2001). Hence, the application of suitable methods should make it feasible to systematically screen for beneficial mutations.

Recent advances in molecular biology allow the processing of multiple samples, which permits the analysis of multiple genetic markers in many individuals. Genome scans to test for the effect of directional selection rely on the concept of hitchhiking (Maynard Smith and Haigh 1974; Kaplan *et al.* 1989), which describes the phenomenon that neutral variation flanking the selected site is also affected when beneficial mutations increase in frequency. Thus, a genome scan using a sufficiently high density of neutral markers could be used to identify genomic regions subjected to recent selective sweeps; this approach was recently termed hitchhiking mapping (Harr *et al.* 2002). Several genome scans based on microsatellite variation have already been performed for a range of organisms (Huttley *et al.* 1999, 2000; Payseur *et al.* 2002; Schlötterer 2002; Vigouroux *et al.* 2002; Wootton *et al.* 2002). A problem is to identify loci that differ from the rest of the genome, suggesting selection. Commonly used measures for inferring selection are increased linkage disequilibrium between loci (Hudson *et al.* 1994; Huttley *et al.* 2000; Kohn *et al.* 2000; Wootton *et al.* 2002), reduced polymorphism (Nurminsky *et al.* 2001; Harr *et al.* 2002; Kim and Stephan 2002; Schlötterer 2002; Wootton *et al.* 2002) or a skewed allele frequency spectrum at individual loci (Braverman *et al.* 1995; Payseur *et al.* 2002; Vigouroux *et al.* 2002).

Here we report a genome scan in *D. melanogaster*, specifically designed to identify genomic regions involved in adaptation to novel habitats. *D. melanogaster* originated in sub-Saharan Africa and colonized the rest of the world only ~10,000 years ago (David and Capy 1988). Previous studies have suggested that this habitat expansion involved the spread of beneficial mutations in non-African populations (Begun and Aquadro 1993; Kirby and Stephan 1996; Kauer *et al.* 2002) and furthermore African and non-African populations may also differ because of recent selection pressure imposed by man such as insecticide resistance (Daborn *et al.* 2001). By comparing putative ancestral African populations and derived European populations, we aimed to identify

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genomic regions in *D. melanogaster* in which an allelic variant had been selected during/after the "out of Africa" colonization. In total, variability at 205 microsatellites was studied on the third and the *X* chromosome.

MATERIALS AND METHODS

Microsatellites: We surveyed 102 *X* chromosomal microsatellite loci and 103 microsatellites located on the third chromosome. For most loci, primers were designed using sequences available from the Drosophila genome project or the Drosophila whole-genome shotgun sequence (releases 1 and 2, http://flybase.bio.indiana.edu/). Microsatellites that were cloned in our lab were, like the above sequences, obtained from non-African flies. Only loci with an uninterrupted repeat structure longer than eight repeat units were chosen for primer design. All loci were typed in two African populations from Zimbabwe and various European populations. The main set of loci was typed without prior evidence for selection. After the first screen additional loci were genotyped in candidate regions for selection. A full list of the loci, populations, and basic statistics is available as online supplementary material (Table S1 at http://www.genetics.org/supplemental/). Loci that were also typed in the previous study of Harr *et al.* (2002) are indicated in Table S1. Primer sequences, annealing temperatures, repeat motifs, and cytological positions are available from the authors' web page (http://i122server.vu-wien.ac.at/). Microsatellite analysis followed standard protocols (Schlötterer and Zangerl 1999).

Fly strains: Zimbabwe flies were sampled from two locations, Sengwa Wildlife Reserve (ZS) and Harare, the capital of Zimbabwe (ZH), and were kindly provided by C. F. Aquadro and C.-I Wu. In previous studies we found different African populations, mainly from Kenya, to be very similar in variability levels to the populations from Zimbabwe (Harr *et al.* 2002; Kauer *et al.* 2002). Population structure is weak ($F_{ST} < 0.025$) and all populations share very high variability levels. The two Zimbabwean populations should, therefore, provide a reasonable sample for the ancestral population. To take into account inbreeding in these isofemale lines we calculated heterozygosity and variance in repeat number by averaging over 200 random data sets. In each data set, one allele was discarded from all inbred individuals. This procedure is implemented in the Microsatellite-Analyzer (MSA) software (Dieringer and Schlötterer 2003). European flies were from Poland (Katovice, 2000; collected by J. Gorczyca), Germany (Friedrichshafen, 1998 and Neustadt/Mannheim, 2000; collected by B. Harr, M. Kauer, and B. Zapfel, respectively), Switzerland (Nyon and Gotheron, 1998; collected by J. David), Russia (Moscow, 1998; collected by J. David), Austria (Vienna1, 1999 and Vienna2, 2000; collected by B. Harr and C. Schlötterer, respectively), Italy (Naples, 2001 and Rome, 1998; collected by C. Schlötterer), France (Prunay, 1998; collected by J. David), The Netherlands (Texel, 1999; collected by D. Slezak), and Denmark (Copenhagen, 1999; provided by V. Loeschke).

For each European population, 30 F_1 individuals were used. For the African populations a minimum of 20 individuals were typed for each locus.

Variability measures: Two measures of microsatellite variability were used: variance in repeat number (Goldstein and Clark 1995) and expected heterozygosity or gene diversity (Nei 1978). Both measures were corrected for small sample sizes by multiplying by $n/(n-1)$, where n is the number of chromosomes that were analyzed. For monomorphic loci, we assumed that one additional allele differed from the others (to avoid division by zero in the calculation of the ratio of European to African variabilities—see below); for variance in repeat number, we assumed that this allele differs from the other alleles by one mutation step (*e.g.*, 2 bp for dinucleotide repeats).

Measures to detect positive selection: Reductions in variability below neutral expectations at individual loci can be indicative of positive selection (Lewontin and Krakauer 1973; Maynard Smith and Haigh 1974; Galtier *et al.* 2000; Kim and Stephan 2002; Schlötterer 2002). We were interested in detecting such reductions in variability in non-African *D. melanogaster* populations, as these reductions may form the footprint of a selective sweep associated with the out of Africa habitat expansion. We used the ln RV and ln RH statistic to search for loci with levels of variability below neutral expectations. The test statistics consider the joint empirical distribution of all loci and identify loci that differ significantly in variability from the remainder of the genome. For each locus, the ratio of the genetic variabilities of two populations is calculated (Schlötterer 2002). Thus all loci have the same expectation irrespective of locus-specific mutation rates. Computer simulations indicate that, if the data do not contain a large number of invariant loci, the test statistics are relatively insensitive to demographic events such as bottlenecks, as demography affects all loci to a similar extent (Schlötterer 2002; C. Schlötterer and D. Dieringer, unpublished results).

The variance-based ln RV is calculated as

$$\ln[E(\mathrm{RV})] = \ln\left[E\left(\frac{\tfrac{1}{2}\theta_{\mathrm{Pop1}}}{\tfrac{1}{2}\theta_{\mathrm{Pop2}}}\right)\right] = \ln\left[E\left(\frac{(2N_{e_{\mathrm{Pop1}}}\mu)}{(2N_{e_{\mathrm{Pop2}}}\mu)}\right)\right] \cong \ln\left[\frac{E(V_{\mathrm{Pop1}})}{E(V_{\mathrm{Pop2}})}\right] \quad (1)$$

with $V = \theta/2$ (Moran 1975), where $\theta = 4N_e\mu$, N_e is the effective population size, and μ is the mutation rate. The corresponding equation for gene diversity is

$$\ln[E(\mathrm{RH})] = \ln\left[E\left(\frac{\theta_{\mathrm{Pop1}}}{\theta_{\mathrm{Pop2}}}\right)\right] = \ln\left[E\left(\frac{((1/1-H_{\mathrm{Pop1}})^2-1)1/8\mu}{((1/1-H_{\mathrm{Pop2}})^2-1)1/8\mu}\right)\right] \cong \ln\left[\frac{E((1/1-H_{\mathrm{Pop1}})^2-1)}{E((1/1-H_{\mathrm{Pop2}})^2-1)}\right], \quad (2)$$

where H is related to θ by the formula $H = 1 - (1/(1+2\theta)^{1/2})$ (Ohta and Kimura 1973).

For the remainder of the text, we use ln $R\theta$ for both ln RV and ln RH. Computer simulations indicate that under neutrality ln RV and ln RH values follow a Gaussian distribution (Schlötterer 2002; C. Schlötterer and D. Dieringer, unpublished results). Note that this assumption also holds when the number of loci used is smaller (*i.e.*, 120) than that of the original article (Schlötterer 2002; data not shown). Furthermore, we used computer simulations to verify that the assumption of normality for the ln $R\theta$ test statistic also holds when an ancestral and a recently derived population are compared (see web supplement, Table S2 at http://www.genetics.org/supplemental/). Given that ln $R\theta$ values are approximately normally distributed, the probability that a given locus deviates from neutrality can be obtained from the density function of a standard normal distribution. Hence, the observed ln $R\theta$ values need to be standardized by the mean and standard deviation of ln $R\theta$ values from putatively neutrally evolving loci typed in the same populations. The standardized distribution of ln $R\theta$ has therefore a mean of zero and a standard deviation of one. After standardization, 95% of the loci are expected to have values between 1.96 and −1.96. Those loci for which ln $R\theta$ values fall outside of this interval are considered as putatively selected loci. Coalescence-based

computer simulations (C. Schlötterer and D. Dieringer, unpublished results) demonstrate a higher power for ln RH than for ln RV to detect selected loci, as ln RH has a smaller variance than ln RV. On the basis of the simulations, we mainly used the ln RH test statistic for the inference of positive selection. C. Schlötterer and D. Dieringer (unpublished results) also noted that the type I error can be reduced two- to threefold when both test statistics, ln RH and ln RV, are considered jointly (*i.e.*, when the test is significant for both ln RV and ln RH).

We applied two methods to adjust significance levels of ln $R\theta$ for multiple testing, Bonferroni correction, and the combination of ln RV and ln RH (see above). While both methods are certainly valid for ruling out false positives, they are extremely conservative. The goal of this study, however, was to provide candidate loci for positive selection that deserve a more detailed analysis, and we therefore report all significant loci.

Identification of out of Africa sweeps: The main goal of this study was the identification of loci that show strongly reduced variability in European populations. To ensure the identification of a putative selective sweep associated with the habitat expansion of *D. melanogaster*, rather than local adaptation of a European population, we analyzed multiple European populations. For each locus, we took the arithmetic mean of variabilities over all populations in the two groups (European and African populations). The test statistics ln RV and ln RH are based on these averages. As we focused on positive selection in European populations, we concentrated on loci with significantly reduced variability.

To determine significance levels for the reduction of variability at individual loci, the empirical distribution of ln $R\theta$ values has to be standardized (see above). When most of the loci evolve neutrally and only a small number of loci are subject to directional selection, the mean and the standard deviation of the empirical ln $R\theta$ distribution can be used and the selected loci should fall into the lower tail of the distribution. When a substantial fraction of the analyzed loci have been affected by directional selection in the same population, this procedure is problematic because the whole distribution would be shifted to negative values and therefore only loci with the most extreme ln $R\theta$ values would fall into the lower tail of the distribution. Alternatively, a set of neutrally evolving loci could be used for standardizing. In this study we found the distribution of ln $R\theta$ values on the *X* chromosome to be shifted to negative values (see results). Previous studies also suggested that *X* chromosomal loci may be influenced by selection more than autosomal ones (Andolfatto 2001b; Kauer *et al.* 2002). While the shift toward negative values of the *X* chromosomal ln $R\theta$ distribution could be also caused by demographic events (see discussion), we used two approaches to standardize the ln RV and ln RH distributions. First we standardized both chromosomal distributions by their own mean and standard deviation (standardization procedure 1). With this treatment demographic factors such as a bottleneck or differential reproductive success of males and females (Caballero 1994), which could potentially affect *X* and autosomes to a different extent (Wall *et al.* 2002), cannot bias the results. To account for the possibility that the *X* chromosome might have been more affected by selection than the autosome was, we also standardized the *X* chromosome with the mean and standard deviation of ln $R\theta$ of the third chromosome (standardization procedure 2). This second procedure, which *a priori* assumes more selection on the *X* chromosome, is not appropriate if the two types of chromosomes have been differentially affected by demographic events (*i.e.*, a bottleneck and/or differential reproductive success of males and females). Thus, the two methods of standardizing therefore provide a conservative and a nonconservative estimate of the number of candidate loci.

Using an analytical approach, we estimated whether demographic events could theoretically explain the difference between *X* and autosomal variation. Furthermore, we used coalescent simulations to estimate the influence of standardization procedure 2 on the number of false positives.

Analytical estimation of the relative variabilities of *X* chromosomes and autosomes: Ignoring new mutations, the genetic variability at time point T (θ_T) can be expressed as a function of the variability level at time point 0 in the past (θ_0), the new effective population size (N_e), which is assumed to remain constant, and the time (t) that elapsed between time points 0 and T:

$$\theta_T = \theta_0 \exp\frac{-t}{2N_e}. \quad (3)$$

This equation can be used to estimate the relative loss of variability on *X* chromosomes and autosomes after a bottleneck by taking into account the difference of (N_e) between the chromosomes. The population is not assumed to be in equilibrium, so that θ_0 is arbitrary.

Solving (3) for ($-t/2N_e$) yields

$$\frac{-t}{2N_e} = \ln\frac{\theta_T}{\theta_0} = \ln \text{RH}. \quad (4)$$

From (4) it follows that the expected ratio of ln θ_T/θ_0 on the *X* compared to an autosome is given by

$$\frac{(-t/2N_e)_X}{(-t/2N_e)_A} \approx \frac{(N_e)_A}{(N_e)_X} = k. \quad (5)$$

In Equation 5 t cancels out. Hence the relative loss of variability for *X* chromosomes and autosomes between time points 0 and T depends only on the ratio of the effective population sizes. For the same distribution of reproductive success for the two sexes the expectation is 1.33 irrespective of the time of the bottleneck (due to the absence of new mutations). Equations 4 and 5 offer the advantage that the loss of variability due to a bottleneck can be approximated by the ln $R\theta$ test statistic, which is easily obtained from experimental data. Thus, the ratio of ln $R\theta$ of the autosomes and *X* chromosomes is conservatively estimated by Equation 5.

The expected ratio of the effective population sizes of the chromosomes for a discrete-generation model can be calculated as

$$k = \frac{N_A}{N_X} = \frac{8(N_{ef} + 2N_{em})}{9(N_{ef} + N_{em})}, \quad (6)$$

where N_{ef} and N_{em} are the effective population sizes of females and males, respectively (Caballero 1994). This ratio in Equation 6 is bounded between 0.889 and 1.778 and equals 1.33 if $N_{ef} = N_{em}$.

Coalescent simulations based on population bottlenecks and differential effective population sizes of chromosomes: We used computer simulations to evaluate the consequences of various demographic scenarios. In a first set of simulations we assumed a constant ancestral effective population size of $N_e = 10^6$ for autosomes and 0.75×10^6 for *X* chromosomes. At time point t, a bottleneck instantaneously reduced the population size by a factor f. After the bottleneck the population increases exponentially in size until it reaches the current population size of 10^6 for autosomes and 0.75×10^6 for *X* chromosomes. The microsatellite mutation rate was set to $\mu = 5 \times 10^{-6}$ (Schug *et al.* 1998a; Harr and Schlötterer 2000; Vazquez *et al.* 2000). The time point t of the bottleneck was scaled by $4N_e$, which differed for autosomal and *X* chromo-

TABLE 1

Mean microsatellite variabilities in European and African populations

	Heterozygosity		Variance in repeat no.	
	Europe	Africa	Europe	Africa
X chromosome	0.51 (0.2)	0.81 (0.13)	12.34 (18.96)	26.65 (42.67)
Chromosome 3	0.53 (0.18)	0.71 (0.14)	3.46 (4.55)	5.6 (8.58)

Standard deviations are shown in parentheses.

somal loci. To account for this we multiplied t for the X chromosomal simulations by the factor 1.33, which corresponds to the ratio of autosomal to X chromosomal population sizes assuming equal distributions of reproductive success for the two sexes. In a second set of simulations, we assumed that X chromosomes have a higher variability level ($\theta = 4N_e\mu$) than autosomes in the ancestral populations while the distribution of reproductive success was assumed to be the same for the two sexes after the population size reduction. Finally, we simulated scenarios where more variability is present on the X chromosomes in the ancestral population but the effective population size for females is lower than that of males after the bottleneck. These scenarios were simulated only for those variability levels that were most similar to the ones observed in the empirical data set (*i.e.*, $\theta_X = 3\theta_A$ in the ancestral population). Summary statistics for all simulations are shown in Table S3 at http://www.genetics.org/supplemental/. Coalescent simulations were performed with a modification of the Makesamples software (Hudson 2002), which incorporates a stepwise microsatellite mutation model (Ohta and Kimura 1973; T. Wiehe, unpublished results). The number of mutations occurring on a branch was converted into microsatellite mutations by adding or removing (with equal probability) one repeat unit for each mutation. To calculate ln RH, one set of data was generated using the ancestral settings without demography and one data set was generated with demography. Monomorphic loci were treated identically to experimental loci with no variability.

Coalescent simulations for evaluating the influence of nonstepwise mutations on ln RH and ln RV: We relied on a commonly used coalescent-based computer simulation algorithm (Hudson 1990), modified to take into account the stepwise mutation behavior of microsatellites (see above). In addition to stepwise changes in repeat number, we also simulated insertions/deletions (indels) occurring in the flanking sequence. The indel size for most simulations was taken from a uniform distribution between 1 and 20 repeat units; for a subset of simulations the indel size was taken from a uniform distribution between 1 and 10 repeat units. In our simulations we allowed for different mutation rates for microsatellites (slippage) and indels. We simulated different frequencies of nonstepwise mutations and also different maximum step sizes. In each simulation run, 1 locus out of 100 was subjected to directional selection, and 1000 replicas were simulated for each parameter combination. Selection was simulated as an instantaneous reduction in the population size. All simulation runs assumed a selective sweep, which occurred $0.05 \times 2N_e$ generations ago and reduced variability by a factor of 0.01. Summary statistics for all simulations are shown in Table S4 at http://www.genetics.org/supplemental/.

Allele excess: Allele excess was determined with the Bottleneck program (Cornuet and Luikart 1996). Bottleneck provides P values for single loci and also deviations from the strict stepwise mutation model (SMM) can be included [two-phase model (TPM)]. Note that the program Bottleneck calculates "heterozygote deficiency" as a measure of allele excess. Here, however, we use the term "allele excess" as a synonym for heterozygote deficiency.

Genetic distance and F_{ST}: Genetic distances (defined as 1 − proportion of shared alleles) and unbiased estimators of F_{ST} (Weir and Cockerham 1984) between populations were calculated with the software Microsatellite-Analyzer (Dieringer and Schlötterer 2003). Significance levels for F_{ST} values were calculated by permuting genotypes among populations (10,000 times) and were corrected for multiple tests (Sokal and Rohlf 1995).

Recombination rates: Recombination rates (in percentage of recombination per kilobase and generation) of genomic sequence and generation were calculated as outlined in Comeron *et al.* (1999) with a program kindly provided by J. M. Comeron. We did not include loci with recombination rates <0.0001% recombination per kilobase after adjusting for zero recombination in males (*i.e.*, multiplying by 0.67 for the X chromosome and by 0.5 for the third chromosome). The rationale for this was that, in genomic regions with low recombination rates, hitchhiking events affect very large regions, thus making the identification of the target of selection impossible (Schlötterer and Wiehe 1999). This selection criterion mainly excluded centromeric and telomeric regions.

RESULTS

Consistent with previous reports (Begun and Aquadro 1993; Andolfatto 2001b; Kauer *et al.* 2002), African flies were more variable than European ones (Table 1). Mean microsatellite variabilities were higher than recently reported (Kauer *et al.* 2002). Furthermore, no correlation between recombination rate and microsatellite variability was detected, irrespective of whether chromosomes were analyzed separately or jointly (data not shown). The discrepancy between the data reported here and our previous report (Kauer *et al.* 2002) can be attributed to the lack of microsatellites located in genomic regions with low recombination rates in this study (see materials and methods). The correlation of recombination rate and microsatellite variability that was recently found by Kauer *et al.* (2002) was mainly due to very low levels of variability in regions of very low recombination rate.

In our analysis, we averaged microsatellite variabilities across populations. As the set of populations analyzed differed among loci, this could have biased our analysis. Consistent with previous reports (Begun and Aquadro 1993; Caracristi and Schlötterer 2003), differentia-

TABLE 2

Genetic differentiation between populations on the *X* chromosome

D/F_{ST}[a]	Rome	Friedrichshafen	Gotheron	Nyon	Copenhagen	ZH	ZS
Rome		0.04	0.03	0.04	0.06	0.23	0.25
Friedrichshafen	0.22		0.04	0.05	0.07	0.23	0.24
Gotheron	0.21	0.22		0.04	0.07	0.23	0.24
Nyon	0.20	0.21	0.19		0.04	0.24	0.25
Copenhagen	0.22	0.25	0.25	0.22		0.24	0.25
ZH	0.65	0.66	0.66	0.67	0.67		−0.001
ZS	0.67	0.67	0.67	0.68	0.68	0.42	

Country origin of populations: Denmark (Copenhagen), France (Gotheron), Germany (Friedrichshafen), Italy (Rome), Switzerland (Nyon), Zimbabwe (ZH/Harare and ZS/Sengwa).

[a] Above diagonal, F_{ST} values (all values are significant, $P < 0.01$); below diagonal, genetic distance (1 − proportion of shared alleles).

tion among European populations was much lower than that between European and African populations (Tables 2 and 3). *X* chromosomal loci were more differentiated than loci on chromosome 3 between European and African populations. This difference cannot be attributed to the choice of populations, as different European populations gave similar results for loci located on the same chromosome (Tables 2 and 3). Variability levels were also very similar among European populations.

Influence of nonstepwise mutations and indel polymorphisms on ln RH and ln RV: Before applying the ln $R\theta$ test statistics to our data we wanted to examine a critical aspect of the two test statistics used, ln RH and ln RV: their robustness to deviations from the strict stepwise mutation model as described by Ohta and Kimura (1973). C. Schlötterer and D. Dieringer (unpublished results) showed that ln RH and ln RV are not perfectly correlated when a strict stepwise mutation model is assumed. The correlation between ln RH and ln RV in our data is lower than that found by C. Schlötterer and D. Dieringer (unpublished results) for a strict stepwise mutation model (*X* chromosome, $r = 0.59$; chromosome 3, $r = 0.46$; simulation under SSM, $r \approx 0.7$–0.8, $P < 0.01$, Spearman rank correlation), suggesting some deviation from the strict stepwise mutation model. In simulations that allow for some mutations of multiple microsatellite repeat units (TPM; Di Rienzo *et al.* 1994), the correlation between ln RH and ln RV is lower than that for SSM. Thus, a two-phase microsatellite mutation model may be sufficient to explain the low correlation between ln RH and ln RV in our data. On the other hand indel polymorphisms in the flanking sequence of the microsatellite could also have a similar effect. Such indel polymorphisms are frequent in Drosophila (Colson and Goldstein 1999), so we performed computer simulations to estimate the influence of indels in the flanking sequence of a microsatellite. Consistent with the results of C. Schlötterer and D. Dieringer (unpublished results) for the two-phase model, we found that ln RH is quite insensitive to indel polymorphisms in the flanking sequence, whereas the power of ln RV drops (Table S4 at http://www.genetics.

TABLE 3

Genetic differentiation between populations on chromosome 3

D/F_{ST}[a]	Moscow	Texel	Prunay	Vienna1	Katovice	Naples	Neustadt	Vienna2	ZH	ZS
Moscow		0.05	0.03	0.08	—	—	—	—	0.12	0.16
Texel	0.27		0.04	0.08	—	—	—	—	0.12	0.16
Prunay	0.22	0.24		0.07	—	—	—	—	0.13	0.17
Vienna1	0.26	0.30	0.25		0.00	0.01	0.02	—	0.11	0.17
Katovice	—	—	—	0.21		0.04	0.02	0.01	0.14	0.18
Naples	—	—	—	0.28	0.18		0.01	0.04	0.12	0.17
Neustadt	—	—	—	0.35	0.16	0.15			0.14	0.16
Vienna2	—	—	—	—	0.17	0.21			0.11	0.17
ZH	0.47	0.48	0.50	0.46	0.42	0.39	0.40	0.40		0.01
ZS	0.51	0.55	0.55	0.53	0.48	0.45	0.42	0.48	0.28	

Country origin of populations: Austria (Vienna1 and Vienna2), France (Prunay), Germany (Neustadt), Italy (Naples), Netherlands (Texel), Poland (Katovice), Russia (Moscow), Zimbabwe (ZH/Harare and ZS/Sengwa).

[a] Above diagonal, F_{ST} values (all values are significant, $P < 0.01$); below diagonal, genetic distance (1 − proportion of shared alleles).

org/supplemental/). Interestingly, the indel mutation rate had almost no effect on the power of both ln RV and ln RH. However, the power of ln RV decreased with an increasing mean indel size (Table S4). The lower power of the ln RV test statistic is the outcome of an increased variance of ln RV values among loci.

Indel mutations become even more problematic when only a small number of loci are affected. As loci with indel mutations have a higher variance in ln RV, they are more frequently located in the tails of the distribution when analyzed jointly with loci varying only in microsatellite repeat number. Hence, indels in the flanking sequence not only reduce the power of ln RV to detect selective sweeps, but also increase the type I error rate. Therefore we relied mainly on ln RH for the identification of candidate loci for positive selection.

Identification of candidate loci: Consistent with previous computer simulations (Schlötterer 2002; C. Schlötterer and D. Dieringer, unpublished results) we found that ln RV, and especially ln RH values, were approximately normally distributed. Nonstandardized ln $R\theta$ values show more negative values on the *X* than on the third chromosome (mean/SD of ln RH, *X*, $-2.37/1.37$; third chromosome, $-1.18/0.94$), indicating a larger loss of variability on the *X* chromosome than on the third chromosome (Kauer *et al.* 2002). Furthermore the *X* chromosomal distribution is broader, as indicated by the higher standard deviation (Figure 1).

As outlined in material and methods, we pursued two different approaches to identify candidate loci for positive selection. In standardization procedure 1 we treated each chromosome separately and standardized the ln $R\theta$ values by the mean and standard deviation from all loci mapping to the same chromosome. Using this approach, we identified a conservative set of candidate loci. In standardization procedure 2, the statistical significance of the ln $R\theta$ values of individual loci on the *X* chromosome was determined by standardizing with the distribution of ln $R\theta$ of the third chromosome. In the absence of demographic events, this procedure is not expected to bias the results and may be even favorable when a larger number of selective sweeps is expected on the *X* chromosome. Demographic events, however, may lead to a more pronounced loss in variability at *X*-linked loci, so that a larger number of false positives may be obtained.

Standardization procedure 1: When both chromosomes were standardized with their own distribution of ln RH and ln RV, seven loci on the *X* chromosome and eight loci on the third chromosome showed a significant reduction in variability by either ln RH or ln RV or both (Table 4, Figure 1). Using ln RH, four loci were located in the lower tail and two loci in the upper tail of the *X* chromosomal distribution. On the third chromosome, seven significant loci were identified, five of which were in the lower tail of the distribution. The ratios of significant loci in the lower and the upper tail of the ln RV distribution were 4:2 on the *X* chromosome and 3:1 for chromosome 3. No difference in the power of the two test statistics was observed and only one locus on the *X* chromosome was significant for both ln RH *and* ln RV tests. After adjusting the α-value for multiple testing by Bonferroni correction (*i.e.*, ln RH and ln RV < -3.67, Table 4) none of the loci in this set remained significant.

Standardization procedure 2 for the X chromosome: A total of 30 loci on the *X* were identified by either ln RH or ln RV using this method (Table 4, Figure 1). Because of the larger reduction of variability on the *X* chromosome, all 28 loci identified with ln RH were located in the lower tail and none in the upper tail of the third chromosomal ln RH distribution. Using ln RV 10 loci were found in the lower tail and 4 loci in the upper tail of the distribution. Eight loci identified with ln RH remained significant after Bonferroni correction.

Considering that deviations from the strict stepwise mutation model have a strong impact on the ln RV test statistic, and given that flanking sequence indels occur frequently in Drosophila (Colson and Goldstein 1999), we considered only those loci with a significant ln RV as candidates for a selective sweep when ln RH also indicated a loss of variability. On the basis of this criterion we rejected two loci on the third chromosome (3L16575599gt and 3R5316419ta) as false positives. Both loci show large allele gaps in the African but not in the European population. In the absence of indel polymorphisms, the repeat number at any microsatellite allele can be determined by subtracting the flanking sequence length (obtained from the published genomic sequence of *D. melanogaster*) from the PCR product length. For these two loci we inferred a microsatellite length of 21 and 51 repeats. Given that long microsatellite alleles are rare in *D. melanogaster* (Schug *et al.* 1998b; Bachtrog *et al.* 1999; Harr and Schlötterer 2000), we regard it as likely that an insertion in the flanking sequence has occurred. For three other loci (X3642495gct, 66-95-3, and 3L2299865), which were significant by the ln RV test statistic, we also observed a loss of variability with ln RH (*i.e.*, ln RH < -1.48, Table 4). Despite the relatively weak support for these loci we included them as putative candidates for a selective sweep associated with the out of Africa habitat expansion of *D. melanogaster* to avoid type 2 error. As mentioned above the remaining eight loci were significant on both test statistics.

All candidate loci are shown in Figure 1, where the confidence limits for both standardization procedures are also drawn. Visual inspection suggests no obvious spatial clustering of significant loci on the third chromosome and of the nonconservative *X* chromosomal set. Three of the four significant *X* chromosomal loci (based on the conservative standardization procedure 1) are located in relatively close proximity to each other.

As microsatellite mutation rates are dependent on repeat length (Harr and Schlötterer 2000; Schlötterer 2000), an important prerequisite for the applica-

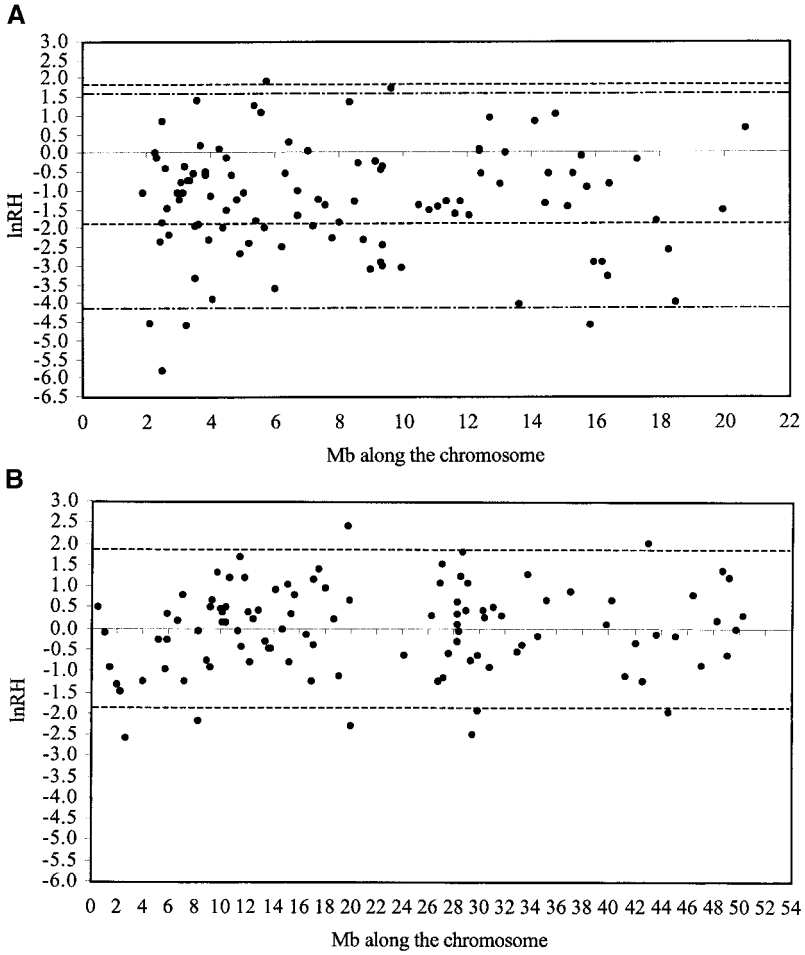

FIGURE 1.—Plot of ln RH along chromosomes. For all loci ln RH values are shown. (A) *X* chromosome. (B) Chromosome 3. Loci are aligned according to their chromosomal position; dashed lines indicate the limits of the 95% confidence interval (−1.96, +1.96) for standardization with loci from the third chromosome. The dashed-dotted lines in A indicate the 95% confidence interval for the standardization with *X* chromosomal loci.

tion of the ln RH and ln RV test statistic is that the repeat number does not differ between African and European *D. melanogaster* populations. For the candidate loci, the inferred mean repeat number of European alleles was not significantly different from the mean repeat number in the African populations ($P > 0.8$, sign test). Furthermore, the mean repeat length in European populations did not differ between the candidate loci and the others ($P > 0.2$, Mann-Whitney *U*-test).

Allele excess and genetic distance of candidate loci: An excess of rare alleles is often taken as evidence for selection (TAJIMA 1989; BRAVERMAN *et al.* 1995; PAYSEUR *et al.* 2002; VIGOUROUX *et al.* 2002). Computer simulations indicate that microsatellite loci with low gene diversity are biased toward an excess of rare alleles even under neutrality (C. SCHLÖTTERER and M. O. KAUER, unpublished results). Thus, we did not consider this test statistic to identify candidate loci, but compared it to our set of candidate loci on the basis of ln RH and ln RV results. Assuming a single SSM we found 12 loci with a significant excess of alleles. Eight of these loci were included in the nonconservative set of candidate loci and 1 in the conservative set. The mean allele excess of the nonconservative candidate loci is significantly higher than that for the rest of the *X* chromosomal loci ($P < 10^{-3}$ for SSM and $P < 10^{-4}$ for TPM, Mann-Whitney *U*-test). The same trend can be seen on the third chromosome, but power is very low because there are only 6 candidate loci ($P = 0.086$ for SSM and $P = 0.028$ for TPM, Mann-Whitney *U*-test).

A selective sweep removes allelic variation around a selected site. Thus the genetic distance between a selected and neutrally evolving population is increased at a locus affected by a selective sweep. As absolute genetic distances were found to be superior to relative measures of genetic distance (*e.g.*, F_{ST}) for the comparison of variability in selected and neutrally evolving regions (CHARLESWORTH 1998), we used the proportion of shared alleles as the genetic distance measurement. Mean genetic distances between European and African populations were higher for the nonconservative set of candidate loci than for the rest of the loci, but the difference is not statistically significant on the *X* chromosome (chromosome 3, $P = 0.008$; *X* chromosome, $P = 0.29$; Mann-Whitney *U*-test).

Analysis of the genomic region flanking a candidate locus: This study's purpose was to identify regions in the genome of *D. melanogaster* that are reasonable candidates for a thorough examination of their adaptive value in European populations. In the analysis above we pre-

TABLE 4

Candidate loci for positive selection

Locus[a]	Mb	Arm	Band	ln RH	ln RV	Heterozygosity[b] Africa	Heterozygosity[b] Europe	Variance in repeat no.[b] Africa	Variance in repeat no.[b] Europe	Allele excess (bottleneck)[b,d] TPM	Allele excess (bottleneck)[b,d] SSM	Genetic distance[b]: *D*
						X chromosome						
P3B02gt[c,e,f]	*2.51*	*X*	*3c*	*−5.81*	*−6.34*	*0.85*	*0.03*	*22.11*	*0.02*	Monomorphic	Monomorphic	*0.68*
X3219363gt[c,e]	*3.22*	*X*	*3d*	*−4.60*	*−2.24*	*0.94*	*0.31*	*30.98*	*1.61*	*−5.74****	*−8.48****	*0.81*
X15830711gt[c,e]	15.83	*X*	14a	−4.57	−0.54	0.92	0.24	153.69	46.75	−1.24	−1.84	0.76
X2102441ct[c,e]	2.10	*X*	2f	−4.56	−1.77	0.97	0.55	25.15	2.14	−0.07	−0.57	0.70
X13624957[c,f]	*13.62*	*X*	*12c*	*−4.02*	*−3.69*	*0.83*	*0.10*	*3.91*	*0.05*	*−0.47*	*−0.52*	*1.00*
X18472039ca[c,f]	*18.47*	*X*	*17d*	*−4.01*	*−3.91*	*0.76*	*0.06*	*8.33*	*0.08*	*−1.38*	*−1.55*	*0.51*
X4071888gt	4.07	*X*	4b	−3.88	−1.44	0.92	0.33	43.41	5.20	−3.53	−5.61*	0.68
X5973753gt	5.97	*X*	5d	−3.63	−1.80	0.94	0.51	32.25	2.65	−2.08	−3.71*	0.70
X3516772ga	*3.52*	*X*	*3f*	*−3.34*	*−2.37*	*0.92*	*0.43*	*8.40*	*0.38*	*−0.53*	*−1.03*	*0.67*
DS09020	16.39	*X*	15a	−3.28	−1.68	0.91	0.42	7.49	0.70	−1.57	−2.67*	0.81
X8956947gt	8.96	*X*	8d	−3.09	−1.54	0.96	0.70	69.87	7.58	−0.29	−1.43	0.83
X9928573gt	*9.93*	*X*	*9b*	*−3.06*	*−2.99*	*0.88*	*0.33*	*25.71*	*0.62*	*−1.51*	*−2.35*	*0.72*
P08E01gt	9.33	*X*	8e	−3.02	−0.65	0.83	0.22	6.80	1.85	−2.75*	−3.85*	0.52
X16203512gt	16.20	*X*	14d	−2.93	0.48	0.75	0.13	1.11	0.97	−1.46	−1.86	0.82
X9312943	9.31	*X*	8e	−2.93	0.26	0.87	0.34	3.89	2.72	−2.65*	−3.76*	0.95
X15959225ca	15.96	*X*	14c	−2.91	−1.07	0.75	0.13	4.73	0.83	−0.48	−0.61	0.64
X4944599ca	4.94	*X*	4d	−2.68	−1.63	0.91	0.50	11.51	1.13	−0.04	−0.44	0.65
X18283112ta	18.28	*X*	17c	−2.59	1.36	0.92	0.57	8.40	18.36	−0.18	−0.81	0.76
X6213328ca	6.21	*X*	5f	−2.51	−0.11	0.91	0.54	14.96	7.16	−2.45*	−4.21*	0.69
X9325355	9.33	*X*	8e	−2.43	−1.81	0.81	0.26	6.10	0.50	−0.52	−0.96	0.76
X5179712gt	5.18	*X*	4f	−2.39	−0.44	0.92	0.61	18.87	6.41	−1.72	−3.19*	0.71
P3B02 atc[c,f]	*2.42*	*X*	*3b*	*−2.37*	*−3.88*	*0.68*	*0.12*	*10.09*	*0.10*	*−0.78*	*−0.86*	*0.46*
X8756567gt	8.76	*X*	8c	−2.33	−1.30	0.84	0.36	9.06	1.26	−0.61	−1.02	0.52
DS00146	*3.96*	*X*	*4b*	*−2.32*	*−2.62*	*0.87*	*0.43*	*8.07*	*0.28*	*0.03*	*−0.30*	*0.50*
X7809164ca	7.81	*X*	7d	−2.27	−1.09	0.84	0.36	6.71	1.15	−1.88	−2.74	0.69
DS06335a	2.70	*X*	3c	−2.17	−0.16	0.93	0.67	11.65	5.26	−0.74	−1.82	0.66
X4364768gt	4.36	*X*	4c	−1.99	−0.83	0.87	0.47	3.57	0.81	−0.34	−1.00	0.54
DS00589	5.67	*X*	5c	−1.98	0.96	0.88	0.52	5.87	8.50	0.09	−0.31	0.74
X3642495gct	3.64	*X*	3f	−1.90	−2.03	0.83	0.39	11.61	0.75	−0.53	−1.17	0.65
66-95-3	2.66	*X*	3c	−1.49	−1.97	0.67	0.22	4.74	0.33	−0.86	−1.22	0.43
						Chromosome 3						
3L2674504gt	2.67	3L	63A	−2.56	−0.84	0.87	0.39	6.36	1.42	−1.32	−1.88	0.59
3R5511972ca	5.51	3R	85E	−2.49	−0.27	0.67	0.10	0.54	0.22	−1.25	−1.52	0.72
3L20028475ca	20.03	3L	77a	−2.29	−1.41	0.77	0.23	6.21	0.77	−0.87	−1.40	0.56
3L8253482ca	8.25	3L	66c	−2.15	−0.71	0.78	0.25	1.79	0.46	−1.50	−2.30	0.76
3R20604755ta	20.60	3R	96b	−1.98	−1.64	0.86	0.47	10.82	1.06	−0.94	−1.60	0.79
3L16575599gt	16.58	3L	73c	−0.12	−2.73	0.84	0.70	67.35	2.13	−0.05	−0.81	0.61
3R5316419ta	5.32	3R	85d	−0.74	−2.43	0.60	0.26	8.78	0.38	−1.39	−2.04	0.26
3L/2299865	2.30	3L	12b	−1.48	−2.23	0.67	0.22	3.81	0.20	−2.26	−3.26*	0.39

[a] Ordered by ln RH; loci that are significant with ln RH *and* ln RV are italic.
[b] Averages over all European populations.
[c] ln RH or ln RV significant after Bonferroni correction.
[d] Significant values are indicated as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[e] Significant with ln RH when standardized with ln RH distribution from the *X* chromosome, *i.e.*, "conservative" candidates on the *X*.
[f] Significant with ln RV when standardized with ln RV distribution from the *X* chromosome, *i.e.*, "nonconservative" candidates on the *X*.

sented both nonconservative and conservative estimations of the number of loci that may have been affected by selection. One approach to verifying a candidate region takes advantage of the fact that a selective sweep reduces variability in the genomic region flanking the selected site (Maynard Smith and Haigh 1974; Wiehe 1998; Kim and Stephan 2002). While some variation in variability among genomic regions is expected under neutrality, a selective sweep renders neighboring sites more correlated than under neutrality (Kaplan *et al.* 1989;

Kim and Stephan 2002). Therefore determining variability levels around candidate loci could provide a tool for distinguishing between a neutral and a selection scenario (Nurminsky *et al.* 2001; Harr *et al.* 2002; Kim and Stephan 2002; Wootton *et al.* 2002). Recently, Harr *et al.* (2002) analyzed linked microsatellites in 850 kb of genomic sequence and identified three putative out of Africa sweeps. For each sweep the authors described a "valley" of reduced variability around the putative target of selection. On the basis of the results of Harr *et al.* (2002), we estimated that a genomic region of up to 100 kb is affected by a selective sweep. Thus, we include in Table S5 (http://www.genetics.org/supplemental/) all microsatellite loci that were characterized within a 100-kb region around a candidate locus. Some of the loci in these 100-kb regions were genotyped without knowledge of the selective sweep (*i.e.*, before the availability of the full genomic sequence of *D. melanogaster*), and we also specifically designed PCR primers for loci falling into the 100-kb region around candidate loci (Table 4).

Table S5 shows that some of the candidate loci fall into the same genomic region and so may indicate the same putative selective sweep. This clustering would reduce the number of independent candidate sweep regions on the *X* chromosome from 30 to 27 (nonconservative set before correction for multiple tests). Consistent with Harr *et al.* (2002) we observed several regions for which variability was significantly reduced for more than one locus (*e.g.*, regions 6, 20, 22, 23, 27, and 32 in Table S5). For some other regions we detected one significant candidate locus, which was flanked by microsatellites with reduced variability (*i.e.*, negative ln RH values) but lacking statistical significance (*e.g.*, regions 3 and 25).

Note that the regions around P3B02gt, 66-95-3, and 3L2299865gt have already been reported and analyzed in detail by Harr *et al.* (2002) but have been typed here using a different set of European populations. Interestingly, two other groups have independently inferred a putative selective sweep in the region around P3B02gt (J. Pool and C. Aquadro, personal communication; D. De Lorenzo and W. Stephan, personal communication).

A detailed discussion and a list of genes in candidate regions can be found in the web supplement accompanying this article (Tables S5 and S6; http://www.genetics.org/supplemental/).

DISCUSSION

In this microsatellite variability screen, we have found a more pronounced reduction in variability in non-African *X* chromosomes than on autosomes. This unbalanced reduction of microsatellite variability could arise from a bottleneck associated with the habitat expansion of *D. melanogaster* (Fay and Wu 1999; Wall *et al.* 2002), biased distributions of reproductive success between the sexes (Charlesworth 2001), or from multiple selective sweeps (Maynard Smith and Haigh 1974), or a combination of these. While selective sweeps affect individual sites, the first two neutral scenarios are genome-wide effects, which affect variability levels at all loci with stochastic variation among them (Hudson *et al.* 1987; Galtier *et al.* 2000; Andolfatto 2001a).

Influence of bottlenecks and skewed reproductive success: Due to the different reduction of microsatellite variability on the chromosomes, we identified very different numbers of candidate loci with our two standardization procedures. While all of the loci in the conservative set are good candidates for positive selection outside of Africa, in the nonconservative set there may be a higher number of false positives than indicated by the nominal α-value of 0.05. This number depends on the demographic scenario that was associated with the colonization of non-African habitats by *D. melanogaster*. Therefore, to evaluate whether our data could be explained under neutrality, we explored a range of demographic models analytically and with coalescence simulations.

Analytical approach: Using the analytical approach outlined in materials and methods (Equations 3–6), we estimated whether the different behavior of *X* chromosomes and autosomes could be explained by a bottleneck and/or skewed sex ratios. Because of the problematic properties of ln RV for nonstepwise mutations (see results) we relied on ln RH. Assuming no sex differences in the distribution of reproductive success outside of Africa, it follows from Equation 5 that the expectation for ln RH is identical for both chromosomes when autosomal ln RH values are multiplied by 1.33. Importantly, this expectation is independent of the relative variability levels before the bottleneck (*i.e.*, the ratio of θ_0 for the chromosomes in Africa). Therefore multiplying autosomal ln RH values by 1.33 assumes an equal distribution of reproductive success for the two sexes only outside of Africa (between time points 0 and *T*). In contrast to this expectation, we found that the mean of ln RH values for the *X* chromosome are significantly more negative than the ln RH values of autosomal loci (*X*, −2.37; *A*, −1.57; $P < 0.0001$, *t*-test). Thus, relative to the autosome the *X* chromosome lost more variability than expected. This result is not affected by the different levels of variability on *X* chromosomes and autosomes in the African population (Begun and Aquadro 1993; Andolfatto 2001b; Kauer *et al.* 2002). Nevertheless, it applies only if males and females have the same distribution in reproductive success in non-African populations.

Similarly, to estimate the influence of our standardization procedure 2, we calculated the number of significant *X* chromosomal ln RH values when standardized with the mean and the standard deviation of ln RH values from the third chromosome multiplied by 1.33. Standardizing in this way yields twice as many significant

ln RH values on the *X* chromosome (11 loci) as on the autosome (5 loci). These 11 *X* chromosomal loci are the ones with the most negative ln RH values in Table 4.

Another factor that could influence the distribution of ln RH is differential reproductive success of males and females. An effective surplus of males in Europe (Bouletreau 1978; Charlesworth 2001; between time points 0 and *T*) would reduce the effective population size of the *X* relative to the autosome, therefore changing the expectation in Equation 5. We tested the influence of skewed effective population sizes of chromosomes by assuming effective male:female ratios of 5:1 and 10:1 ($k = 1.63$ and 1.69, respectively). The ratio of chromosomal variability levels (k) was calculated using Equation 6; k was then used as the expectation in Equation 5 and the ln RH values on autosomes were corrected accordingly. After correcting for a 5-fold excess of males the mean ln RH of the *X* is still significantly more negative than that on the autosome (*X*, −2.37; *A*, −1.92; $P = 0.03$, *t*-test); after correction for a 10-fold excess of males the difference is marginally significant (*X*, −2.37; *A*, −2.00; $P = 0.08$, *t*-test).

With the analytical analyses we explored in a simple way (ignoring new mutations) the combined effect of a bottleneck and skewed sex ratios on the relative loss of variability on the *X* and the autosome outside of Africa. Assuming that different levels of variability among *X* chromosomes and autosomes are caused by different distributions of reproductive success for males and females, it has to be noted that an inverse difference must be present among African and non-African populations, as in Africa *X* chromosomes are more variable. Note that the analytical analyses implicitly assumed a bottleneck outside of Africa, because otherwise no variability would have been lost. The results from these analyses indicate that our data can be explained under certain demographic scenarios.

Coalescence simulations: Despite the fact that *D. melanogaster* microsatellite mutation rates are low (Schug *et al.* 1997; Schlötterer 2000) and non-African variation appears to be a subset of African variation (Andolfatto 2001b; Schlötterer and Harr 2002), we evaluated the impact of demographic scenarios, which included mutations after the bottleneck. As outlined in materials and methods, for standardization procedure 2 we used ln RH values from the third chromosome to standardize *X* chromosomal ln RH values. This procedure can bias the test statistic toward a higher number of nonneutral loci in non-African populations when demographic events were associated with the habitat expansion of *D. melanogaster*. To quantify this effect, we performed coalescent simulations for *X*-linked and autosomal loci under a range of demographic scenarios. Table S3 summarizes these simulations and shows the ratio of false positives in the postbottleneck population on the basis of the standardization using autosomal loci relative to the standardization using *X* chromosomal loci; a ratio of 2 would indicate that standardization method 2 (with autosomes) leads to twice as many "significant" loci as the conservative standardization method 1.

Computer simulations that assumed the same distribution of reproductive success for males and females in Africa did not result in a large excess of false positives when standardization procedure 2 was used (Table S3, 1a–1h). Nevertheless, this set of simulations failed to capture the higher *X* chromosomal variability in Africa. Therefore, for another set of simulations we assumed different θ-values for *X* chromosomes and autosomes (Table S3, 2a–4g). The best fit to the observed African variation was obtained when θ of the *X* chromosome was 2–4 times as high as θ on the autosome (Table 1, Table S3). This is not surprising as the observed mean value of θ (based on heterozygosity) of the *X* chromosome in our data is ~2.5 times the one on the autosome in Zimbabwe [Table 1, where heterozygosity (H) is related to θ by the formula $H = 1 - (1/(1 + 2\theta)^{1/2})$ (Ohta and Kimura 1973)]. For some demographic scenarios (*e.g.*, Table S3, 3g, 3c, and 4c) we found ln RH and the heterozygosity of the derived population to be very similar among simulated and experimental data (Table 1 and Table S3). Importantly, the number of false positives was strongly increased when we applied standardization procedure 2 to data sets generated under these demographic models. An aspect of the experimental data that these simulations could not reproduce is the variance of ln RH. While with a higher impact of selection on the *X* a higher variance of ln RH could be expected on the *X* chromosomes (Kauer *et al.* 2002), no difference could be noted between *X* chromosomes and autosomes under these models.

Finally, in simulations 5a–5h (Table S3) we combined a threefold excess of variation (θ) for African *X* chromosomes relative to autosomes (as for simulations 3a–3h in Table S3) with an unequal distribution of reproductive success of the two sexes in non-African populations (Table S3, 5a–5h). The effective population sizes of males and females in the postbottleneck population were set to 5:1. Three aspects could be highlighted in these simulations: (i) some parameter combinations closely matched the observed levels of variability in African and non-African chromosomes; (ii) the number of false positives increased when standardization procedure 2 was applied; and (iii) for some scenarios the variance in ln RH was increased on the *X* chromosome, although to a lesser extent than in the empirical data.

Analytical analyses and simulations indicated that the standardization of *X* chromosomal data with autosomal data may be associated with an error leading to an overestimation of the number of selected loci on the *X* chromosome. The magnitude of this error can be so large as to explain a large number of candidate loci we found on the *X* chromosome when using standardization procedure 2. The actual error that is made could,

however, be estimated only if the true demographic scenario was known. An exhaustive likelihood approach where the probability to observe the data assuming different demographic scenarios and also incorporating selection will be a worthwhile task for future analysis but is beyond the scope of this study. Another factor that our simulations may not have captured is a different mutation rate on *X* chromosome and autosome. This could explain the difference in microsatellite variability in Africa and could in principle bias the distribution of the variability reduction in non-African populations. A conservative estimation of the number of candidate loci on the *X* is given by standardization procedure 1.

Positive selection in non-African populations of *D. melanogaster*: An alternative explanation for the larger reduction of variability on the *X* chromosome is a higher impact of selection on the *X* chromosome. This could be the result of hemizygosity of the *X* chromosomes in males or the result of more beneficial alleles on the *X* chromosomes (Aquadro *et al.* 1994; Kauer *et al.* 2002). In support of a nonneutral interpretation, Kauer *et al.* (2002) found the loss of variability outside of Africa to be most pronounced in regions of low recombination rate on the *X* chromosome but not on the autosomes. The higher variance of ln RH on the *X* chromosome than on autosomes that could not be reproduced by simulations could also be attributed to more selection on the *X* chromosome, as many selected loci would provide more extreme values in the distribution of ln RH (Kauer *et al.* 2002).

Although, as noted above, an exhaustive examination of demographic scenarios remains to be done, an empirical approach to disentangle the false positives from truly selected loci could be to gather more information about all candidate loci. As presented in results, a first step in this direction is a detailed analysis of variability in the genomic region flanking the candidate loci.

A question of great interest would be to extract the rate of adaptation of *D. melanogaster* to non-African habitats from our data. This goal is difficult to address even when the effects of demography are ignored, as the power of our approach is dependent on the impact of hitchhiking. This impact can be different for the *X* chromosome and autosomes (Orr and Betancourt 2001). Thus, apart from demographic effects, it is possible that the higher number of candidate loci on the *X* chromosome may be due to a higher power to detect hitchhiking on the *X* chromosome, whereas it is likely that both chromosomes carry an equal number of beneficial mutations. Furthermore, additional, elusive parameters, such as the size of the region affected by selection, are required to calculate the fraction of loci affected by selective sweeps. Hence, to extrapolate the rate of adaptation from our data could be misleading. Finally, demography could also inflate the number of selected loci, and different chromosomes may be differently affected by this.

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