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African and North American populations of *Drosophila melanogaster* are very different at the DNA level

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UNDERSTANDING genetic evolution within species requires an accurate description of variation within and between populations and the ability to distinguish between the potential causes of an observed distribution of variation. In the cosmopolitan species *Drosophila melanogaster*, previous studies suggested that gene flow within and between continents is extensive[1] and that most of the nuclear gene variation is found within, rather than among, populations[2,3]. Here we present evidence that a population from Zimbabwe is more than twice as variable as those from the United States of America at the DNA sequence level, that most variants are not shared between the two geographic regions, and that there are nearly fixed differences between the Zimbabwe and USA samples in genomic regions experiencing low recombination rates. It appears that there is an unappreciated degree of population structure in *D. melanogaster* and that equilibrium models of molecular evolution are inappropriate for this species.

In Table 1 we present our four-cutter restriction site polymorphism data from seven X-linked gene regions in *D. melanogaster* chromosomes sampled from Zimbabwe; we also show our data and previously published data from the same genes in samples from the USA[4–7]. With the exception of *ac*, each gene is more variable in Zimbabwe than in the USA. Overall, there are more than twice as many segregating sites in Zimbabwe (167) as in the USA (77). The difference in proportion of segregating sites in the two samples is highly significant (Fisher's exact test, $P<0.0001$; this test is appropriate because the sample sizes for Zimbabwe and the USA are roughly equal) and the populations are significantly differentiated ($P<0.001$) at each of the seven genes examined[8]. Only 44 of a total of 200 polymorphic restriction sites (22%) scorable in both Zimbabwe and the USA are observed in both geographic regions. This difference is not attributable solely to rare 'private' alleles. For example, in the USA the mean frequencies of *white* and *G-6pd* locus variants present in the USA but absent from Zimbabwe are 0.16 (±0.042) and 0.23 (±0.058), respectively. The contrasting Tajima *D* values[9] (Table 1) for the two samples (especially in regions of 'normal' crossing-over) indicate that there is a tendency for Zimbabwe to harbour more low-frequency variants than the USA, providing further evidence of different evolutionary processes in these populations. But the difference in variability is not simply a consequence of the presence of rare alleles in Zimbabwe which are absent in the USA. Even for estimates of nucleotide diversity, π, which are only weakly affected by rare variants, the Zimbabwe sample is two- to sixfold more heterozygous for most loci.

Our results contrast with previous comparisons of nuclear gene variability in African and USA samples of *D. melanogaster*. Restriction site data suggested that the *y-ac-sc* and *G-6pd* loci were slightly more variable in Botswana than in the USA; however, the high levels of linkage disequilibrium and small number of bases surveyed made it difficult to assess the significance of the observation[10,11]. Levels of allozyme variation in Benin and USA samples of *D. melanogaster* (Table 2) were virtually identical and 57 of 84 allozyme variants (68%) were shared between geographic regions[2,12]. The difference in the proportion of shared variants in our restriction site data versus the allozyme data is highly significant (Fisher's exact test, $P<0.0001$; this test is conservative given the different sample sizes for the USA and Africa allozyme data). Thus, our four-cutter data are the first good evidence that there is a vast reservoir of previously unknown nuclear DNA polymorphism segregating in Africa.

Although it is thought that *D. melanogaster* left Africa to colonize temperate regions within the past several thousand years[13], earlier data did not support the hypothesis that a wholesale loss of nuclear gene variability accompanied the colonization. Our data, however, are consistent with the notion that a considerable loss of variation occurred in some populations during the history of the *D. melanogaster* lineage. The data are compatible with several hypotheses, including a partial bottleneck in the lineage(s) from which USA populations are derived and the notion that only a subset of 'ancestral' types were selectively favoured to leave Africa during the colonization process. Whatever the case, it is no longer realistic to view *D. melanogaster* populations as being near equilibrium.

Earlier interpretations of allozyme data from *D. melanogaster* attributed the few frequency differences between populations to selection on allozyme variants prevailing over extensive gene flow[1,2]. Because a large majority of observed polymorphic restriction sites in our data are located in flanking or intronic sequences, most of the differences between Zimbabwe and the USA may be explained in terms of mutation and drift rather than selection. Furthermore, our results cannot be explained solely by differences in the frequency of protein electrophoretic variants between populations. For example, even within a single electromorph class at *Pgd* (Fast), the Zimbabwe (sample size $n=49$) and USA ($n=32$) samples shared no four-cutter haplotypes and only 7 of 28 polymorphic sites (data not shown). There are at least two (not mutually exclusive) potential explanations for the contrast between the DNA data (Table 1) and the allozyme data (Table 2). First, there may be significant population structure within Africa; we note that our Zimbabwe sample has not been surveyed for allozyme variation. Second, we cannot rule out the possibility that restriction site and allozyme variation are experiencing substantially different evolutionary dynamics (such as selection on allozyme variation).

The Zimbabwe four-cutter data provide novel and qualitatively different inferences about selection from those possible from allozyme frequencies. As had been seen in a heterogeneous sample[14], nucleotide heterozygosity is positively correlated with regional rates of recombination (Fig. 1). Hitch-hiking effects of advantageous or deleterious alleles have been proposed to contribute to this pattern[14–17]. In particular, the *ac* and *su(f)* regions appear to have significantly less variation than expected under a neutral model (Table 2 legend). Although several shared polymorphic sites in Zimbabwe and the USA are found at different frequencies in the two samples, the most extreme cases

TABLE 1 Four-cutter restriction site data from *D. melanogaster* samples collected in Zimbabwe and the USA

Gene	Sites scored		Polymorphic sites			$\hat{\theta}(3N\mu)$		$\hat{\pi}$		Tajima *D*		F_{ST}	cM per band
	Zim.	USA	Zim.	USA	shared	Zim.	USA	Zim.	USA	Zim.	USA		
y	102	80	8	2	0	0.0026	0.0008	0.0017	0.0009	−0.433	0.469	0.56	<0.003
ac	68	62	2	2	1	0.0010	0.0011	0.0012	0.0011	0.789	0.260	0.54	<0.003
su(f)	171	108	6	2	0	0.0011	0.0005	0.0011	0.0002	0.338	−0.842	0.60	<0.003
Pgd	200	200	25	14	9	0.0042	0.0021	0.0018	0.0024	−1.497	0.922	0.25	0.010
G-6pd	226	175	35 (48)	16 (17)	9	0.0076	0.0030	0.0048	0.0025	−0.888	−0.009	0.30	0.033
vermilion	80	76	25	9	9	0.0118	0.0045	0.0082	0.0042	−0.804	−0.273	0.32	0.067
white	285	271	66 (73)	32 (33)	16	0.0094	0.004	0.0065	0.004	−0.714	0.136	0.28	0.093

Loci are listed in order of increasing recombination (centimorgans (cM) per band). Homozygous X-chromosome lines were made with the *FM7a* balancer. The Zimbabwe sample ($n=50$) was collected at the Sengwa Wildlife Preserve in September 1990. The USA *Pgd* ($n=53$) and *vermilion* ($n=35$) samples were from North Carolina and California, respectively. Ten four-cutter restriction enzymes were used (*AluI*, *DdeI*, *HaeIII*, *HhaI*, *HinfI*, *MspI*, *RsaI*, *Sau3AI*, *ScrFI* and *TaqI*) to make blots as previously described[20]. USA data from *y*, *ac*, *su(f)*, *G-6pd* and *white* are from a set of X-chromosomes collected from North Carolina ($n=20$) and Texas ($n=27$) as previously described[4,7]. For *y*, *ac*, *su(f)*, *G-6pd* and *white* the set of enzymes used differed slightly from the Zimbabwe sample. For the column 'Polymorphic sites', numbers not in parentheses show the sites that could be scored in both USA and Zimbabwe given the enzymes used and the genomic regions surveyed; numbers in parentheses show the total number of polymorphic sites observed. Calculation of θ, π, *D* and F_{ST} were as described[9,21,23]. $\hat{\theta}$ Is an estimate of nucleotide variability based on the proportion of polymorphic nucleotides. Assuming a neutral equilibrium model, $\hat{\theta}$ equals the parameter $3N\mu$ (for X-linked genes) where *N* and μ are the effective population size and the neutral mutation rate, respectively. $\hat{\pi}$ Is an estimate of nucleotide heterozygosity or the average pairwise difference per nucleotide between two randomly selected sequences. Tajima's *D* provides an estimate of the magnitude of the difference between the estimates of nucleotide variation provided by $\hat{\theta}$ and $\hat{\pi}$ and is expected to equal zero under a neutral equilibrium model. Tajima's *D* becomes more negative as the number of rare mutations increases and becomes more positive as the number of intermediate frequency mutations increases. F_{ST} provides an estimate of population differentiation based on the proportion of total heterozygosity found within versus between populations. We used the HKA test[24] to assess the hypothesis that Zimbabwe *ac* and *su(f)* polymorphism within *D. melanogaster* and divergence to *D. simulans*[6,7] compared to a sample of 5′ *Adh*[25] are consistent with a strictly neutral model. The HKA test is significant for both *ac* ($\chi^2=4.05$, $P<0.05$) and *su(f)* ($\chi^2=12.83$, $P<0.001$); the best interpretation is that there is less variation at *ac* and *su(f)* than expected under a neutral model of molecular evolution. This is a conservative test for reduced polymorphism at *ac* and *su(f)* because the Zimbabwe population appears to be considerably more variable than Kreitman's sample. The *ac* and *su(f)* gene regions also showed a significant reduction in variability in the USA[6,7]. cM per band was estimated by dividing the genetic distance between markers which flank the gene of interest by the number of polytene bands as determined from Bridge's maps[26]. cM per band are multiplied by 0.666 to correct for the absence of recombination in males[14]. The value for cM per band for *vermilion* is greater than in our previous report[14]. This discrepancy results from a more detailed analysis of the recombination rate in the region which revealed that *vermilion* is in a region where recombination appears to increase rapidly. We believe the value presented in this report is more accurate. cM per band for *y*, *ac* and *su(f)* are uncertain because of poor genetic and/or physical data and are probably overestimates.

of differentiation are found in regions of reduced recombination (Tables 1 and 3). The joint observations of genomic regions with significantly reduced variation within populations and nearly fixed differences between populations are best explained by independent hitch-hiking events[18]. Whether the selective histories of Zimbabwe and USA samples differ because of isolation, as opposed to varied environments, is an important, unanswered question. A potential problem for a simple hitch-hiking interpretation is the lack of skewness toward rare variants in regions of reduced crossing-over predicted by some models[19]; the 16 polymorphisms observed in *y*, *ac* and *su(f)* from Zimbabwe have less skewness than the polymorphisms located in regions of 'normal' crossing-over. Further theoretical research is necessary to explain contrasting patterns of variation in genomic regions experiencing different recombination rates.

Our data provide convincing evidence that *D. melanogaster*, as a species, is far more variable than previously thought, and that most of this variation is not segregating in the populations from which our evolutionary inferences (effective population size, frequency of lethal mutations, transposable element copy number and so on) and models are derived. It is no longer tenable to think of *D. melanogaster* as essentially panmictic for nuclear genes. Furthermore, it is no longer realistic to assume that *D. melanogaster* populations are near equilibrium or that conclusions derived from studies of USA (or similar) samples are even roughly true for the species as a whole. □

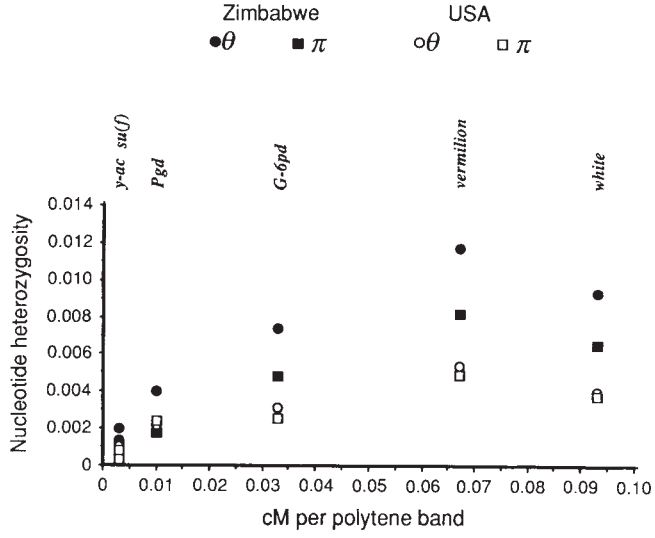

FIG. 1 Scatterplot of nucleotide heterozygosity in Zimbabwe and USA *D. melanogaster* versus cM per polytene band. Data are from Table 1.

TABLE 2 Allozyme data from USA and African samples of *D. melanogaster*

	Massachusetts (USA) ($n=30$)	Texas (USA) ($n=30$)	Benin (West Africa) ($n=28$)
Proportion polymorphic loci	0.43 (0.81)	0.36 (0.81)	0.39 (0.77)
Average number of alleles	1.65 (2.48)	1.55 (2.52)	1.66 (2.64)
Heterozygosity	0.13 (0.28)	0.12 (0.31)	0.11 (0.27)

Summary statistics from previously published allozyme surveys[2,12]. Numbers not in parentheses are from a random sample of 117 loci, and numbers in parentheses are from a sample of 26 polymorphic loci.

TABLE 3 Restriction sites in regions of reduced recombination showing large frequency differences between Zimbabwe and the USA

Gene	Site	Site frequency (+) Zimbabwe	USA
y	*HaeIII* 4444	0.00	0.79
ac	*TaqI* 36	1.00	0.15
su(f)	*DdeI* 2242	0.00	0.91

USA data and site designations are as previously described[6,7].

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Macaque V1 neurons can signal 'illusory' contours

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We describe here a new view of primary visual cortex (V1) based on measurements of neural responses in V1 to patterns called 'illusory contours' (Fig. 1*a*, *b*). Detection of an object's boundary contours is a fundamental visual task. Boundary contours are defined by discontinuities not only in luminance and colour, but also in texture[1–3], disparity[4] and motion[5–7]. Two theoretical approaches can account for illusory contour perception. The cognitive approach emphasizes top-down processes[8,9]. An alternative emphasizes bottom-up processing. This latter view is supported by (1) stimulus constraints for illusory contour perception[10–14] and (2) the discovery by von der Heydt and Peterhans[15–17] of neurons in extrastriate visual area V2 (but not in V1) of macaque monkeys that respond to illusory contours. Using stimuli different from those used previously[15,16], we found illusory contour responses in about half the neurons studied in V1 of macaque monkeys. Therefore, there are neurons as early as V1 with the computational power to detect illusory contours and to help distinguish figure from ground.

We measured responses of 25 neurons in para-foveal V1 of anaesthetized monkeys to four types of patterns sharing the same mean luminance: sinusoidal luminance gratings (Fig. 1*c*); luminance edges (Fig. 1*d*); half-screen patches of sinusoidal luminance grating, in which the bar ends define an illusory contour oriented perpendicularly to the bars and located at the boundary between the grating patch and a blank portion of the stimulus screen at the same mean luminance (Fig. 1*e*); and two half-screen patches of grating abutting one another, in which the two patches are identical and in antiphase (Fig. 1*f*). Results obtained using the pattern in Fig. 1*f* resemble those shown in Fig. 1*e* (for example, the cell in Fig. 2*a* responded similarly to both).

Because the grating is periodic, it is natural to express its position relative to receptive field location as spatial phase: the starting spatial phase angle of the sinusoidal grating relative to the stimulus frame. For example, in Fig. 1*e* the spatial phase is 0 degrees. As phase varies, the position of grating bars with respect to fixed cell receptive fields varies so that, when the illusory contour is drifted, different values of black and white are swept across a receptive field.

We found responses to illusory contours drifted across a cell's receptive field in the preferred direction for a luminance edge (that is, when grating bars defining the illusory contours were perpendicular to the optimal orientation for an edge). A variety of responses to illusory contour patterns was observed. One type is illustrated in Fig. 2*a*: this direction-selective complex cell fired two bursts per period, one to each of the 'left-handed' and 'right-handed' boundaries of the central patch in Fig. 1*e*, *f*. Comparison of responses to luminance (Fig. 1*d*) and illusory contours (Fig. 1*e*, *f*) showed that the two bursts were synchronized to the passage of the illusory contour over the cell's receptive field. The cell in Fig. 2*a* signals the presence of a contour in its receptive field independent of contrast polarity: the stimulus when the illusory contour leaves the receptive field is opposite in sign to when it enters, but the cell's response is excitatory to both entry and exit. Our measure of this frequency-doubled response is the Fourier amplitude of the second harmonic (F2) of the post-stimulus time histogram. Another type of cell responded to just one of the two contours passing over the receptive field, illustrated by the response shown in Fig. 2*b*.

If a frequency-doubled response signifies the presence of illusory contours crossing a cell's receptive field, its magnitude should not depend on the precise position (spatial phase) of the illusory contour along the long axis of the receptive field. Figure 2 shows how response changed for two cells as spatial phase varied. The simple cell in Fig. 2*b* responded to the leading edge of the one-sided pattern from 1.75π to 0.25π radians, and to the trailing edge from 0.75π to 1.25π radians. This implies that the cell was responding simply to a bright bar-end entering the receptive field or to a dark bar-end leaving it. But the response of the cell shown in Fig. 2*a* was the same at all spatial phases, implying that it responded to contour and not simply to bar-endings.

An index of response-dependence on spatial phase was derived: the greatest (pairwise) difference in response, divided by the sum of the maximum and minimum response. Figure 3 shows the distribution of the index across complex cells (*a*) and simple cells (*b*). For most complex cells this index was less than 0.5; the indices of simple cells were distributed uniformly between 0–1. This implies that many cortical cells responded to extended contour, rather than to individual grating bar endings. Of the nine cells with phase modulation index of less than 50%, the median relative contrast sensitivity (grating threshold/illusory contour threshold) was 0.76 (range, 0.32–4.0). This means that the illusory contours were effective stimuli. We did not measure the length of the neurons' receptive fields, but spatial phase invariance implies nonlinear spatial summation along the grating-defined contour. A modification of the Spitzer–Hochstein[18] model for cortical complex cells may account for the illusory contour responses we observed. Misaligning that model's subunits in the axis parallel to the cell's preferred orientation, so that each subunit has a different centroid of sensitivity in the 'long axis' of the receptive field, will yield spatial phase insensitivity.

Responses to illusory contours defined by line gratings (as in Fig. 1*b*) were compared with those defined by sine gratings in eight neurons. The linewidth used was 1.2 arcmin, chosen to be like line-grating stimuli used in refs 15–17. The median ratio of peak responses to line/sine-defined illusory contours was 0.2. The linewidth in the line-defined illusory contour seems to be a

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