Temporal and Spatial Heterogeneity of mtDNA Polymorphisms in Natural Populations of *Drosophila mercatorum*

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ABSTRACT

Restriction endonuclease analysis of mtDNA was used to examine the genetic relatedness of several geographically separated isolines of the *Drosophila mercatorum* subgroup. In addition, we examined the temporal and spatial distribution of two mtDNA restriction site polymorphisms produced by the enzymes *Bst*EII and *Bst*NI at a single locality—Kamuela, Hawaii. Due to small sample sizes of some collections and the undesirable dependance of the estimation of polymorphism frequency on its variance, an arcsin square root transformation of the frequency data was used. We also use an F_{st} estimator of our transformed frequencies to demonstrate considerable spatial and temporal differentiation within the Kamuela population. In contrast, isozyme data from the same population reveals no pattern of differentiation. The temporal and geographic heterogeneity and population subdivision detected with mtDNA analysis also is consistent with the known dispersal behavior and ecological constraints of this species. The mtDNA data in conjunction with the isozyme data show that the population structure of the Kamuela *D. mercatorum* is close to the boundary line separating panmixia from subdivision, a conclusion that could not be made from isozyme data alone.

DROSOPHILA mercatorum consists of two subspecies, *D. m. mercatorum* and *D. m. pararepleta*. Both subspecies are endemic to Central and South America. *D. m. mercatorum* has, however, assumed a more cosmopolitan distribution, as it is also found in North America (Baton Rouge, Louisiana and Rochester, New York) and in Hawaii. The Hawaiian population of *D. m. mercatorum* at Kamuela has been well characterized by isozyme analysis (TEMPLETON *et al.* 1976; CLARK, TEMPLETON and SING 1981), ecological analysis (JOHNSTON and TEMPLETON 1982) and morphological analysis of a complex developmental phenotype called abnormal abdomen (*aa*) (TEMPLETON and JOHNSTON 1982; TEMPLETON, CREASE and SHAH, 1985). In this paper we report on the examination of the Kamuela populations, as well as some preliminary analysis of the subspecies *D. m. pararepleta* and the full species but close relative *D. paranenesis* with a relatively new approach, restriction site analysis of mtDNA.

The utility of restriction site analysis in phylogenetic studies has been well documented (BROWN 1980; BROWN, GEORGE and WILSON 1979; TEMPLETON 1983; FERRIS, WILSON and BROWN 1981). Restriction site analysis to examine population structure or population subdivision has been much less utilized (AVISE and LANSMAN 1983; AVISE, LANSMAN and SHADE 1979; TAKAHATA and PALUMBI 1985), but there are at least two reasons why it might be useful. First, if one chooses a DNA sequence that evolves rapidly, it is reasonable to assume that levels of polymorphism will be high, and hence there will be more genetic markers available for tracing population level phenomena. With many isozyme analyses, levels of variability are very low, making it difficult to infer population structure. MtDNA appears to be evolving at a relatively rapid rate, at least in mammals (BROWN, GEORGE and WILSON 1979) and probably in some species of Drosophila (DESALLE, GIDDINGS and KANESHIRO 1985) and should afford an adequate DNA sequence for the analysis of population level phenomena.

Second, mtDNA should be useful in analyzing population structure as it is effectively inherited as a maternal haploid. Even if males and females were more or less equivalent in their attributes affecting population structure, the joint effects of haploidy and maternal inheritance mean that the effective population sizes that measure the genetic impact of population subdivision and gene flow should be about four times less for mtDNA than for nuclear gene markers. Consequently, the mtDNA markers can yield observable amounts of genetic differentiation at levels of gene flow and population size that would totally obscure any measurable subdivision for nuclear genes.

We wanted to address two questions of evolutionary importance in *D. mercatorum*. First, we were interested

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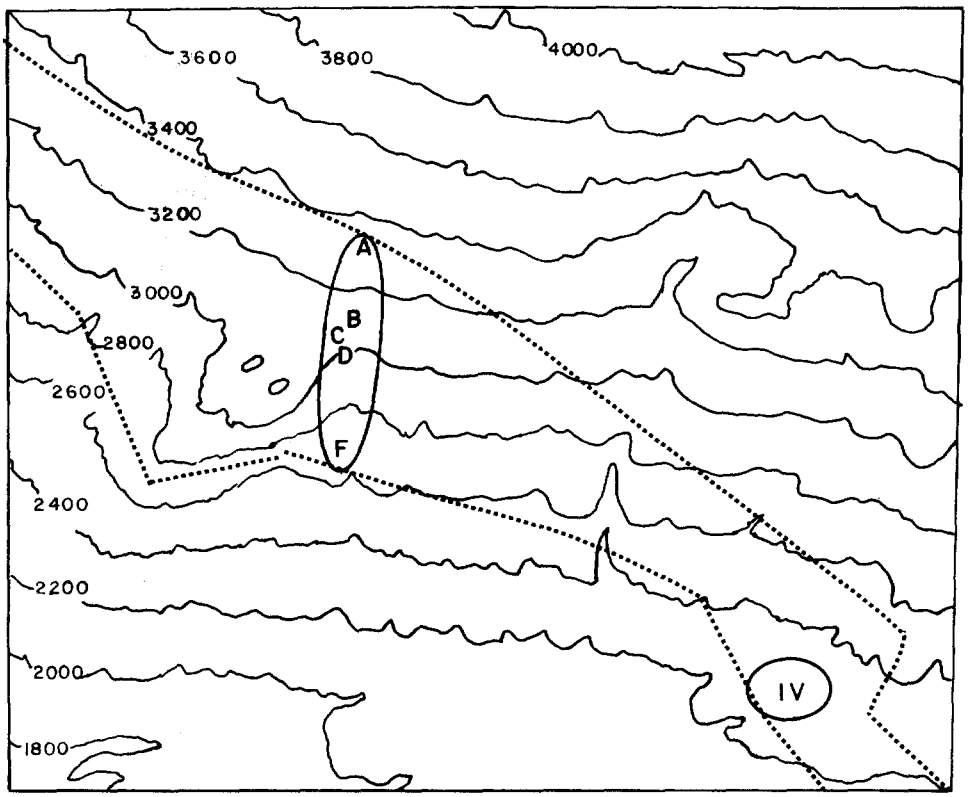

FIGURE 1. Map of the study site near Kamuela, Hawaii. The contour lines show elevation above sea level in feet in 200-ft increments. The dotted line shows the approximate distribution of cactus *O. megacantha*, the sole food plant for *D. mercatorum* in this area. Sites A through F represent a transect over the entire humidity gradient on which the cactus exists. (Site E, intermediate between D and F, was destroyed in 1982 during the construction of a pipeline.) Site IV is lower in elevation, but is normally very dry, like site F. Unlike sites A through F, site IV lies in the saddle between Kohala and Mauna Kea.

in seeing if mtDNA restriction site analysis could resolve some of the genetic relationships of the already established cosmopolitan *D. mercatorum* isolines. Second, within the well characterized population at Kamuela, Hawaii, we were interested in seeing if mtDNA restriction site analysis could add to our understanding of population structure and population dynamics.

MATERIALS AND METHODS

Flies and fly stocks: The following *D. mercatorum* strains were examined in the initial characterizations of mtDNA; Oahu 1 from Oahu, Hawaii (MO01), K28 from Kamuela, Hawaii (MK28), K23 from Kamuela, Hawaii (MK23), Man11 from Managua, Nicaragua (MM11), SBr15 from San Salvador (MSBR) and *D. m. pararepleta* from Brazil (MPAR). A single *D. paranensis* isoline (PRNA) was also characterized.

We subsequently concentrated on a detailed examination of mtDNA variability at one geographical location, Kamuela, Hawaii. The collection site at Kamuela consists of a 1-km transect of cactus patches (*Opuntia megacantha*) situated on the slope of the cinder cone Puu Kawaiwai in the Kohala mountain range. The transect has been divided into several regions designated by the letters A (3400 ft above sea level) through F (2500 ft above sea level). In addition, flies were collected in the saddle region between Kohala and Mauna Kea just outside the town of Kamuela on the Kawaihae Road. This site is designated by the Roman numeral IV and is located about 3 km from A–F at an altitude of 2100 ft. There is a high density of cactus patches between A–F and IV (Figure 1). Twenty-four wild caught females each from the 1981 and 1982 collections at Kamuela were allowed to establish isofemale lines. Wild caught females from the 1982, 1983 and 1984 collections were singly test crossed to the abnormal abdomen tester strains (TEMPLETON, CREASE and SHAH 1985) and then frozen at −80° for later examination of mtDNA restriction fragment polymorphism.

DNA isolation and manipulation: We used the CNA (cytoplasmic nucleic acid) preparation of LANSMAN *et al.* (1981) for isolation of DNA from isolines. For isolation of DNA from single flies we used the method of COEN *et al.* (1982). Restriction digestion, electrophoresis, Southern transfer of nucleic acids to nitrocellulose, hybridization of nick translated probes, and autoradiography were done as described in DESALLE, GIDDINGS and TEMPLETON (1985).

Due to a high degree of similarity of *D. melanogaster* mtDNA and *D. mercatorum* mtDNA, we have used highly purified *D. melanogaster* mtDNA as probe for detecting restriction fragment polymorphisms. We have also used cloned portions of the *D. melanogaster* and *D. yakuba* mtDNA genome as probe (CLARY *et al.* 1982; DESALLE, GIDDINGS and TEMPLETON (1985).

Restriction site analysis: Twenty-five restriction enzymes were used in the initial characterization of *D. mercatorum* mtDNA. Of these 25 restriction enzymes, only 16 cut the mtDNA molecule at least once. These 16 enzymes (Figure 2) were therefore used in all of the preliminary Kamuela population screens and the examination of the various laboratory strains. Restriction maps were constructed by combining double digestion and probing with different cloned segments of the mtDNA molecule.

Examination of the restriction site polymorphisms in the Kamuela natural population revealed that only two restriction enzymes produced fragment polymorphisms (Figure 3). These two enzymes, *BST*NI and *Bst*EII, were used in the mass population screens of single individuals in the following way. DNA isolated from a single fly was aliquotted into two restriction reactions, one for *Bst*NI and one for *Bst*EII. The reactions were then performed according to the specifications of the supplier (New England Biolabs).

Isozyme analysis: An isozyme survey of five polymorphic

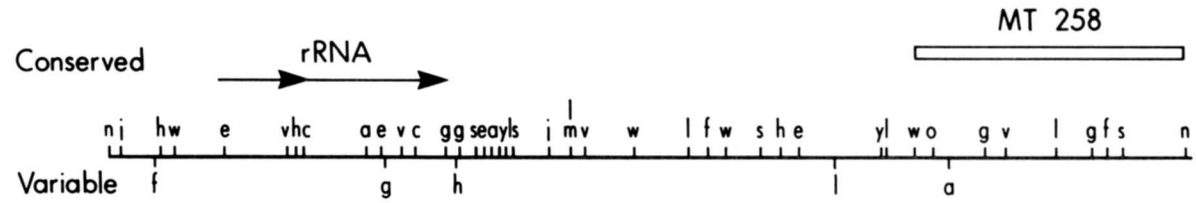

FIGURE 2.—Restriction map of *D. mercatorum* mtDNA. Solid arrows indicate the relative position of the mitochondrial ribosomal RNA genes. The solid bar above the restriction map indicate the relative limit of hybridization of the Drosophila mtDNA probes used in this study. mt259 was cloned from *D. melanogaster*. Conserved sites are shown above the restriction map and variable sites are shown below. Restriction site abbreviations are as follows: a = *Ava*II, c = *Cla*I, d = *Bcl*I, e = *Eco*RI, t = *Bst*EII, g = *Bst*NI, h = *Hin*dIII, j = *Eco*Rv, l = *Hin*cII, m = *Hpa*I, n = *Nru*I, o = *Nco*I, p = *Pvu*II, s = *Sac*I, v = *Xba*I and w = *Xmn*I.

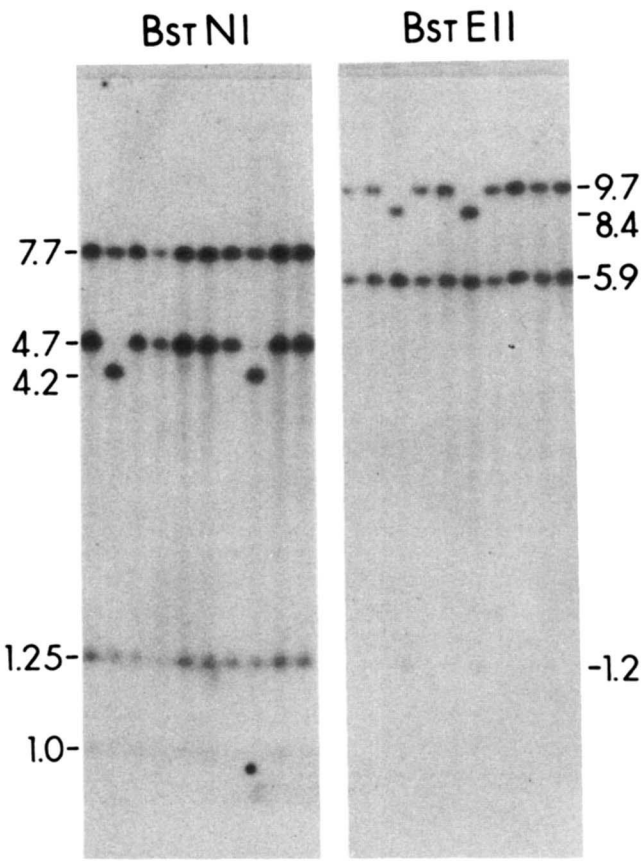

FIGURE 3.—Examples of the rare *Bst*EII (right panel) and *Bst*NI (left panel) restriction fragment polymorphisms. Sizes of the fragments are given in kilobases. Ten individuals have been examined in this figure for the two restriction enzymes by splitting the DNA made from a single fly two ways for the two reactions. The rarer *Bst*EII variant is produced from the more common pattern by the presence of an extra *Bst*EII site that cleaves the 9.7-kb fragments to two new fragments (8.4 and 1.2 kb) both of which are visible in the right panel. The rare *Bst*NI variant is produced from the more common pattern by the presence of an extra *Bst*NI site that cleaves the 4.7 kb fragment into two new fragments (4.2- and 0.4-kb fragment). The smaller of the two fragments (0.4 kb) has been run off of the gel pictured in the left panel. We routinely score the presence of this rare *Bst*NI polymorphism by the presence of the 4.2-kb band.

loci was carried out on the Kamuela *mercatorum* population samples in 1980 and 1981. The five loci are: esterase A (Est A), esterase B (Est B), acid phosphatase (Acph), alcohol dehydrogenase (Adh) and glucose-6-phosphate dehydrogenase (G-6-PD). The last locus is *X*-linked, the remainder are autosomal. Genetic variability at these loci was scored using the starch gel electrophoresis techniques described in CLARK TEMPLETON and SING (1981). Under electrophoresis, each of these loci is found to be segregating for slow (*S*) and fast (*F*) migrating alleles. In addition, Est A has a rare third allele (*XS* for "extra slow").

Analysis of population polymorphism, genetic distance and population subdivision: Levels of variability and genetic divergence for the mtDNA analysis were estimated by the techniques outlined in EWENS (1983). These techniques yield a pairwise estimate of genetic difference for any two mtDNA haplotypes examined called $\hat{d}$ and an overall estimate of population variability called $\hat{d}$.

Geographical heterogeneity in the frequencies of the various mitochondrial haplotypes and isozyme alleles was analyzed as follows. Suppose that n_i haploid genomes are sampled at site "i", x of which show the variant type of interest. The standard maximum likelihood estimator of the frequency of this variant in the population being sampled is simply x_i/n_i, with an expected sample variance of pq/n_i where p is the true frequency of the variant in the population and $q = 1 - p$. Note that this estimator of the frequency has a variance that depends upon the parameter that it is estimating, namely, p. This undesirable dependence of the estimator variance upon the parameter being estimated can be eliminated by an arcsin, square-root transformation. In particular, we used the transformation that corrects for small sample sizes as many of our samples have n's less than 50. The transformation used is from FREEMAN and TUKEY (1950).

$$a_i = \{(\arcsin\sqrt{x_i/(n_i + 1)} + \arcsin\sqrt{(x_i + 1)/(n_i + 1)}\}/2. \quad (1)$$

Statistic (1) is asymptotically normal with a mean of arcsin $\sqrt{p}$ and variance of $1/(4n_i)$. Note that the sampling variance of the estimator a_i is determined entirely by the sample size.

Now suppose that a total of r collecting sites are sampled, with the data being transformed with Equation 1. Geographical heterogeneity between sites is then detected through the statistic

$$V = 4\sum_{i=1}^{r} n_i(a_i - \bar{a})^2 \quad (2)$$

where $\bar{a} = \sum_{i=1}^{r} n_i a_i/N$ and $N = \sum_{i=1}^{r} n_i$.

Under the null hypothesis of no geographical heterogeneity (all $p_i = p$), the asymptotic normality of the a_i's yield that statistic (2) is distributed as a chi square with $r - 1$ degrees of freedom.

If statistic (2) indicates that there is significant geographical variation in the frequency of a variant haplotype, the total variance of the transformed frequencies can be subdivided into a sampling and a between population compo-

TABLE 1

Genetic distances (*d*) of the *D. mercatorum* and single *D. paranensis* isolines used in the initial study of mtDNA in the *mercatorum* subgroup

	MM11	MSBR	MK23	MK28	MO01	MR01	MPAR	PRNS
MMII	0.0000	0.0019	0.0038	0.0019	0.0074	0.0056	0.0074	0.0381
MSBR		0.0000	0.0019	0.0000	0.0056	0.0037	0.0056	0.0391
MK23			0.0000	0.0019	0.0073	0.0019	0.0037	0.0376
MK28				0.0000	0.0056	0.0037	0.0056	0.0391
MO01					0.0000	0.0055	0.0073	0.0400
MR01						0.0000	0.0019	0.0385
MPAR							0.0000	0.0377
								0.0000

Isolines are abbreviated as in the MATERIALS AND METHODS.

nent. First, the total variance is estimated by the statistic $rV/[4N(r-1)]$. Under the null hypothesis of no geographical variation, the expected total variance is simply the average sampling variance; that is, $r/(4N)$. If there is between population heterogeneity in haplotype frequencies, this variance will be inflated, and $rV/[4N(r-1)] - r/(4N) = r[V-(r-1)]/[4N(r-1)]$ estimates the between population variance (hereafter called the *Wahlund variance*) in transformed frequencies. The proportion of the total variance that is due to between population heterogeneity is therefore $[V-(r-1)]/V$. Note that this *estimated* proportion can take on negative values. This is biologically meaningless, so we have truncated our estimates at the origin. Since many readers are more familiar with untransformed frequencies, we also calculate the usual F_{st} values which estimate the between population variance in untransformed frequencies divided by pq, where p is the average untransformed haplotype frequency in the total population. We used the formulae given by WEIR and COCKERHAM (1984) to estimate F_{st}, since this estimator is rigorously corrected for various sampling biases. The unbiased nature of the estimator means that negative values of F_{st} are possible, but such negative estimates are probable only when the true F_{st} is close to zero. WEIR and COCKERHAM (1984) do not provide a statistical framework for testing hypotheses about F_{st}. Fortunately, the null hypothesis of statistic (2) (*i.e.*, no geographical heterogeneity in frequencies between sites) is equivalent to the null hypothesis of $F_{st} = 0$. Hence, we test the significance of the F_{st} values with statistic (2).

RESULTS

Analysis of laboratory strains of *D. mercatorum* and *D. paranensis*: A restriction map for *D. mercatorum* is shown in Figure 2. Pairwise percent divergence of the various strains revealed that genetic distances with respect to mtDNA restriction sites are very small (Table 1). Within *D. m. mercatorum* the percent divergence ranged from 0.0000 to 0.0074, or in terms of minimal mutational distance, from 0 to 3 restriction site differences. There were no clear geographic relationships of the percent divergences observed for the *D. mercatorum* lines, except that the Oahu-1 line appeared to be more distant from the other *D. mercatorum* lines on the average. The single *D. mercatorum pararepleta* isoline examined was very similar to the *D. m. mercatorum* lines as shown by the small percent divergences in Table 1. *D. paranensis* was, however, relatively divergent from all *D. mercatorum* lines (*d* ranging from 0.0376 to 0.0400).

Analysis of isolines established from wild populations at Kamuela for the 1981 and 1982 collections: Isolines were established from wild caught females from the 1981 and 1982 collections at Kamuela (isolines from each year). We assayed each isoline for restriction site variability for the sixteen restriction enzymes that are shown in Figure 2. Only two restriction enzymes (*Bst*NI and *Bst*EII) produced restriction patterns that were variable in the several *D. m. mercatorum* isolines collected at Kamuela in 1981 and 1982 (Figure 3). The two restriction polymorphisms are not coupled; that is, the rarer variants are never found in the same mtDNA genome. The frequencies of these polymorphisms are shown in Table 2.

Analysis of single wild caught flies from the Kamuela populations from the 1981 through 1984 collections: Because of the low level of variability in the Kamuela population, wild flies caught in subsequent years were assayed for the two restriction enzymes that were polymorphic in the 1981 and 1982 isolines (Table 2). The *Bst*EII polymorphism is more widespread than the *Bst*NI, with the latter found predominantly at cactus site IV.

Estimates of the levels of variability at the population level by year for the entire Kamuela population can also be made by assuming that the other 14 restriction enzymes that were not variable for the 1981 and 1982 collections were also nonvariable for subsequent collections. Table 3 shows that the population levels of variability for 1981 through 1984 are relatively stable and very low (in all collections less than 0.2%).

Degree of population subdivision: Table 4 presents the statistical analysis for geographical heterogeneity in the frequency of mtDNA variants using the data given in Table 2. As can be seen, many of the V statistics yield highly significant chi-square values, particularly from 1982 on where the sample sizes are larger. Thus, there is much evidence for between population heterogeneity in this data set.

TABLE 2

Frequencies of the *Bst*NI and *Bst*EII mtDNA polymorphisms in wild caught flies from Kamuela

	Cactus patch	Frequency of *Bst*NI	Frequency of *Bst*EII	N^a
Summer 1981	A	0.00	0.00	1
	B	0.00	0.25	4
	C	0.00	0.00	9
	D	0.00	0.00	5
	E–F	0.00	0.00	5
	IV	0.00	0.00	4
Winter 1981	B	0.00	0.00	13
Summer 1982	A	0.00	0.00	1
	B	0.00	0.00	30
	C	0.00	0.08	12
	F	0.00	0.13	15
	IV	0.15	0.07	129
Summer 1983	B	0.00	0.52	127
	C	0.00	0.19	26
	D	0.00	0.00	4
	F	0.00	0.13	40
	IV	0.33	0.11	9
Spring 1984	A	0.00	0.75	4
	B	0.00	0.28	18
	C	0.00	0.40	10
	D	0.00	0.26	19
	F	0.00	0.11	18
	IV	0.17	0.00	24
Summer 1984	B	0.00	0.26	65
	C	0.00	0.34	59
	D	0.00	0.21	28
	F	0.04	0.13	51
	IV	0.37	0.00	107

[a] N is the sample size for a particular collection.

Because sites A–F are in close proximity to one another, whereas site IV is 3 km away from the A–F transect, we next used the V statistic to see if there is any heterogeneity in haplotype frequencies within the A–F transect (that is, the data from site IV were removed from the analysis). The results of this analysis are shown in Table 5. With only one exception, the chi-square values are consistent with the hypothesis of geographical homogeneity along the A–F transect. In contrast, when one tests for homogeneity between site IV and the pooled A–F transect, most of the chi-square values are highly significant (Table 6). Hence, the vast majority of the detected geographical heterogeneity exists between site IV *vs* A–F, with the A–F transect being relatively homogeneous.

We can also use the data in Table 2 to test the temporal homogeneity within a geographical site using the same statistics but with "i" now indexing a sampling time rather than a geographical site. The results of this temporal analysis are shown in Table 7, and significant temporal heterogeneity over this four year period was detected for sites B and IV, with the other sites being temporally homogeneous.

Table 8 shows the estimated allele frequencies of

TABLE 3

Estimates of the proportion of nucleotides that are polymorphic in the population $\hat{d}$ from Kamuela for the 1981–1984 collections

Year	$\hat{d}$
1981	0.00142
1982	0.00114
1983	0.00181
1984	0.00122

the five isozyme loci scored of the 1980 and 1981 collections. Table 9 gives the V statistic analysis and estimated F_{st} values. In great contrast to the mtDNA results, only one of ten isozyme contrasts yielded a significant V value (Acph in 1980), and the F_{st} estimators pooled across loci were extremely small (0.00015 in 1980 and −0.00647 in 1981) and not significantly different from zero.

DISCUSSION

The mtDNA analysis of strains of *mercatorum* subgroup flies indicates a high degree of genetic relatedness among the geographically separate strains. This high relatedness is similar to mtDNA in three other continental *Drosophila* species examined, *D. melanogaster*, *D. simulans* and *D. virilis* (SHAH and LANGLEY 1979; HUDSON 1982). The high level of similarity among the geographically diverse *D. mercatorum* lines is, however, in marked contrast with the high levels of variability and divergence observed for the mtDNA of several species of Hawaiian picture wing Drosophila (DESALLE, GIDDINGS and TEMPLETON, 1985; DESALLE, GIDDINGS and KANESHIRO 1985).

The single strain of the subspecies *D. m. pararepleta* is very similar in its mtDNA to the *D. m. mercatorum* strains, while *D. paranensis*, a close relative and member of the *mercatorum* subgroup, is very different in its mtDNA from all the *D. mercatorum* strains. Because of this high degree of similarity among the *D. mercatorum* lines, neither cladistic nor distance analysis is particularly informative in determining the evolutionary relationships of the *D. m. mercatorum* lines. In fact, the subspecies *D. m. pararepleta* is not sufficiently different from the *D. m. mercatorum* lines to infer the phylogeny of these two subspecies with any statistical confidence.

Although the mtDNA restriction site analysis was not phylogenetically informative, it did prove a powerful indicator of population structure for the *D. m. mercatorum* at Kamuela, Hawaii. These flies were most likely introduced in the early 1800s with the introduction of the cactus *O. megacantha* as food for cattle (TEMPLETON and JOHNSTON 1982). The observation that only three mtDNA haplotypes were found at Kamuela along with the low estimates of levels of

TABLE 4

Test for heterogeneity over the entire Kamuela collecting site

Collection/ Enzyme	Chi square	Degrees of freedom	F_{st}	Probability
Su'81/*Bst*NI	0.00	5	0.000	NS
Su'81/*Bst*EII	2.11	5	0.108	NS
Su'82/*Bst*NI	13.12	4	0.069	$P < 0.025$
Su'82/*Bst*EII	5.20	4	0.005	NS
Su'83/*Bst*NI	11.98	4	0.446	$P < 0.025$
Su'83/*Bst*EII	32.96	4	0.219	$P < 0.005$
Sp'84/*Bst*NI	7.10	5	0.111	NS
Sp'84/*Bst*EII	21.06	5	0.187	$P < 0.005$
Su'84/*Bst*NI	89.08	4	0.303	$P < 0.005$
Su'84/*Bst*EII	68.75	4	0.151	$P < 0.005$

This table lists the collection by year and season (summer = Su; spring = Sp) and the restriction enzyme used. Chi-square estimates, degrees of freedom, probabilities and F_{st} were estimated as outlined in the text.

TABLE 5

Tests for heterogeneity at upper level collection sites (*i.e.*, Cactus site IV removed from analysis)

Collection/ Enzyme	Chi square	Degrees of freedom	F_{st}	Probability
Su'81/*Bst*NI	0.00	4	0.000	NS
Su'81/*Bst*EII	2.10	4	0.119	NS
Su'82/*Bst*NI	0.00	3	0.00	NS
Su'82/*Bst*EII	4.88	3	0.046	NS
Su'83/*Bst*NI	0.00	3	0.000	NS
Su'83/*Bst*EII	30.47	3	0.222	$P < 0.005$
Sp'84/*Bst*NI	0.00	4	0.000	NS
Sp'84/*Bst*EII	6.38	4	0.090	NS
Su'84/*Bst*NI	3.58	3	0.025	NS
Su'84/*Bst*EII	6.24	3	0.027	NS

TABLE 6

Test for heterogeneity of Cactus site IV *vs.* all upper elevation cactus

Collection/ Enzyme	Chi square	Degrees of freedom	F_{st}	Probability
Su'81/*Bst*NI	0.00	1	0.000	NS
Su'81/*Bst*EII	0.002	1	0.009	NS
Su'82/*Bst*NI	17.68	1	0.103	$P < 0.005$
Su'82/*Bst*EII	0.13	1	0.007	NS
Su'83/*Bst*NI	12.25	1	0.849	$P < 0.005$
Su'83/*Bst*EII	2.66	1	0.106	NS
Sp'84/*Bst*NI	10.16	1	0.265	$P < 0.005$
Sp'84/*Bst*EII	14.76	1	0.187	$P < 0.005$
Su'84/*Bst*NI	81.45	1	0.418	$P < 0.005$
Su'85/*Bst*EII	62.59	1	0.195	$P < 0.005$

polymorphism for this population (Table 2) is consistent with either a small founder size for the initially introduced population or very low levels of variability in the parent population. The low levels of variability from the widely separated strains suggests that variability across this species for mtDNA is very low, and that the even lower degree of variability at Kamuela is probably the result of a combination of small founder size and low levels of variability in the parent population. It is also significant that the rare *Bst*NI and *Bst*EII polymorphisms are found only at Kamuela, but this may be due to the small sample sizes at localities other than Kamuela.

TABLE 7

Tests for year to year heterogeneity at cactus patches with relatively large population sizes for the 4-yr collecting period

Collection site/ Enzyme	Chi square	Degrees of freedom	Probability
B/*Bst*EII	53.63	5	$P < 0.005$
B/*Bst*NI	0.00	5	NS
C/*Bst*EII	9.17	4	NS
C/*Bst*NI	2.00	4	NS
IV/*Bst*EII	24.46	4	$P < 0.005$
IV/*Bst*NI	18.41	4	$P < 0.005$

CLARK, TEMPLETON and SING (1981) have shown that the levels of variability at the isozyme level of the wild *D. mercatorum* at Kamuela are very similar to the levels observed for continental species of Drosophila. It appears that although there is a small amount of mtDNA variability in *D. mercatorum*, and in particular in the Kamuela population, the nuclear genes as assayed by isozyme analysis are relatively variable. This result is consistent with the prediction of CARSON and TEMPLETON (1984) that founder effects will be more extreme for mtDNA than for nuclear genes because of the maternal haploid inheritance of mtDNA.

A detailed analysis of the spatial and temporal distribution of mtDNA variability reveals several interesting observations. As seen by the results of Tables 4 and 6, the mtDNA variants can detect significant geographical heterogeneity. Not surprisingly, most of this heterogeneity is observed between sites IV *vs.* A–

TABLE 8

Estimated allele frequencies for five isozyme loci

Cactus patch	Est A				Est B			Acph			Adh			G-6-PD		
	S	*F*	*XS*	*N*	*S*	*F*	*N*	*S*	*F*	*N*	*S*	*F*	*N*	*S*	*F*	*N*[a]
Summer 1980																
A	0.8125	0.1750	0.1250	40	0.4000	0.6000	40	0.1500	0.8500	40	0.0000	1.0000	40	0.5100	0.4900	45
B	0.8141	0.1474	0.0385	78	0.3947	0.6053	76	0.1288	0.8712	66	0.0192	0.9808	78	0.6111	0.3889	36
C	0.7768	0.1964	0.0268	56	0.4537	0.5463	54	0.0417	0.9583	60	0.0250	0.9750	60	0.5658	0.4342	76
IV	0.7971	0.1667	0.0362	69	0.5075	0.4925	67	0.0417	0.9583	72	0.0507	0.9493	69	0.4950	0.5050	101
Summer 1981																
A	1.0000	0.0000	0.0000	2	0.5000	0.5000	2	0.2500	0.7500	2	0.0000	1.0000	2	0.5000	0.5000	4
B	0.7500	0.2004	0.0100	50	0.2755	0.7245	49	0.0833	0.9167	36	0.0192	0.9808	52	0.5000	0.5000	68
C	0.8103	0.1897	0.0000	58	0.3860	0.6140	57	0.1058	0.8942	52	0.0690	0.9310	58	0.5422	0.4578	83
D	0.9400	0.0600	0.0000	25	0.3800	0.6200	25	0.1250	0.8750	24	0.0400	0.9600	25	0.4242	0.5758	33
E	0.7895	0.2105	0.0000	19	0.3750	0.6250	20	0.0750	0.9250	20	0.0000	1.0000	19	0.4412	0.5588	34
IV	0.8000	0.2000	0.0000	10	0.4500	0.5500	10	0.0000	1.0000	10	0.0500	0.9500	10	0.2667	0.7333	15

[a] Since G-6-PD is *X*-linked, the sample size here is the number of haploid genomes examined. In all other cases, the sample size is the number of diploid individuals examined.

TABLE 9

Tests for heterogeneity and estimated F_{st} values for the isozyme data over the entire Kamuela collection site

Collection/Locus	Chi square[a]	Degrees of freedom	Probability	F_{st}
1980/Est A	0.63	3	NS	−0.01
1980/Est B	4.27	3	NS	0.00
1980/Acph	13.74	3	$P < 0.005$	0.03
1980/Adh	6.71	3	NS	0.03
1980/G-6-PD	1.81	3	NS	−0.01
1980/All loci				0.00
1981/Est A	10.34	5	NS	0.01
1981/Est B	4.60	5	NS	−0.01
1981/Aph	5.38	5	NS	−0.01
1981/Adh	5.94	5	NS	−0.00
1981/G-6-PD	4.52	5	NS	−0.01
1981/All loci				−0.01

[a] In calculating the chi square for the esterase A locus, the two rarer alleles, *F* and *XS*, were pooled.

F, the most distant geographically. Even this distance is relatively modest, being only 3 km. Moreover, there are no geographical or habitat barriers separating IV from A–F, and fly populations are distributed continuously between them. Consequently, the mtDNA subdivision is most likely due to isolation by distance.

In contrast, the results given in Table 9 indicate that the Kamuela populations of *D. mercatorum* are behaving as a single panmictic unit with respect to their nuclear genetic systems with no detectable population subdivision whatsoever. The contrast between Tables 4 (mtDNA) and 9 (nuclear loci) is dramatic, but not entirely surprising in light of the theoretical work of BIRKY, MURUYAMA and FUERST (1983). They have shown that, all else being equal between the sexes, the maternal hapolid inheritance of mtDNA reduces the effective amount of gene flow by a factor of 4 with respect to a diploid nuclear genetic system. Moreover, this inherent fourfold difference can be accentuated if there is a male bias in gene flow. Fortunately, much is known about dispersal in this population (JOHNSTON and TEMPLETON 1982), so the potential for such a bias can be directly evaluated.

These dispersal studies reveal that flies will move between cactus patches only on days with wind speeds less than 10 km/hr. Because of the trade winds and the geographical location of this study area in and near the saddle between Mauna Kea and Kohala, this area is normally very windy. By examining the weather records collected at the nearby Kamuela airport over the past several years, only about 50 days per year have wind speeds less than 10 km/hr. Moreover, these low winds must occur either in early morning or late evening, as these are the only times flies will disperse even on windless days. Consequently, the opportunities for dispersal are limited. Even given the opportunity, only about a third of the flies actually move to a different cactus patch on a windless day, and the average amount of movement is only about 60 m/windless day, given dispersal (JOHNSTON and TEMPLETON 1982). Most important, there are no sex biases in any of this dispersal behavior. Given a windless day, the average proportion of males dispersing is 0.27 ± 0.04 (SD), and of females dispersing is 0.35 ± 0.07. Given dispersal, the average male travels 57 ± 53 m and the average female 66 ± 63 m. Hence, there is no significant difference in dispersal behavior between the sexes.

However, equal dispersal does not mean equal gene flow. Virtually all adult females in the natural population are inseminated, and this includes dispersing females (J. S. JOHNSTON and A. R. TEMPLETON, unpublished observations). Hence, female dispersal causes the flow of female and male-derived genomes, whereas male dispersal results only in the flow of male-derived genomes. Consequently, a system of equal

dispersal of males and females leads to a male-biased gene flow pattern if the dispersing females are inseminated, as is the case here. Moreover, multiple insemination appears to be very common with little sperm precedence (A. R. TEMPLETON and J. S. JOHNSTON, unpublished observations). Combining these effects, it is likely that for every one mitochondrial genome that is transferred between cactus patches, about 6–8 haploid nuclear genomes are transferred. A six- to eightfold difference in the effective amount of gene flow could easily explain the difference seen between mtDNA and nuclear genes with respect to population subdivision. For example, the dispersal observed in nature primarily involves transfers between nearby cactus patches (JOHNSTON and TEMPLETON 1982), and so a "stepping stone" model of gene flow seems appropriate. Such models show that if the effective number of individuals participating in gene flow per deme, Nm, is less than one, then "significant differentiation" should result (KIMURA and MARUYAMA 1974). These individuals are diploid in the model, so significant differentiation is expected when the effective number of haploid genomes transferred is less than 2. If the effective transfer of haploid genomes is between 2 and 8 ($1 \leq Nm \leq 4$) "important differentiation" can still be expected, but above 8 ($Nm \geq 4$) little or no differentiation is expected. Consequently, a six- to eightfold difference in the effective amount of gene flow for mitochondrial *vs.* nuclear genomes could easily place the mitochondrial genomes within the parameter space associated with significant or important differentiation while simultaneously placing the nuclear genomes in an area associated with no differentiation.

The joint observations on mitochondrial *vs.* nuclear genetic subdivision can also be used to place some bounds on the likely values for Nm in nature. The observed F_{st} values for the nuclear markers are consistent with virtually any value of Nm greater than 4. On the other hand, the mtDNA analysis clearly shows that the constrained dispersal pattern of the Kamuela *D. mercatorum* results in such restricted gene flow that 3 km are sufficient to show extreme genetic differentiation, and that occasionally significant differentiation can exist between cactus patches only 60 m apart (*i.e.*, B and C during the summer of 1983 for the *Bst*EII variant). These results imply an Nm value of 1 or less for mitochondrial markers (*i.e.*, less than 2 haploid genomes). Taken together with an estimated six- to eightfold difference in effective gene flow, these results imply that the Nm values for the diploid nuclear system fall between 4 and 8 and those for the mitochondrial system between 0.5 and 1 (1 and 2 in haploid units). These figures are not precise estimates given the number of assumptions, but what is clear from considering the joint pattern of mitochondrial *vs.* nuclear genetic subdivision is that the Kamuela *D. mercatorum* population, although panmictic for the nuclear markers, is very close to the border line separating panmixia from subdivision. Thus, although there is sufficient gene flow to cause panmixia for neutral or uniformally selected nuclear markers, the gene flow is limited enough to allow effective differentiation on mtDNA markers or on clinally selected nuclear markers, a conclusion drawn by JOHNSTON and TEMPLETON (1982) strictly from dispersal data.

The temporal analysis also indicates that significant genetic heterogeneity exists within some sites over time. Interestingly, the two sites showing significant temporal heterogeneity are B and IV, the two elevational extremes for the range of *D. mercatorum*. (Although *D. mercatorum* can occasionally be found at site A, many years it cannot, and even when it is found at A it is very rare.) These two sites have been strongly influenced by weather fluctuations during the study period. The year 1981 was one of severe drought (TEMPLETON and JOHNSTON 1982) that greatly diminished the population densities of *D. mercatorum*. Site IV, normally being the driest, experienced very severe depressions in density. In contrast, the spring and summer of 1982 were the most humid and wet on record (A. R. TEMPLETON and J. S. JOHNSTON, unpublished data). Although the lower elevation populations recovered in density, the extremely wet conditions at the upper elevations drove the densities of *D. mercatorum* to levels even lower than that during the drought. Indeed, it required several days of collecting to obtain the B collection, whereas during more normal years a collection of that size could easily be obtained in 30 min. The year 1983 was one of rapid fluctuation between wet and dry spells, and 1984 was close to the long-term average for the area, except for site IV which experienced more humid conditions than normal due to abnormal wind patterns that affected the saddle area but not the upper elevation sites. Hence, sites B and IV represent the ecological extremes for *D. mercatorum* in this area, and the densities fluctuate markedly from year to year at these extremes. As a consequence, these sites would be the most affected by drift effects and near-extinction recolonization cycles, both of which could cause rapid fluctuations in haplotype frequencies.

These results indicate that mtDNA is indeed a sensitive indicator of population structure, particularly when combined with genetic surveys on nuclear markers. Using the mitochondrial markers, we have successfully detected both geographical and temporal heterogeneity in the Kamuela populations of *D. mercatorum* that is consistent with the known dispersal behavior and ecological constraints of this species. By overlaying the mitochondrial results on the isozyme survey of nuclear markers, we have shown that the

population structure of the Kamuela *D. mercatorum* is close to the boundary line separating panmixia from subdivision, a conclusion that could not be drawn from isozyme data alone.

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