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GENETIC VARIABILITY IN THE KILAUEA FOREST POPULATION OF *DROSOPHILA SILVESTRIS*

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ABSTRACT

In this report, we investigate the chromosomal and genetic characteristics of one of the populations of an endemic Hawaiian fly, Drosophila silvestris. This species is found only on the Big Island of Hawaii and typically occurs in montane rain forest areas such as that encompassed by the Kilauea Forest Reserve. Our collections from this forest were made along one of the transects within the 200-acre Tract which has been under intensive study by the IBP since 1970. Samples of D. silvestris were collected several times between September 1971 and December 1972 and analyzed with respect to both their allozymic and chromosomal constitutions, in order to detect any possible seasonal changes in the genetic composition of the population. Although the samples were not particularly large and the study not a very long-term one, the available data do not support the occurrence of marked cyclic fluctuations in the genetic characteristics of the population, but rather suggest that both the chromosomal polymorphisms and the genetic polymorphisms show considerable temporal stability, at least in the short term. The observed genetic stability of the Kilauea Forest population of D. silvestris is consistent with the population dynamics of the fly as well as with the general ecological stability of the habitat and lack of marked seasonal fluctuations in climate.

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INTRODUCTION

Drosophila silvestris is endemic to the Big Island of Hawaii, occurring in suitable montane rain forest areas between altitudes of 3,400 ft and 5,300 ft. It is a comparatively young species in the island ecosystem, and although rapidly evolved, it may well be still adapting and still undergoing various evolutionary processes. In Kilauea Forest, the species is relatively abundant, and therefore this population was selected for study of its genetic variability. We were particularly interested in any possible seasonal effects and in addition, in any indications of longer-term changes.

The species *D. silvestris* is known to be highly polymorphic chromosomally (Carson and Stalker, 1968), and in this respect is amongst the minority of Hawaiian *Drosophila* species. Previous studies of chromosomal inversion polymorphisms in continental *Drosophila* species have shown that in some cases, the frequencies of particular gene arrangements show cyclic fluctuations in association with seasonal climatic changes (Dobzhansky, 1948; Epling *et al.*, 1953, 1957; Brncic, 1972). The chromosomal polymorphism in these temperate species thus operates as a flexible system and appears to be very important in the adaptation of the population to its environment (Dobzhansky, 1962). It was one of the aims of this study to determine whether inversion polymorphisms behaved similarly in *Drosophila* species from tropical environments.

It should be noted that the presence of inversion polymorphisms is a very important feature in the genetic structure of a species, with far-reaching effects on the levels of recombination and thus available genetic variation. Chromosomally monomorphic and chromosomally polymorphic species might be expected to respond somewhat differently to environmental changes, and genetic variability in the latter might be affected to some extent by the degree of polymorphism in a population and also by the flexibility or stability of those polymorphic systems. Actually, the precise relationship between chromosomal polymorphisms and genic polymorphisms is not at all understood. In the few instances in which both have been studied concurrently in natural populations, it has usually been found that the two kinds of polymorphism behave differently, in some respects at least. In populations of *D. willistoni* for example, Ayala *et al.* (1971) found that there was little or no correlation between the inversion and genic polymorphisms, which showed contrasting behavior in continental vs. island populations. In other cases, there may be some correlation between the two kinds of variation, although the relationship observed might depend in part on the distribution of the genes studied relative to the

inverted and non-inverted regions in the genome. The species D. pseudoobscura and D. persimilis provide a case in point. It has long been known that populations of both species show temporal changes in the frequencies of several chromosomal polymorphs, and now preliminary evidence has been presented to show that two loci in each of these species undergo significant temporal changes in allelic frequencies which might also follow a cyclic pattern (Dobzhansky et al., 1973).

In this study, our main objective was to determine whether the Kilauea Forest population of D. silvestris was subject to any temporal changes in its genetic composition. We therefore studied both the allozymic variation and the chromosomal variation of the population over time. It is hoped that the data obtained from this population and from the species as a whole might secondarily make some contribution towards an elucidation of the relationship between the two kinds of variation.

MATERIAL AND METHODS

The population of D. silvestris in Kilauea Forest was sampled five times during a 15-month period extending from September 1971 to December 1972. Adult flies were collected with the aid of banana baits distributed over several areas within the Forest Reserve. Collections made at different points by various collectors were pooled together in order to give an adequate sample. Thus there are no effective data available on possible microspatial variations in the genetic characteristics of the population, and the combined data of each sample describe the average genetic composition of the "total population"at that point in time.

The chromosomal data were obtained via isolation of the collected females and scoring of the salivary gland chromosomes of larval progeny reared from these "isofemales." Between 65% and 70% of field-collected females were fertile when brought into the laboratory - the remainder were probably newly emerged and not yet fertilized at the time of collection. Analysis of seven or more larvae from a family provided information on the chromosomal constitutions of both parents, and each family thus analyzed resulted in a sample of four chromosomes from the population. The first larval method sampled only two chromosomes per isofemale from the original population.

The chromosomal data obtained by both the methods just described sampled male and female genomes equally. However, the separate contributions of the two sexes to the genetic constitution of the population could not be compared (by contrast with the allozyme data which sampled male and female individuals directly). Due

to the lack of a chromosomally homozygous tester stock throughout most of the study, field-collected adult males could not usually be analyzed chromosomally. There are data available on four males from the September 1971 sample, however: these were obtained via interspecific matings to a closely related and chromosomally homozygous species (D. planitibia) and analysis of the hybrid larval progeny.

The allozyme data for the first three samples were obtained via electrophoretic analysis of the same samples of field-collected adults, permitting direct correlations between the chromosomal and electrophoretic data. As noted above, the electrophoretic studies used both males and females, the females being analyzed after oviposition and completion of the progeny testing for chromosomal data. The allelic variation in males and in females of the sample could thus be compared.

For electrophoresis, the wild-caught flies were individually homogenized in 0.05 ml of 0.074 M Tris - 0.008M citrate buffer and the supernatant of each fly absorbed by two wicks of Whatman No. 3 filter paper. These wicks were then separated and applied to two horizontal starch gels combining different buffer systems. Following electrophoresis, the gels were each sliced horizontally four times, and these slices stained separately for one or more of ten enzymes. (For details of the different enzymes and their locus designations, see TABLE 10). The two buffer combinations used in the study are as follows: Buffer System A - Gel: pH 8.9, 0.0076M Tris - 0.005M citrate; Electrodes: pH 8.7, 0.269M borate - 0.1M sodium hydroxide, and Buffer System C - Gel: pH 8.5, 0.074M Tris - 0.008M citrate; Electrodes: pH 8.1, cathode = 0.343M Tris - 0.079M citrate, anode = 0.458M Tris - 0.0104M citrate. The staining methods used have been modified from those described by Ayala et al. (1972) and Selander et al. (1971) and are given in detail in Steiner and Johnson (1973), together with information on the buffer of choice for each enzyme. Alleles at a particular locus were numbered according to the relative mobilities of their allozymes on the specified electrophoretic buffer systems. The allele producing the most frequent allozyme was designated 1.00 and alleles corresponding to faster or more slowly migrating bands were arbitrarily assigned values indicative of their respective mobilities.

Heterozygosities (both genic and chromosomal) were calculated as the proportion of individuals in the sample which were heterozygous at that locus or for that particular inverted sequence. The estimates of genic heterozygosities were based on the raw genotypic data, whereas those for the chromosomal heterozygosities were based on the deduced parental chromosomal types obtained from the larval family segregations.

RESULTS

a) The chromosomal data from Kilauea Forest

The Kilauea Forest population of D. silvestris was found to be polymorphic for 6 of the 7 inversion sequences known within the species, and fixed for the standard sequence of the 3r inversion on chromosome 3. The polymorphic inversions were 2o on chromosome 2, 3m on chromosome 3, and $4k^2$, 4t, $4l^2$ and $4m^2$ on chromosome 4. The 2o and $4m^2$ sequences were present as very low frequency polymorphisms, and were not recorded in some of the smaller samples. The remaining inversions were present at substantial frequencies.

The inversion frequency data for the five samples - September and December 1971, and May, November and December 1972 - are based on 31, 29, 48, 20 and 12 successful isofemale lines respectively (plus 4 males for the September 1971 sample). These data are presented in TABLES 1 and 2. The frequency estimates in TABLE 1 are based on scores of the first larval progeny of each isofemale line (cf. FIGURE 1); those in TABLE 2 are based on complete family information which was available for the first three samples only. There are some inconsistencies in the frequencies estimated by the two methods. The second method, which is based on larger sample sizes, might be expected to give the more reliable frequency estimates. However, there is evidence of some multiple insemination of females in D. silvestris, and this occurrence would reduce the validity of frequency estimates from complete family data, the degree of bias being a function of the extent to which multiple inseminations occur in the natural population. Since this is presently unknown, the major conclusions will be based only on first larval data.

The heterogeneity between the five samples in inversion frequencies was tested for by means of the G-test (Sokal and Rohlf, 1969) applied independently to each inversion. TABLE 3 presents the first larval data on all inversions and the G values calculated from these data. These were then compared with X^2 with four degrees of freedom to give the associated probabilities which are also given in the table. In every case, the value of G was less than 9.488, the critical value of $X^2_{4d.f.}$ at the 5% level. Thus the five samples are homogeneous with respect to the frequency of each of the inversions, and there is no evidence of any significant variation in inversion frequencies in the Kilauea Forest population over the time period studied.

Some additional heterogeneity tests were done between pairs of samples using 2 x 2 tests of independence to calculate the G-statistic, and in most cases applying Yates' correction (i.e. where the size of the two samples together was less than

TABLE 1. Chromosomal inversion frequency data from repeated samplings of the Kilauea Forest population of Drosophila silvestris. Frequencies are based on first-larval data only.

Date of sample	Ref. No.	No. of chroms. Sampled	Inversion frequencies (%)						
			2o	3m	3r	$4k^2$	4t	41^2	$4m^2$
Sept. 27, 1971	Q48	70	1.43	34.29	-	54.29	88.57	8.57	1.43
Dec. 7, 1971	Q54	58	3.44	32.75	-	55.17	77.58	18.96	0
May 22, 1972	R14	96	2.08	30.21	-	40.63	83.33	18.75	4.17
Nov. 20, 1972	R59	40	2.50	40.00	-	52.50	77.50	17.50	0
Dec. 16, 1972	R68	24	0	25.00	-	54.17	66.67	20.83	0
Nov. & Dec. 1972 combined		64	1.56	34.38	-	53.13	73.44	18.75	0
Overall mean frequency		288	2.08	32.64	-	53.13	81.25	16.32	1.74

TABLE 2. Chromosomal inversion frequency data for the Kilauea Forest population of D. silvestris. Frequencies are estimated from complete family information.

Date of sample	No. of chroms. sampled	Inversion frequencies (%)						
		2o	3m	3r	$4k^2$	4t	$4l^2$	$4m^2$
September 27, 1971	130	2.3	30.0	-	53.1	83.1	12.3	1.5
December 7, 1971	108	3.7	40.9	-	50.9	73.1	21.3	0.9
May 22, 1972	132	3.0	27.3	-	48.5	81.1	18.9	3.0

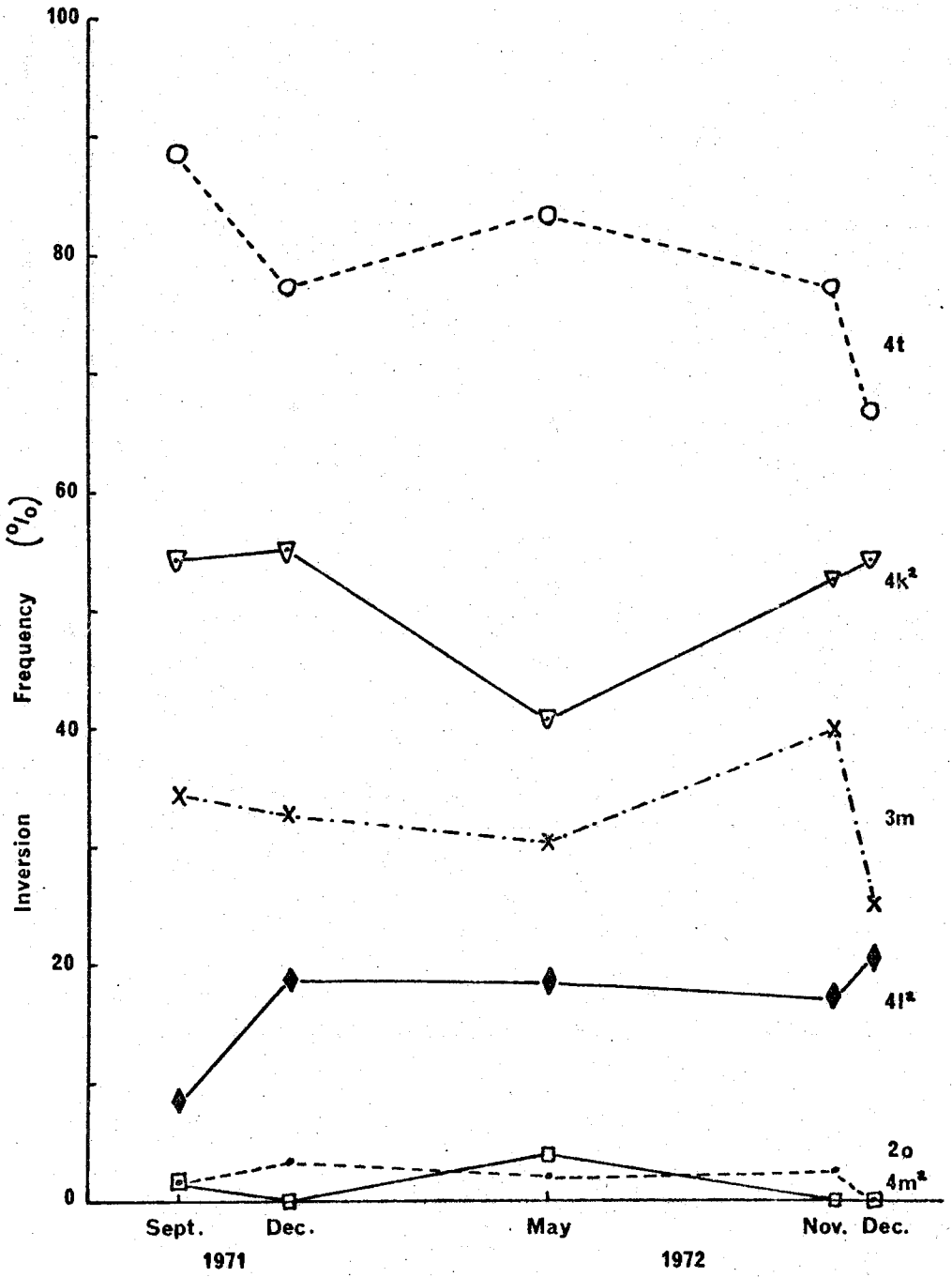

FIG. 1. Temporal variation in the chromosomal inversion frequencies for six polymorphic sequences in the Kilauea Forest population of D. silvestris.

TABLE 3. Comparison of the distribution of chromosomal sequences in successive samples of D. silvestris from Kilauea Forest (first larval data only). Possible heterogeneity between the samples was tested for each inversion separately via calculation of the G statistic and comparison of this with the critical value of $\chi^2_{4d.f.}$

Chromosome arrangement	Sept. 1971	Dec. 1971	May 1972	Nov. 1972	Dec. 1972	G_H	P
2+	69	56	94	39	24	1.651	= 0.80
2o	1	2	2	1	0		
3+	46	39	67	24	18	1.968	0.70 < P < 0.80
3m	24	19	29	16	6		
3+	70	58	96	40	24	-	-
3r	0	0	0	0	0		
$4+_2$	32	26	47	19	11	0.319	0.98 < P < 0.99
$4k^2$	38	32	49	21	13		
4+	8	13	16	9	8	6.775	0.10 < P < 0.20
4t	62	45	80	31	16		
$4+_2$	64	47	78	33	19	4.667	0.30 < P < 0.50
41^2	6	11	18	7	5		
4+	69	58	92	40	24	6.710	0.10 < P < 0.20
$4m^2$	1	0	4	0	0		
No. of chromosomes sampled	70	58	96	40	24		

200). TABLE 4 presents the calculated G-values for each of the polymorphic inversions for seven sets of comparisons. The asterisks indicate frequency variations between samples which are significantly different or very close to the borderline of significance.

The first comparison between consecutive September and December 1971 samples indicates that three inversions (3m, 4t and 41^2) show marked variation between samples when total family data are used to estimate frequencies. However, the frequency differences are just on the borderline of significance. An identical result is obtained when these samples are compared using the 2 x 2 contingency X^2 test. The comparisons using first larval data from the same samples do not indicate any significant differences. Nonetheless, the magnitude of the difference between these two consecutive samples is appreciable, particularly for the frequencies of 4t and 41^2. It could be that this difference had some biological significance which could have been substantiated by larger sample sizes. Alternatively, it could result merely from sampling effects in one or other or both of the samples. The exceptionally low frequency of 41^2 in the September 1971 sample is notable, but no explanation can be offered at this time.

The next comparison between the December 1971 and May 1972 samples again shows no significant differences in inversion frequencies when first larval data are used, but a different result for one inversion using total family data. In this instance, the 3m inversion shows a large frequency difference between samples which is significant at the 5% level. This effect arises from the discrepancy in the two estimates of the frequency of 3m in the December 1971 sample, and is therefore probably spurious. Thus the December 1971 and May 1972 samples can be considered to be not effectively different in inversion frequencies.

The fifth comparison indicates no significant difference between the November 1972 and December 1972 samples (collected three weeks apart), so the data from these two very small samples can be pooled to give a more accurate estimate of the inversion frequencies in the population at the end of the study period (cf. TABLE 1). These average frequencies were then used for the sixth and seventh comparisons to test whether there had been any overall shift in inversion frequencies, despite the apparent homogeneity between the samples taken all together (TABLE 3).

Comparisons between the samples taken at the beginning of the study period (September 1971) and at the end (November - December 1972) showed that six of the inversions showed no significant deviations in frequencies, whereas on inversion,

TABLE 4. G values calculated for 2 x 2 tests of independence to test the heterogeneity in inversion frequencies between pairs of samples of D. silvestris from Kilauea Forest. Asterisks indicate cases which differ at the levels of significance shown.
* $0.05 < P < 0.10$
** $0.02 < P < 0.05$

Sample comparison	Frequencies estimated from	Inversions					
		2o	3m	$4k^2$	4t	41^2	$4m^2$
September 1971 and December 1971	total family data	0.401	3.114*	0.109	3.443*	3.468*	0.182
September 1971 and December 1971	first larval data	0.027	0	0.006	2.042	2.138	0.009
December 1971 and May 1972	total family data	0.083	5.006**	0.142	2.123	0.205	1.403
December 1971 and May 1972	first larval data	0	0.023	0.110	0.445	0.032	1.300
November 1972 and December 1972	first larval data	0.066	0.922	0.017	0.428	0.0002	-
September 1971 and Nov.-Dec. 1972	first larval data	0.444	0.029	0.002	4.140**	2.183	0.002
December 1971 and Nov.-Dec. 1972	first larval data	0.007	0	0.002	0.103	0.041	-

4t, showed a mean trend towards a decrease in frequency (from 88.6% to 73.4%). This difference did not appear in the annual comparison between the December 1971 and November - December 1972 samples, suggesting that the former result was due in part to the previously demonstrated discrepancy between the September 1971 and December 1971 samples.

The complete family data available for the September and December 1971 samples and for part of the May 1972 sample permit a more complete analysis of the genetic structure of the Kilauea Forest population, both with respect to the mating structure in relation to each of the chromosomal inversions, and with respect to the associations between linked inversions. From the segregations obtained amongst the progeny of an isofemale, it is usually possible to deduce the genotypes involved in the parental mating. Thus these data provide information on:

(1) the genotypes present in the sampled adult population and

(2) the actual matings which occurred within the population - (progeny from these would have contributed to the zygotes of the following generation).

In a few instances, progeny analysis revealed that the isolated female had been inseminated by males of different genotypes. These cases were rather rare. However, instances of multiple insemination may be more frequent, since they would not always be detected, especially when the two males involved were of identical genotypes. The occurrence of multiple insemination would not only distort the inversion frequency estimates as already mentioned, but also the genotypic frequencies and the frequencies of particular mating combinations. Most Drosophila species are only inseminated once and this had been assumed to be the normal situation for females of Hawaiian species. For the present report, data from multiply inseminated females has been excluded from the analysis, (except for the contribution of the first larva to the inversion frequency estimates). It is thus hoped that the effects of possible multiple insemination can be disregarded in the subsequent analyses.

Mating analysis:

The randomness of mating in the Kilauea Forest population of D. silvestris (or any departure from random mating) was examined in two ways. Firstly, by comparison of the observed genotypic array in each sample with that expected if the population is in Hardy-Weinberg equilibrium: and secondly, by analysis of the data on mating combinations. Both tests indicate that mating is completely random with respect to each of the inversions. The data are not presented here in full - only that from the December 1971 sample are given by way of example.

TABLE 5 presents the observed and expected distributions of genotypes for this sample, together with the X^2 values, all of which are far from the critical value (5.991 at the 5% level of significance). For the chromosome 4 inversions, the expected numbers of each genotype were calculated ignoring the linkage relationships between these inversions. However, each separate inversion shows relatively good fit to the expected Hardy-Weinberg proportions.

The second analysis of the frequencies of all the possible mating combinations (TABLE 6) also shows excellent agreement of the observed mating frequencies with the expectations derived from the hypothesis of random mating.

Chromosomal heterozygosity:

The genotypic data on the combinations of chromosomal sequences in the Kilauea Forest population also permitted estimates of the chromosomal heterozygosity of the population. The heterozygosity for each inversion was calculated as the proportion of individuals in the sample which were heterozygous for that inversion. The mean chromosomal heterozygosity for the population (H) was taken as the unweighted mean of the heterozygosities for each of the inversions (including the non-polymorphic inversion 3r with zero heterozygosity). The data are given in TABLE 7 and show a mean chromosomal heterozygosity for the Kilauea Forest population of 0.232. This is somewhat intermediate for the species, with other populations of D. silvestris showing chromosomal heterozygosities both above and below this level. From TABLE 7, it would appear that there is a trend toward increasing chromosomal heterozygosity over time, suggesting that there could be some other changes in the genetic makeup of the population apart from inversion frequency changes. However, the available genotypic data from the May 1972 sample are undoubtedly biased and not a true random sample of genotypes from the population at that time. The family data for May 1972 were taken from a specially selected set of isofemales which showed heterozygosity for some of the chromosome 4 inversions in the first larva and chromosomal and/or allelic segregation amongst the subsequent progeny. Thus a higher heterozygosity would automatically obtain in this sample, and it should therefore be excluded from these comparisons.

The heterozygosities in the September 1971 and December 1971 samples appear to be very similar. Statistical comparisons of the two sets of data via 2 x 2 contingency X^2 tests (TABLE 7) show that indeed there are no significant deviations between samples in the heterozygosities of six of the seven inversions. Only the 3m inversion shows a heterozygosity level which is significantly higher in the December sample than in the September sample. This is directly related to the

TABLE 5. Distribution of chromosomal genotypes in the December 1971 sample of *D. silvestris* from Kilauea Forest, and X^2 tests of the goodness of fit to the expected genotypic arrays, based on the estimated inversion frequencies.
ST/ST - homozygote for the standard chromosomal sequence,
ST/IN - inversion heterozygote,
IN/IN - homozygote for the inverted sequence
o - observed numbers of each chromosomal genotype
e - numbers expected for Hardy-Weinberg equilibrium

Genotype \ Invers.	2o		3m		$4k^2$		4t		$4l^2$		$4m^2$	
	o	e	o	e	o	e	o	e	o	e	o	e
ST/ST	49	48.2	15	18.2	13	12.1	4	3.6	34	31.0	49	49.1
ST/IN	2	3.7	31	25.1	23	25.0	18	19.7	12	16.8	1	0.9
IN/IN	1	0.1	6	8.7	14	12.9	28	26.7	4	2.3	0	0
Totals	52	52	52	52	50	50	50	50	50	50.1	50	50
$X^2_{2d.f.}$	0.182		2.787		0.321		0.254		2.918		0.011	

TABLE 6. Analysis of the mating combinations between the various chromosomal genotypes in the December 1971 sample of D. silvestris from Kilauea Forest. The agreement of the observed mating frequencies with expectation was tested for by the χ^2 test for each of the inversions.

Chromosome 2 - inversion 2o

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	22	21.50
+/+ x o/+	2	3.30
+/+ x o/o	1	0.06
o/+ x o/+	-	0.13
o/+ x o/o	-	0.005
o/o x o/o	-	0.00005
	25	24.99505

$X^2_{1d.f.} = 0.083$ $0.70 < P < 0.80$

(five low frequency classes pooled)

Chromosome 3 - inversion 3m

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	2	3.17
+/+ x m/+	8	8.78
+/+ x m/m	3	3.04
m/+ x m/+	10	6.08
m/+ x m/m	3	4.21
m/m x m/m	0	0.73
	26	26.01

$X^2_{5d.f.} = 4.059$ $0.50 < P < 0.70$

Chromosome 4 - inversion $4k^2$

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	1	1.5
+/+ x k^2/+	7	6.0
+/+ x k^2/k^2	4	3.1
k^2/+ x k^2/+	5	6.3
k^2/+ x k^2/k^2	6	6.5
k^2/k^2 x k^2/k^2	2	1.7
	25	25.1

$X^2_{5d.f.} = 0.686$ $0.98 < P < 0.99$

Chromosome 4 - inversion 4t

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	0	0.13
+/+ x t/+	2	1.42
+/+ x t/t	2	1.93
t/+ x t/+	2	3.87
t/+ x t/t	12	10.51
t/t x t/t	7	7.14
	25	25

$X^2_{5d.f.} = 1.503$ $0.90 < P < 0.95$

TABLE 6 Continued.

Chromosome 4 - inversion 41^2

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	12	9.59
+/+ x 1^2/+	8	10.38
+/+ x $1^2/1^2$	2	1.41
1^2/+ x 1^2/+	1	2.81
1^2/+ x $1^2/1^2$	2	0.76
$1^2/1^2$ x $1^2/1^2$	0	0.05
	25	25

$X^2_{5d.f.} = 4.225$ $0.50 < P < 0.70$

Chromosome 4 - inversion $4m^2$

Mating combination	Observed nos.	Expected nos.
+/+ x +/+	24	24.11
+/+ x m^2/+	1	0.88
+/+ x m^2/m^2	0	0.004
m^2/+ x m^2/+	0	0.008
m^2/+ x m^2/m^2	0	0.00006
m^2/m^2 x m^2/m^2	0	0.00000
	25	25.0020

$X^2_{1d.f.} = 0.014$ $0.90 < P < 0.95$

(5 low frequency classes pooled)

TABLE 7 Estimates of the chromosomal heterozygosity (H) of the D. silvestris population in Kilauea Forest. H is given as the population mean for each sample ($\underline{H}$) and also individually for each inversion.

Chromosomal heterozygosity

Inversion / Sample	September 1971	December 1971	May 1972	Average heterozygosity Sept., Dec. and May samples combined	1 χ^2 1 d.f.
2o	0.047	0.038	0.111	0.059	0.057
3m	0.355	0.574	0.390	0.439	4.742*
3r	0	0	0	0	-
$4k^2$	0.468	0.442	0.565	0.488	0.007
4t	0.323	0.365	0.391	0.356	0.079
41^2	0.226	0.250	0.341	0.266	0.006
$4m^2$	0.016	0.019	0.068	0.032	0.348
n	62	52	43	157	
Mean heterozygosity $\underline{H}$	0.2048	0.2414	0.2585	0.2320	

1 - 2 x 2 contingency χ^2 comparison of the September, 1971 and December, 1971 heterozygosities.

* - significant at 0.05

higher frequency of the 3m sequence in the December sample as estimated from total family data (TABLE 2), this difference between samples being close to the borderline of significance (TABLE 4).

Associations of chromosome 4 inversions:

The fourth chromosome of D. silvestris contains four inversions, and when all four are polymorphic within a population, as in the Kilauea Forest population, theoretically there should be 16 different associations of chromosome 4 sequences present within the population. Some of the combinations would be present at extremely low frequencies and may not be found unless very very large samples are taken. An analysis of the inversion associations in the Kilauea Forest population showed that eight fourth chromosome sequences were present in the frequencies given in TABLE 8. The inversion linkage associations in the two samples (September and December 1971) appear to be distributed fairly similarly. A test of their homogeneity in terms of the frequency distributions of the various classes of chromosomal sequences gave a G-value of 17.072, indicating that the two samples are significantly different at the 5% level but not at the 1% level ($X^2_{0.05\ 7d.f.} = 14.067$). No further tests were done to identify the source of this heterogeneity: both samples are comparatively small and several of the eight classes are represented at very low frequencies, so that sampling effects could easily bias any statistical test. However, visual comparison of the two distributions of fourth chromosome sequences suggests that the heterogeneity between them is largely due to the apparent temporal difference in the frequency of the $+ + 1^2 +$ sequence, which, as a matter of fact, is one of the more common associations in the population. This discrepancy could be attributed to the difference in the frequency of the 41^2 sequence between the September and December samples. It has already been noted that the frequency of 41^2 in the September sample is highly anomalous, and this fact is responsible for the frequency difference observed here. In all probability, the constitution of the Kilauea Forest population has remained essentially the same with respect to its inversion associations on chromosome 4, as it has in the frequencies of individual inversions.

b) The chromosomal data from Olaa Forest

Of the several known populations of D. silvestris on Hawaii, that in Olaa Forest is the closest geographically to the population in Kilauea Forest. By contrast with the latter location, D. silvestris is comparatively rare in Olaa Forest. Consequently, sample sizes are always extremely small, and inadequate for

TABLE 8. Associations of chromosome 4 inversions observed in the September, 1971 and December, 1971 samples of D. silvestris from Kilauea Forest.

Association	September 1971 No. of chroms.	September 1971 Frequency	December 1971 No. of chroms.	December 1971 Frequency	Mean frequency
+ + + +	2	0.016	3	0.030	0.022
k^2+ + +	2	0.016	2	0.020	0.018
+ t + +	45	0.362	31	0.319	0.343
+ + 1^2+	10	0.080	15	0.154	0.113
k^2t + +	58	0.467	42	0.432	0.452
k^2+ 1^2+	4	0.032	3	0.030	0.031
+ t + m^2	2	0.016	0	-	0.009
k^2t + m^2	1	0.008	1	0.010	0.009
	124		97		

G_H = 17.072 $\quad$ 0.01 < P < 0.02

statistical manipulations. Nonetheless, it was of interest to use the available data to compare the genetic and chromosomal characteristics of the two adjacent populations.

Since seasonal fluctuations in inversion frequency are apparently negligible in Hawaiian flies (as demonstrated by the Kilauea Forest data), it was considered legitimate to pool the data available from repeated samplings over time in order to estimate the approximate inversion frequencies characteristic of a particular population. Furthermore, where sample sizes are extremely small, total family data are used in preference to first larval data. In a low density population, sexual contacts might be infrequent, and in all likelihood the majority of females would be inseminated only once, if at all. Thus the bias resulting from multiple inseminations can be disregarded in such populations. The data from Olaa Forest were treated in accordance with these assumptions.

The inversion frequency data for the Olaa Forest population of D. silvestris are presented in TABLE 9. Visual comparison of the mean frequencies for each inversion in this population with the mean frequencies in the Kilauea Forest population (TABLE 1) indicates some marked differences in their chromosomal compositions. The major and most reliable differences are in the frequencies of the two chromosome 3 inversions. In the Kilauea Forest population, the standard sequence of the 3m inversion is the more common, with the inverted sequence being found in only 33% of third chromosomes: in the Olaa Forest population, the inverted sequence 3m is by far the more abundant sequence, being present at a frequency of approximately 97%, rather close to fixation. Secondly, the 3r inversion is present at low frequency in the Olaa Forest population, whereas it is completely absent from the Kilauea Forest population, never having been recorded in a total sample of 434 chromosomes scored from this population during this study. The small sample of 30 chromosomes taken from the Olaa Forest population does not permit any further definite statements about chromosomal differences between the two populations. However, it might be surmised that there are additional inversion frequency differences (e.g. divergence in the frequencies of $4k^2$ and $4l^2$) which may eventually be substantiated by collection of more data from Olaa Forest.

c) The allozyme data from Kilauea Forest

The electrophoretic analysis of the Kilauea Forest population of D. silvestris entailed 12 gene loci from 11 enzyme systems, the details of which are given in TABLE 10. Three samples were analyzed, i.e. the September 1971, December 1971 and

TABLE 9. Chromosomal inversion frequencies in the Olaa Forest population of D. silvestris based on total family data.

Date of sample	Ref. No.	No. of chroms. Sampled	Inversion frequencies (%)						
			2o	3m	3r	$4k^2$	4t	$4l^2$	$4m^2$
Dec. 8, 1971	Q55	10	0	100	0	60.0	80.0	0	0
June 26, 1972	R27	12	0	100	8.3	91.7	83.4	0	0
Sept. 25, 1972	R49	8	0	87.5	0	75.0	87.5	0	0
Overall mean frequency		30	0	96.7	3.3	76.7	83.3	0	0

TABLE 10. Enzyme systems and loci studied in the electrophoretic analysis of the Kilauea Forest population of *D. silvestris*.

Buffer system (*cf.* Methods)	Enzyme	Designation	No. of loci studied
A	Leucine aminopeptidase	*Lap*	1
A	Octanol dehydrogenase	*Odh*	1
A or C	Phosphoglucomutase	*Pgm*	1
A or C	Alcohol dehydrogenase	*Adh*	1
C	Malate dehydrogenase	*Mdh*	2
C	Isocitrate dehydrogenase	*Idh*	1
C	Malic enzyme	*Me*	1
C	Glutamate oxaloacetate transaminase	*Got*	2
C	Hexokinase	*Hk*	1
C	α - glycerophosphate dehydrogenase	*α -gpd*	1

May 1972 collections, which together amounted to a total of 604 genomes from the population. (For some of the loci, the total sample sizes were less than this, either because one rather than three samples were analyzed for that locus, or because certain loci could not be scored in a few of the individuals in some samples.) TABLE 11 presents the available allelic frequency data for the twelve loci analyzed from the Kilauea Forest *silvestris* population. Three of the loci (*Pgm-1*, *Me-1* and *Got-1*) were represented in the population as high frequency polymorphisms with more than one allele present at a frequency in excess of 5%. The variation at eight of the remaining loci was that typical of low frequency polymorphisms with one predominant allele, and in addition, one or two minor alleles. The hexokinase locus (*Hk-1*) which is sex-linked in *D. silvestris*, was fixed in all samples for the 1.00 allele.

The genotypic frequency data for the *Pgm-1*, *Me-1* and *Got-1* loci are given in TABLES 12, 13 and 14 respectively, with the data on male and female individuals of each sample shown separately. Only these three loci which show significant heterozygosity were suitable for detailed statistical analyses. Comparisons of the allozymic variation between males and females of each sample and the total heterogeneity between males and females for each of the three loci were evaluated by estimation of the contingency X^2 values, applying Yates' correction where necessary. For the *Pgm-1* locus (TABLE 12), there was no significant heterogeneity between males and females in their genotypic distributions within each of the individual samples. However, when all samples were pooled, some heterogeneity between males and females became apparent with the difference being barely significant at the 5% level and most probably of no biological importance. The data from the single sample available for the *Got-1* locus similarly showed no meaningful differences between male and female individuals with respect to their genotypic arrays at this locus (TABLE 14). Although the male frequency for the common allele ($Got\text{-}1^{1.00}$) was considerably higher than that for the females, the corrected Chi Square was not significant at the 5% level.

Only the *Me-1* locus showed a significant overall difference between males and females which could be attributed to discrepancies between the sexes in one of the three samples, that from the September 1971 collection (TABLE 13). In this sample, the frequency of $Me\text{-}1^{1.05}$ in males was significantly higher than the frequency of this allele in females, due mainly to an excess of 1.05/1.05 homozygotes. Since this effect was not observed in either of the two following samples, it is almost certainly not a constant feature of this locus in the Kilauea Forest population.

TABLE 11 Allozymic variation at twelve loci in the D. silvestris population from Kilauea Forest Reserve.

Locus and Alleles	Allelic frequencies in samples taken at different times			
	9/71	12/71	5/72	Total
Pgm-1	(104)[1]	(156)	(332)	(592)
1.15	-	0.013	0.012	0.010
1.10	-	-	0.006	0.003
1.05	0.144	0.071	0.108	0.105
1.00	0.759	0.814	0.747	0.767
.95	0.038	0.025	0.045	0.039
.90	0.058	0.077	0.081	0.076
Me-1	(106)	(152)	(326)	(584)
1.05	0.358	0.263	0.263	0.265
1.00	0.632	0.724	0.748	0.721
.90	0.009	0.013	0.015	0.014
Got-1	-	-	(346)	(346)
1.05	-	-	0.355	0.355
1.00	-	-	0.636	0.636
.95	-	-	0.009	0.009
Got-2	-	-	(346)	(346)
1.05	-	-	0.029	0.029
1.00	-	-	0.946	0.946
.95	-	-	0.026	0.026
Adh-1	(106)	(152)	(346)	(604)
1.05	0.009	-	0.006	0.003
1.00	0.972	1.00	0.945	0.969
.95	0.019	-	0.049	0.028
Mdh-1	(106)	(152)	(346)	(604)
1.00	0.991	1.00	0.991	0.993
.95	0.009	-	0.006	0.005
.90	-	-	0.003	0.002
Mdh-2	(106)	(152)	(346)	(604)
1.05	-	0.007	0.003	0.005
1.00	1.00	0.993	0.997	0.997
∝-gpd-1	(106)	(152)	(346)	(604)
1.00	1.00	1.00	0.997	0.998
.95	-	-	0.003	0.002
Lap-1	(106)	(152)	(346)	(604)
1.00	1.00	1.00	0.997	0.998
.95	-	-	0.003	0.002
Idh-1	(106)	(152)	(346)	(604)
1.05	-	0.013	0.003	0.005
1.00	1.00	0.987	0.997	0.995
Hk-1	(82)	(97)	(240)	(419)
1.00	1.00	1.00	1.00	1.00
Odh-1	-	-	(346)	(346)
1.05	-	-	0.003	0.003
1.00	-	-	0.997	0.997

1 Number of genomes sampled is enclosed in parenthesis.

TABLE 12 Temporal variation at the Pgm-1 locus in the D. silvestris population from Kilauea Forest Reserve.

Sample Subdivisions	Genotypic frequencies												
	1.15/ 1.00	1.10/ 1.00	1.05/ 1.05	1.05/ 1.00	1.05/ .95	1.05/ .90	1.00/ 1.00	1.00/ .95	1.00/ .90	.95/ .95	.95/ .90	.90/ .90	Totals
9/71													
♀	-	-	-	1	2	3	19	1	2	-	-	-	28
♂	-	-	-	9	-	-	13	1	1	-	-	-	24
Total	-	-	-	10	2	3	32	2	3	-	-	-	52
12/71													
♀	-	-	1	2	-	-	14	1	3	-	-	-	21
♂	-	2	1	2	-	3	41	3	4	-	-	1	57
Total	-	2	2	4	-	3	55	4	7	-	-	1	78
5/72 ♀	-	-	1	12	-	2	28	4	12	1	-	-	60
♂	2	4	-	19	1	-	62	5	10	-	3	-	106
Total	2	4	1	31	1	2	90	9	22	1	3	-	166
Total													
♀	-	-	2	15	2	5	61	6	17	1	-	-	109
♂	2	6	1	30	1	3	116	9	15	-	3	1	187
Total	2	6	3	45	3	8	177	15	32	1	3	1	296

Heterogeneity χ^2 for temporal variation in females = 2.01, df = 2

Heterogeneity χ^2 for temporal variation in males = 0.86, df = 2

Heterogeneity χ^2 for temporal variation in total samples = 6.27, df = 6

χ^2 for male vs. female heterogeneity in sample taken 9/71 = 0.0003, df = 1

χ^2 for male vs. female heterogeneity in sample taken 12/71 = 0.02, df = 1

χ^2 for male vs. female heterogeneity in sample taken 5/72 = 3.41, df = 3

χ^2 for total male vs. female heterogeneity = 3.85, df = 3

TABLE 13 Temporal variation at the Me-1 locus in the D. silvestris population from Kilauea Forest Reserve.

Sample Subdivisions	Genotypic frequencies					Allelic frequencies		
	1.05/1.05	1.05/1.00	1.00/1.00	1.00/.90	Totals	1.05	1.00	.90
9/71								
♀	-	15	14	-	29	0.259	0.741	-
♂	6	11	6	1	24	0.479	0.506	0.021
Totals	6	26	20	1	53	0.358	0.632	0.009
12/71								
♀	1	8	11	1	21	0.238	0.738	0.024
♂	5	20	29	1	55	0.273	0.718	0.009
Totals	6	28	40	2	76	0.263	0.724	0.013
5/72								
♀	4	13	39	1	57	0.184	0.807	0.009
♂	9	38	55	4	106	0.264	0.717	0.019
Totals	13	51	94	5	163	0.263	0.748	0.015
Totals								
♀	5	36	64	2	107	0.215	0.776	0.009
♂	20	69	90	6	185	0.295	0.689	0.022
Totals	25	105	154	8	292	0.265	0.721	0.014

χ^2 for temporal variation in females = 1.38, df = 2

χ^2 for temporal variation in males = 9.21, df = 2 ***

χ^2 for temporal variation in total = 5.39, df = 2

χ^2 for male vs. female heterogeneity in sample taken 9/71 = 6.58, df = 1**

χ^2 for male vs. female heterogeneity in sample taken 12/71 = 0.06, df = 1

χ^2 for male vs. female heterogeneity in sample taken 5/72 = 3.19, df = 1

χ^2 for total male vs. female heterogeneity = 5.04, df = 1**

*** = signif. at 0.01
** = signif. at 0.025
* = signif. at 0.050

TABLE 14 Allozymic variation at the Got-1 locus of D. silvestris in the May, 1972 sample from Kilauea Forest Reserve.

Sample Subdivision	Genotypic Frequencies						Allelic frequencies		
	1.05/1.05	1.05/1.00	1.05/.95	1.00/1.00	1.00/.95	Total	1.05	1.00	.95
5/72									
♀	12	31	1	23	-	67	0.418	0.575	0.007
♂	6	55	-	43	2	106	0.316	0.675	0.009
Total	18	86	1	66	2	173	0.355	0.636	0.009

χ^2 for male vs. female heterogeneity in allele frequencies = 3.12, df = 1

For the moment, the reasons for the observed male-female heterogeneity in the September 1971 sample are a matter for speculation. This sample was the smallest of the three, involving only 24 males, and it is conceivable that the bias results from sampling effects.

The mating structure of the Kilauea Forest population as revealed by the allozymic data proved not to depart from normal random mating, in agreement with the indications given by the chromosomal data (cf. TABLES 5 and 6). When the observed genotypic distributions at several loci were compared with the expected Hardy-Weinberg frequencies, all were found to be consistent with expectation.

Temporal variation:

Of the nine loci sampled repeatedly in this study, only two (Pgm-1 and Me-1) showed sufficient heterozygosity to make statistical tests of the temporal variation worthwhile. For the remaining seven loci, visual comparisons of the data from successive samples showed them to be more or less consistent, with the same allele predominating in all samples for every locus (TABLE 11). In some of these low frequency polymorphisms, the minor alleles were only present in the larger samples. Nonetheless, there was no strong evidence of any major frequency fluctuations at these loci throughout the course of the study. The hexokinase (Hk-1) locus also showed no temporal variation, remaining fixed for the 1.00 allele in all samples.

At the Pgm-1 locus, statistical comparisons of successive samples showed no temporal variation between the total samples, neither for males nor for females of the various samples (TABLE 12).*

At the Me-1 locus, the comparisons showed no temporal variation in the total samples, or in the female component of all samples (TABLE 13). However, there was significant temporal heterogeneity between the successive male samples, which could be attributed to the anomalous male data of the September 1971 sample. Disregarding this subsample, the data from the Me-1 locus agree with data from all other loci tested in indicating that the allozyme frequencies in the Kilauea Forest population of D. silvestris remained effectively stable over the time period studied.

Genic heterozygosity:

The heterozygosities at each locus were calculated from the raw genotypic data as the proportion of individuals in the total samples which were heterozygous at that locus. The data are presented in TABLE 15, together with the unweighted mean

* Because the Pgm-1 locus has six alleles, some of which occur at very low frequencies, a collapsing Chi Square statistic was applied, with the collapsing accomplished by repeated pooling of the less frequent allele categroies until a minimum of five observations were in each block of the contingency table. This procedure necessarily affected the degrees of freedom, as evident in TABLE 12.

TABLE 15. Heterozygosity at twelve loci in the D. silvestris population from the Kilauea Forest Reserve.

Locus	Sample size	Number of Alleles	Proportion of Individuals heterozygous
Pgm-1	296	6	0.385
Me-1	292	3	0.387
Got-1	173	3	0.514
Got-2	173	3	0.098
Adh-1	302	3	0.056
Mdh-1	302	3	0.013
Mdh-2	302	2	0.007
α-gpd-1	302	2	0.003
Lap-1	302	2	0.003
Idh-1	302	2	0.010
*Hk-1	302	1	0.000
Odh-1	173	2	0.006
		unweighted mean =	0.124

* Hk-1 is sex-linked

of the individual heterozygosity values. The highly polymorphic loci (Pgm-1, Me-1 and Got-1) and those with more than two alleles make the greatest contribution to the mean genic heterozygosity which takes the value 0.124 for this population of D. silvestris in Kilauea Forest.

Complete data on the heterozygosities at three loci are given in TABLE 16 for each of the samples and also individually for males and females of each subsample. There are no significant differences between the heterozygosity levels in males and females for any of the samples from all three loci. The temporal variation in genic heterozygosity was tested for at the Pgm-1 and Me-1 loci. In the case of the Pgm-1 locus, the tests indicated no significant temporal variation between successive male subsamples, nor between successive female subsamples, although both showed considerable variation which must have contributed to the apparent overall temporal variation in heterozygosity at the Pgm-1 locus. In the case of the Me-1 locus, the tests showed no overall temporal variation in heterozygosity, not any effective variation in the heterozygosity of males, but rather, significant variation in the heterozygosity levels of females due to successive decreases between the three samples. This result was unexpected in view of the data presented in TABLE 13 which had shown the gene frequencies in females to be relatively constant over time, whereas the frequencies in males had demonstrated substantial variability due to the irregular results of the September 1971 sample. If anything, it might have been predicted that the male heterozygosity levels should show temporal variation while the female heterozygosity levels remained the same. This inconsistency in the heterozygosities at the Me-1 locus can only be attributed to sampling errors.

Although the total numbers of silvestris adults sampled from the Kilauea Forest are substantial, some of the individual samples (especially the September 1971 sample) are not particularly large, and this could at times distort the statistical analysis. This is unfortunate since our statements as to the temporal genetic variation in Kilauea Forest must remain in part provisional until larger samples have been analyzed.

DISCUSSION

The analysis of the allozyme data and the chromosomal data from successive samples of D. silvestris from the Kilauea Forest population provide no strong evidence for the occurrence of temporal changes in the genetic composition of this population. Although there are a few inconsistencies in the allozyme data, in

TABLE 16 Heterozygosity at three loci in D. silvestris from the Kilauea Forest population.

Sample	Subsample	Proportion of Individuals Heterozygous (sample size) Pgm-1	Me-1	Got-1
9/71	Female	0.321 (28)	0.517 (29)	-
	Male	0.458 (24)	0.500 (24)	-
	Total	0.370 (52)	0.509 (53)	-
12/71	Female	0.286 (21)	0.429 (21)	-
	Male	0.246 (57)	0.382 (55)	-
	Total	0.256 (78)	0.395 (76)	-
5/72	Female	0.500 (60)	0.246 (57)	0.478 (67)
	Male	0.415 (106)	0.396 (106)	0.538 (106)
	Total	0.446 (166)	0.344 (163)	0.514 (173)
Total	Female	0.413 (109)	0.355 (107)	-
	Male	0.369 (187)	0.405 (185)	-
	Total	0.385 (296)	0.387 (292)	-

χ^2 for temporal variation in heterozygosity in females Pgm-1 = 4.246, df=2; Me-1=6.808*, df=2
χ^2 for temporal variation in heterozygosity in males Pgm-1 = 5.517, df=2; Me-1=1.055, df=2
χ^2 total temporal variation in heterozygosity Pgm-1 = 8.036**, df=2; Me-1=4.665, df=2

χ^2 for male vs. female heterozygosity in sample 9/71 for Pgm-1 = 0.527, df=1; Me-1=0.023, df=1
χ^2 for male vs. female heterozygosity in sample 12/71 for Pgm-1 = 0.005, df=1; Me-1=0.012, df=1
χ^2 for male vs. female heterozygosity in sample 5/72 for Pgm-1 = 0.801, df=1; Me-1=3.090, df=1; Got-1=.282, df=1

χ^2 for male vs. female heterozygosity in total sample for Pgm-1 = 0.389, df=1; Me-1=0.526, df=1

** significant at 0.025
* significant at 0.050

general, the allelic frequencies at all twelve loci studied remained effectively constant over the nine month period for which samples were analyzed electrophoretically. The heterozygosities at two of the more variable loci also remained more or less uniform. There were however, some minor fluctuations between samples and also some unexpected discrepancies in the allozyme data which undoubtedly result from sampling effects as discussed more fully below.

For the chromosomal inversion polymorphisms, the data showed that there was no major seasonal variation in inversion frequencies in D. silvestris equivalent to that observed in some mainland species of Drosophila. Successive samples taken from Kilauea Forest were effectively homogeneous in several chromosomal characteristics i.e. in inversion frequencies, in heterozygosities, in inversion associations, and also with respect to their mating characteristics. However, despite the overall temporal stability of the Kilauea Forest population, some of the inversions showed minor fluctuations in frequency, some of which approached the borderline of significance when frequency estimates were derived from family data. It is notable that the variability in chromosomal characteristics was on the average greater than that observed in the enzyme systems. The apparent inversion frequency differences were responsible for most of the other differences found in chromosomal heterozygosities and in inversion associations. The 3m polymorphism was the most variable of all, with the 4t and $4l^2$ polymorphisms also showing some deviations during the study period. The remaining sequences - 2o, $4k^2$, $4m^2$ (and the fixed standard sequence of 3r) - were homogeneous in frequency for all the samples and did not appear to show any temporal variation.

The apparent lack of seasonal variation in the genetic characteristics of this population of D. silvestris should not be interpreted to mean that this population is not adapted to its environment, nor should it be implied that the chromosomal polymorphisms have no role to play in this species. There are many other Drosophila species in which the chromosomal polymorphisms are "rigid" (cf. Dobzhansky, 1962) and not subject to cyclic seasonal fluctuations. Furthermore, the tropical environment is by no means comparable to the temperate environments occupied by the continental Drosophila species which display seasonal genetic changes in their populations. The climatic fluctuations in Hawaiian environments are certainly much less extreme than those of temperate environments. In Kilauea Forest, seasonal changes are minimal with only very small differences in mean temperature and humidity between summer and winter, and these seasonal changes may even be exceeded at times by much shorter-term variations which occur sporadically rather than in a regular

seasonal cycle. By all accounts, the Kilauea Forest environment could be classed as a relatively stable one, subject to only minor changes of irregular occurrence. Thus if the environment does not change dramatically, then there would be no pressure on the population to modify its chromosomal and genetic composition in response to demands which do not exist.

A more compelling explanation for the observed lack of seasonal fluctuations in populations of this species relates to its life cycle pattern. D. silvestris is a large and long-lived fly, adults usually surviving for many months. Their longevity in the field would probably be almost equivalent to that in the laboratory, and it is estimated that in the field, there may be only two or perhaps three generations per year, although generations would necessarily be overlapping. This contrasts with most other smaller Drosophila species which have much shorter life cycles, and in which populations may pass through many more generations in the course of a year, and several generations during one particular season. D. silvestris being a species with a much longer generation cycle would not necessarily be expected to be capable of a rapid genetic response to brief changes in environmental conditions. This is in accord with an observation made by Brncic (1970) that seasonal fluctuations in chromosomal polymorphisms may be found "only in species that have rapid developmental cycles and reproduce over many months of the year."

Only two other Hawaiian Drosophila species have been investigated so far with regard to seasonal genetic changes. D. mimica, a species from Hawaii belonging to the modified-mouthparts group, was sampled regularly over an eight month period and the polymorphisms in three enzyme systems studied (Rockwood, 1969). The allele frequencies at two of the loci remained stable, but at the third locus, Acph, significant allele frequency changes were recorded in one population but not in another. The pattern of allelic changes at this locus has been confirmed in a recent, more detailed study of this species (Steiner, unpublished data). This study has also demonstrated significant temporal heterogeneity at five additional loci in D. mimica, and at four loci in populations of the picture-winged species D. engyochracea, also found on Hawaii. The variation at some of these loci shows a short-term cyclical pattern; at one particular locus, the allelic frequency changes appear to be long-term and directional, whilst at the remaining loci, the variations show no consistent pattern. By contrast to D. silvestris, both D. mimica and D. engyochracea are dry-forest species which experience much more variable environments than that of Kilauea Forest. Populations of both these dry-forest

species apparently respond genetically to the seasonal and sporadic fluctuations to which they are exposed in constantly adapting to their environments.

With regard to the chromosomal data obtained from the Kilauea Forest population of D. silvestris, several comments should be made and discussed in greater detail. The first pertains to the differences in inversion frequencies when estimated by the first larval method vs. the total family method. Almost all of the apparent departures from stability arise from data based on the second method (but these kinds of information can only be obtained in this way). The first larval method is unquestionably the more accurate: the total family method is less accurate, being subject to bias from two sources. These derive from the possibility of multiple inseminations as already discussed, and secondly, from possible misinterpretations of progeny segregations. In most cases, seven larvae are adequate to discriminate between a single class result, a 1:1 or a 1:2:1 chromosomal segregation. However, when only seven larvae are scored, errors could arise from normal sampling effects, and also wherever there are substantial differences in the developmental rates of the various genotypes. Sometimes in a three-class segregation, only two of the genotypes appear amongst the first seven larvae, and deductions as to the parental genotypes based on these data alone would accordingly be inaccurate. Whenever there was doubt about the result, several additional larvae were scored if at all possible. This often resolved the question and provided the necessary positive evidence on parental genotypes, eliminating a possible error in the inversion frequency estimates for that sample. Despite these precautions, some bias is inevitable with this method of deduction, and this can lead to discrepancies as great as those found in the two frequency estimates for each of the first three samples (cf. TABLES 1 and 2).

The second comment relates to the nature of the observed temporal fluctuations in inversion frequencies. The data show some variations for some of the inversions, although these changes are mostly too small to be statistically significant. Such fluctuations could well be an artefact resulting from sampling procedures, as indicated earlier. However, the possibility remains that they could be biologically real. At present there can be no discrimination between these two explanations. The observed frequency fluctuations appear to be at random, and not particularly related to climatic variations. However, they could conceivably be due to other factors, e.g. changes in population size. Although D. silvestris seems to maintain a relatively large population in Kilauea Forest, it is almost certain that factors such as the availability of suitable breeding substrates would cause some

fluctuations in density. Since each silvestris female can oviposit several hundred eggs, populations of silvestris have a great capacity for increase under favorable conditions. Within a few months there could be dramatic changes in population density, and probably there would be some associated changes in inversion frequencies. Since no estimates of population size were undertaken in this study, it is impossible to say whether density differences may have contributed to the observed variations in inversion frequencies, but this possibility should be kept in mind. In D. flavopilosa, Brncic (1972) found that cyclical changes in chromosomal inversion frequencies corresponded to fluctuations in population density. However, he considered the correlation trivial, and interpreted it as an independent response by both chromosomal frequencies and density to the weather conditions, and especially to variations in temperature.

The spatial distribution of flies throughout the forest should also be considered as another possible source of the observed minor variations in both the chromosomal and the allozyme data, since the distribution of the population may have significant effects on sampling. Kilauea Forest is an extensive forested area and even if total population size remains constant, the numbers of flies at a particular point within the forest may change significantly with time. The distribution of feeding and oviposition sites varies more or less randomly with time, and a species like D. silvestris which shows relatively high mobility would probably respond to these environmental changes by moving to the most favorable site.

There is another anomaly in the chromosomal data which deserves comment. In view of the apparent internal homogeneity in inversion frequencies of all samples considered together (cf. TABLE 3), it was somewhat unexpected that comparisons between the first and last samples should demonstrate a shift in frequency for one of the inversions, 4t (cf. TABLE 4). This sequence appeared to have undergone a significant decrease in frequency over the time of the investigation. Remarkably, this frequency difference was obtained using estimates from first larval data. As pointed out, this apparent shift in frequency could be accounted for by the difference between the first and second samples (i.e. September and December 1971), so probably did not represent a true and long-term trend towards a decrease in frequency of the 4t sequence. Moreover, the remaining inversions were stable in initial and final frequencies, and these included the other fourth chromosome inversions which may have been expected to show correlated changes because of the linkage relationships between them. Thus it is concluded that there has probably been no shift in the inversion frequencies of this silvestris population during the course

of this study; it has remained quite stable in its chromosomal composition, showing neither major cyclic fluctuations nor indications of progressive long-term changes.

The previous consideration of the possibility of a shift in the frequency of the 4t inversion suggested by the data, draws attention to the difference between the September 1971 and December 1971 samples. Although these are consecutive samples, the difference between them is perhaps greater than the differences between any other pairs of samples. There are in addition several other pieces of data which reflect this difference, and tend to indicate that the September 1971 sample is the one which shows least agreement with all the other samples taken from Kilauea Forest. The frequency of the 41^2 sequence for example, is much lower in the September sample than usual, whilst the frequency of 4t is too high. At the Me-1 locus, the frequency of the 105 allele is in excess in males, but not in females - a difference not shown by any of the following samples. The September sample was a composite one collected by six collectors at different sites and at slightly different altitudes within the forest. The only explanation that can be offered is to suggest that there is local variation within the population in its chromosomal and genetic composition, and that this variation was sampled unevenly, so that the final sample did not represent a true random sample from the population. The validity of this explanation can only be tested by further studies of this Kilauea Forest population involving larger samples from several defined and restricted areas within the forest to see whether there is indeed significant local population subdivision, or whether all the deviations can be attributed merely to sampling effects. The results from the Me-1 locus are best interpreted in terms of the latter explanation. Possibly all of the males collected from one site were sibs from a single family (carrying the 1.05 allele) aggregated together because of the clustering of lek sites shown by males. Females which do not engage in this behavior probably disperse randomly away from the emergence site, and thus rarely contribute to local differences in allele frequencies.

The comparison of the chromosomal compositions of the Kilauea Forest population (TABLE 1) and the adjacent Olaa Forest population (TABLE 9) showed that they were markedly different, varying significantly in the frequencies of several inversion sequences. Lack of adequate allozyme data from Olaa Forest prevented a similar genic comparison. The observation of chromosomal variation between adjacent silvestris populations has been repeated for all of the known populations of the species on Hawaii (Craddock and Johnson, in preparation). The chromosomal polymorphisms show many qualitative and quantitative differences between the various

populations, so that each appears to be a discrete genetic entity with a unique chromosomal composition. Currently there seems to be little gene flow between populations even when these are geographically very close. This population structure has important implications for the dynamics of genetic variation within the species and for its future evolution. The chromosomal polymorphism which characterizes present populations may well be vitally involved in any further changes in the species.

Finally, the question could be posed as to whether the chromosomal polymorphisms in D. silvestris are "flexible" i.e. with geographic and seasonal fluctuations in the frequencies of the various gene arrangements, or "rigid," without such fluctuations (cf. Dobzhansky, 1962). Although this endemic species has a relatively restricted range, there is good evidence of substantial geographic variation between its various populations in their chromosomal compositions (as cited above), but there is so far no evidence of any seasonal fluctuations in any of these chromosomal polymorphisms. The question may be inappropriate (or the terms ill-defined), since it has now been shown that within a single species, Drosophila pseudoobscura, there are neighboring populations in which the third chromosome polymorphisms show either a seasonally rigid or alternatively a flexible pattern (Crumpacker and Williams, 1974; and references cited therein). Thus the chromosomal polymorphisms characteristic of a particular species may behave independently in each population. In the case of the Kilauea Forest population of D. silvestris then, it appears that the chromosomal polymorphisms are seasonally rigid, but this may only be a reflection of the relative stability of this particular environment.

SUMMARY

The Kilauea Forest population of Drosophila silvestris was found to be highly stable in its genetic and chromosomal composition. The polymrophisms at eleven gene loci and for six chromosomal inversions in this population maintained more or less uniform frequencies over the entire period the population was under investigation, and one gene locus and one chromosome sequence remained monomorphic throughout. Contrary to the situation in some mainland Drosophila species, there was no evidence for seasonal fluctuations in relative frequencies of the various gene arrangements of this tropical species. This lack of temporal variation is not unexpected considering the apparent stability of the ecosystem of which this population is a part, together with the comparatively long life cycle of this endemic Hawaiian Drosophila species.

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