VARIATION IN CHROMOSOMAL POLYMORPHISM IN HAWAIIAN POPULATIONS OF *DROSOPHILA IMMIGRANS*¹⁾

YONG K. PAIK AND KEE C. SUNG

Department of Genetics, University of Hawaii,
Honolulu, Hawaii 96822, U.S.A.

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There is very little information on chromosomal polymorphism in "domestic" species of *Drosophila* probably because workers have been unwilling to study this problem because most of these species have small numbers of inversions at low frequencies (Carson 1965); adaptive role of the inversion polymorphism in this group remains rather speculative.

D. immigrans, virtually a domestic and cosmopolitan species, is now found in semi-wild habitats on most continents and in natural states such as in Chile (Brncic 1970) and in the Hawaiian islands (Paik and Sung 1972; others). Several different workers from their studies on the chromosomal polymorphism in this species have shown some evidence of geographic variations in the relative frequencies of the same polymorphic inversions (see Discussion). The need of further studies on the local variations is obviously indicated.

In a large breeding population the frequencies of such "widespread" inversions are determined by selection and migration. But in a small, island-type population, or in one subject to changes in climatic and ecological conditions, the frequency changes due to drift are also important. Apparently, work needs to be done along this line.

In this communication we present the chromosomal polymorphism found in natural populations of *D. immigrans* on three main islands of Hawaii. First, the data obtained from dissimilar habitats within island are compared with each other. Secondly, the differences between different islands, and between the islands and continental populations are discussed.

MATERIALS AND METHODS

D. immigrans is very common in the Hawaiian islands. It is adapted not only for breeding in introduced plants, but also for breeding in niches with endemic plants. Its vertical distribution extends up to about 7000 feet elevation, but at low altitudes near sea level it is rare. This species seems then to have a preference for cold areas. Samples were collected by yeasted banana baits from the islands of Oahu, Hawaii and Kauai (Fig. 1).

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Fig. 1. Map of major islands of Hawaiian archipelago. Arabic figures show the channel distances in miles.

The Oahu samples were from three distinct habitats: the first population (OT-70no) was sampled in late November, 1970, on Mt. Tantalus at 1700 feet; a second collection from this area (OT-71ja) was made in mid-January, 1971. High humidity characterizes the climate of this mountain; characteristic trees of this area are the introduced guava, avocado and christmas berry. Another sample (OM-70oc) was collected at Mauna Kapu at 2300 feet in late October, 1970; a second collection (OM-71ja) and a third collection (OM-71mc) were made in early January and March, 1971 respectively. *Eugenia* and *Schinus* are most common trees, with a few guava trees in a small area. A final sample (OP-71mc) was taken in mid-March, 1971 at Puu Keaau at 1000 feet. Cactus (*Opuntia*) forms the characteristic vegetation of this mountain.

In early April and late December, 1971, populations were sampled on the island of Hawaii along the Mauna Loa Transect and the Kilauea Rain Forest. These samples will be referred to as HS- and HK- respectively. In the first collecting area, samples were taken at six elevations extending from 3000 to 6700 feet; in the Kilauea Forest at 5300 feet. The biotic characteristics of Mauna Loa Transect by elevations are: from 4000-4300 feet, savana ecosystem is formed by *Acacia koa-Sapindus* trees, with scattered *Rubus* colonies. From 5100-6100 feet, a mountain parkland ecosystem is formed by *Acacia koa* colonies, *Styphelia-Dodonea-Vaccinium* scrub communities, and scattered *Rubus*. From 6700-7400 feet, there is an open subalpine *Metrosideros-Coprosma-Vaccinium* scrub forest. In the Kilauea Forest, *Acacia koa* and *Metrosideros* are common, with the tree fern (*Cibotium*), a native *Rubus* and *Ilex* found throughout the forest.

The Kauai samples were taken in the Kokee area at two nearby locations in late January, 1972 and in mid-February, 1973: one collection at Halemanu (at 3800 feet) and the other at Berry Flat Trail (at 4000 feet). *Acacia koa* is the most common tree in Halemanu; *Metrosideros* is predominant in Berry Flat, and blackberries are found scattered throughout both areas. The Halemanu and Berry Flat samples will be referred to as KH- and KT- respectively.

There were two analytical techniques used. The majority of the results were obtained by performing cytological studies on the wild-caught females. Salivary

chromosomes of one F_1 larva were smeared with the aceto-lacto-orcein from each of the female lines, and the slides were fully read for the presence of both hetero- and homokaryotypes ("egg samples"). The other technique was performed by mating the wild males singly to virgin laboratory females homozygous for the "standard" gene arrangement. In this case, salivary chromosomes of 7 F_1 larvae were smeared from each cross ("male samples"). The cultures were kept at $23 \pm 2^\circ\text{C}$ throughout the experiment.

RESULTS

The salivary chromosomes in our samples showed 5 long arms and 1 dot, *i.e.*, a "type 1" configuration; five kinds of Chromosome II inversions were detected from the present study. Three of the five are identical to those which were previously reported by other workers; they are denoted as inversions "A", "B" and "C" in this paper, following the notation used by Brncic. Inversion A is a long median one of the left arm; B is a small subterminal inversion of the right arm; C is a small proximal one of the right arm. One of other two inversions was found from the April sample of Kilauea Forest and the other from the December sample of the HS-5100' population. The new HK-5300' inversion is pericentric, relatively short and symmetric about the centromere, and the HS-5100' inversion is present near the proximal region of the right arm and forms a complex inversion with the inversion C. Each of these new inversions was detected only once. But it seems unlikely that they arose in the laboratory because the F_1 larvae were analyzed shortly after the collection of their mothers. In addition, the new HS-5100' inversion has been found occasionally in our more recent collections in the Mauna Loa Transect.

Table 1 shows the actual frequencies of various heterozygotes for the three common inversions obtained from the egg samples of all collections. From this table one can easily compile the separate frequencies of individuals which are heterozygous for a single inversion for further analysis.

On Oahu, heterokaryotype A (19-28%) was the most common, followed by B (2-9%), and C (0-1.6%) was absent or rare. The relative frequency of three heterokaryotypes is very similar in the samples taken at different times in OT or OM. This indicates stability of the heterokaryotype frequencies throughout the period during which the populations were studied. The frequencies of the total A and total B heterozygous inversions in the two combined OT's, three combined OM's and one OP populations are statistically similar ($P=0.28$ by the chi-square test). The plural (*e.g.*, OT's) is used in the text of this paper and in the tables for samples which have been combined because of statistical homogeneity. The t-test for the mean frequency of heterozygous inversions per larva indicates that the amount of the polymorphism in the OM's (0.35) is significantly higher than that of OT's (0.27) at the 5% level ($t=2.5$, $df=452$).

In HS-4000', HS-5100' and HK-5300' on Hawaii, the frequency of heterokaryotype A (25-42%) ranks first, with B (17-22%) and C (8-22%) having a similar frequency. The mean frequency of heterozygosity per larva in these populations (0.61-0.73) at least doubles those of the Oahu populations. As shown in Table 1, HK-5300'ap is the smallest

Table 1. Frequency of inversion heterozygotes and average number of heterozygous inversions per larva, measured by the egg sample technique

Population	Larvae tested	% Inversion heterozygotes							Average number per larvae (SE)*
		A	B	C	A+B	A+C	B+C	A+B+C	
O T-70no	83	25.3	2.4	1.2	—	—	—	—	0.29±0.05
O T-71ja	120	18.3	3.3	0.8	0.8	—	—	—	0.24±0.04
OM-70oc	53	24.5	1.9	—	1.9	1.9	—	—	0.34±0.08
OM-71ja	158	22.8	6.3	1.3	2.5	1.3	0.6	—	0.39±0.04
OM-71mc	40	22.5	5.0	—	2.5	—	—	—	0.32±0.08
O P-71mc	54	16.7	5.6	—	3.7	1.9	—	—	0.33±0.08
All Oahu:	508	21.6	4.3	0.8	1.8	0.8	0.2	—	0.32±0.02
H S-4000'ap	67	17.9	10.4	16.4	6.0	—	6.0	—	0.69±0.08
H S-4000'dc	167	17.4	9.0	15.6	6.0	0.6	3.0	0.6	0.63±0.05
H S-5100'ap	101	32.7	9.9	6.9	5.9	3.0	3.0	—	0.73±0.06
H S-5100'dc	90	30.0	10.0	4.4	7.8	3.3	—	1.1	0.71±0.08
H K-5300'ap	10	10.0	10.0	10.0	—	10.0	—	—	0.50±0.22
H K-5300'dc	49	22.4	8.2	10.2	10.2	6.1	—	—	0.73±0.14
H S-4300'ap	12	25.0	—	8.3	—	—	—	—	0.33±0.14
H S-6100'ap	11	36.4	—	9.1	—	—	—	—	0.45±0.16
H S-6700'ap	15	26.7	—	6.7	6.7	—	—	—	0.47±0.16
H S-3000'ap	24	12.5	—	8.3	4.2	—	4.2	—	0.38±0.13
All Hawaii:	546	23.3	8.4	10.8	6.2	2.0	2.4	0.4	0.65±0.02
K H-72ja	53	5.7	11.3	13.2	—	—	1.9	—	0.34±0.07
K H-73fb	201	7.4	3.5	20.0	—	1.0	0.5	—	0.34±0.02
K T-72ja	37	13.5	2.7	13.5	2.7	—	—	—	0.35±0.09
K T-73fb	187	11.2	5.3	16.6	0.5	0.5	3.2	—	0.42±0.04
All Kauai:	478	9.2	5.0	17.4	0.4	0.6	1.7	—	0.37±0.03

* Number of heterozygous inversions per larva.

of all the samples, and its data are used on a pooled basis for a statistical reason to be discussed soon. HS-3000'ap, HS-4300'ap, HS-6100'ap and HS-6700'ap appear to be unusual in that Inversion B is absent or rare. What significance is to be placed on these observations is not clear, however. A sample size of each of the four is apparently too small to warrant their inclusion in the other analysis. In each of the adequately sampled populations, the two samples taken eight months apart showed no significant changes in the frequency of heterokaryotypes: the chi-square test for the April and December samples in HS-4000' and HS-5100' shows a P value greater than 0.7 in both cases; the variations between the two HK-5300' samples arose mainly from the excess of heterokaryotype A in the December sample and its deficit in the small April sample, but the difference turns out to be insignificant ($P=0.17$ by the Fisher-Irwin's hypergeometric distribution test). All of these suggest the constancy of heterokaryotype frequencies in one locality over the period of eight months. On the contrary, the relative frequencies of heterozygous inversions between HS-4000's and HS-5100's were highly nonuniform ($P<0.001$). The frequencies in HK-5300's do not appear like

Table 2. Frequency of inversion homozygotes and gene arrangement

Population	% Homozygote			% Arrangement		
	A/A	B/B	C/C	A	B	C
OT-70no	—	—	—	12.7	1.2	0.6
OT-71ja	2.5	—	—	12.1	2.1	0.4
Pooled:	1.5	—	—	12.3	1.7	0.5
OM-70oc	5.7	—	—	19.8	1.9	0.9
OM-71ja	2.5	—	—	15.8	4.7	1.6
OM-71mc	2.5	—	—	15.0	3.8	—
Pooled:	3.2	—	—	16.5	4.0	1.2
OP-71mc	1.9	—	—	12.9	4.6	0.9
HS-4000'ap	3.0	—	—	14.2	11.2	11.2
HS-4000'dc*	2.2	0.9	0.4	15.9	9.7	9.0
Pooled:	2.4	0.7	0.3	15.5	10.0	9.5
HS-5100'ap	4.0	—	—	24.7	9.4	6.4
HS-5100'dc	2.2	—	—	23.3	8.9	3.9
Pooled:	3.1	—	—	24.1	9.2	5.2
HK-5300'(ap+dc)	—	—	—	17.8	8.5	8.5
KH-72ja*	2.3	—	—	7.4	6.8	9.7
KH-73fb	—	—	0.5	4.2	2.0	11.2
KT-72ja*	—	—	—	6.7	4.4	7.8
KT-73fb	—	—	2.0	6.1	4.5	11.8
Pooled:	0.4	—	0.8	5.7	3.9	10.8

* Includes both male samples and the egg samples.

those of HS-4000's or HS-5100's, but the observed differences do not attain the conventional level of significance.

On Kauai, in the January, 1972, collections, the frequency of heterozygous A in KH is only 6% and 16% in KT; the frequency of B is 14% in KH and 6% in KT; the frequency of C in KH is 16% and 14% in KT. The variations between locations, however, are not statistically significant ($P=0.05$ for A; $P=0.11$ for B). On a pooled basis, heterozygous C (14%) is the most common, and A (10%) and B (9%) occur at lower frequencies. In the February of 1973 collections, the heterozygous C (20-21%) is the most frequent in both locations, A (8-12%) occur at intermediate, and B (4-9%) at somewhat lower frequencies; the frequencies between locations are similar ($0.20 > P > 0.10$). The relative frequency of the heterokaryotypes, based on the combined data of the two local populations, is essentially consistent throughout the period, January, 1972-February, 1973 ($0.30 > P > 0.20$). The overall frequency of heterozygous A in this period is 10%, the frequency of B is 7% and the frequency of C is as high as 20%. Apparently, among the most characteristic features of the chromosomal polymorphism in the Hawaiian populations is the high frequency of heterozygous C in the Kauai populations.

The frequencies of inversion homozygotes and the frequencies of gene-arrangements

Table 3. Comparison of the frequencies of gene-arrangements A, B and C in per cent, between the egg samples and male samples in three populations, with the Fisher-Irwin significance probability (P) for the differences

Population tested	Chromosomes examined	A	P	B	P	C	P
H S-4000'71dc							
Male sample	120	17.5	0.29	7.5	0.22	5.8	0.08
Egg sample	334	15.3		9.9		10.2	
KH-72ja							
Male sample	70	11.4	0.05	7.1	0.44	12.9	0.12
Egg sample	106	4.7		6.6		7.6	
KT-72ja							
Male sample	16	—	0.14	12.5	0.04	12.5	0.22
Egg sample	74	8.1		2.7		6.8	

Table 4. Tests for the goodness of fit for inter-island differences in the frequencies of the gene-arrangements A, B and C. Each test is a chi-square with 1 df.

Comparison	Goodness-of-fit		
	A	B	C
All Oahu vs. Kauai's	44.28***	0.93	91.49***
All Oahu vs. HS-4000's	0.33	30.37***	71.50***
All Oahu vs. HS-5100's	13.70***	22.03***	25.99***
All Oahu vs. HK-5300's	1.02	8.45***	36.75***
Kauai's vs. HS-4000's	43.33***	22.07***	0.71
Kauai's vs. HS-5100's	83.87***	14.68***	10.39**
Kauai's vs. HK-5300's	24.28***	5.20*	0.63

Kauai's and HS-4000's include the male samples for the estimate of arrangement frequencies.

* Denotes significance at the 5% level.

** At the 1% level.

*** At the 0.1% level.

have been grouped together in Table 2. Because the three homozygotes were either absent or rare in all populations, the data do not provide new information over that in Table 1. But OT's and OM's, judged by the frequency of arrangement A or B, turn out here to be significantly different from each other ($P=0.04$ for both A and B). This and the earlier results on the mean frequency of heterozygosity per larva between the two populations reinforce each other, suggesting that the differences probably are of practical significance. It should be pointed out that the frequency of each arrangement in egg samples agrees with that of the corresponding arrangement in male samples of the same collection (see Table 3). Therefore, the combined frequencies of the arrangements (as * in Table 2) can probably be acceptable.

We have determined the inter-island differences based on the gene-arrangement frequencies, and the details are listed in Table 4.

DISCUSSION

Our results suggest the following generalization: (1) three inversions are widespread throughout the islands; (2) within each island there is a general similarity from population to population as to the amount and relative frequencies of the inversions; (3) nonetheless, shifts in the frequency of the inversion(s) occur between certain localities; (4) the relative distribution of the inversions are distinct from one island to another.

The frequencies of the heterozygous inversions reported by Richmond and Dobzhansky (1968) for *D. immigrans* populations on Maui, Hawaii are hardly comparable to any of those found by us on the other islands. Although the pattern of the frequency distribution on Maui somewhat resembles that characteristic of the Oahu populations, most of their frequencies (with an exception at Ulupalakua) are considerably higher, ranging from 19-33% heterozygous A, 11-21% B and 0-7% C; with Ulupalakua, much lower and at a level incomparable in any category to that characteristic of Oahu. The large amount of inversion heterozygosity per larva on Hawaii (about 0.6 compared to 0.3 on the other islands), and the presence of the native inversions on this island, all support the present argument for inter-island differences.

The above facts suggest that there exist racial differences from one island to another. The most likely explanation for such striking differences may be that all the same polymorphic inversions react differently to environmental differences which might exist among the islands. If this is true, oceanic channels acting as isolating barriers could probably explain the maintenance of the differences. Our experience in the islands leads us to suggest that the differences among the islands in topography and ecoclimatic conditions and in geologic age (Stearns 1966) are great enough to force such divergence of the polymorphism.

The considerations above, however, do not negate the possible effect of permanent drift and/or historical sampling errors in the variations among islands. Nevertheless, our results indicate that the inversion frequencies remained quite stable in all populations throughout the considerable periods of time. We would like to suggest that permanent drift probably is not very important as the cause of the differences we are discussing. Even if our populations passed through periodic bottlenecks such as Spencer (1947) indicated for the same species in Ohio, then the effect of random drift might have been overridden by slight selective differences of the chromosomal types. But we obviously need more work to validate our tentative conclusion.

Dobzhansky and Pavlovsky (1957) from their laboratory model experiment demonstrated the random "founder effect" on inversion frequencies in small populations. Waddington (1957) explored the feasibility of "intermittent drift" in island-type population, which could occur in the past as a result of sampling error. But both phenomena were not confirmed by Ford (1971) and his co-workers in their extensive field

studies on the frequency of wing-spot patterns in *Maniola jurtina* on the Scilly islands; their remarkable variations among islands have been ascribed to adaptive responses through selection. Our study indicates that the three kinds of "cosmopolitan" inversions are all present on every island; these inversions occur at substantially low frequencies in continental areas (this will be clarified later); as stated earlier this species is essentially domestic in distribution. Taking these information together we would like to suggest that the Hawaiian islands were probably colonized by multiple propagules rather than a small number of founders. It seems, then, unlikely that the geographic variations in the case of our own studies were influenced mostly by the founder effect. But it is still very difficult to preclude the effect of Waddington's drift in the present study, since our survey on every island could not be extended over a period of more than a year. The problem can be settled only by further studies as to the long-range changes in the polymorphism. However, it should be pointed out that the islands studied by us are fairly large, ranging from 600 to 4,000 sq. mi., and stable. Under such conditions, small selective differences on chromosomal types may be sufficient to lower the effect of drift to relative insignificance (Mac Arthur and Wilson 1967).

Our results indicate significant differences in some inversion frequencies between certain localities. The difference between HS-4000' and HS-5100' appears to be environmental differences because of the following reasons: first, these two locals are quite unlike in ecological conditions as stated in Materials and Methods; second, the daily climatic data collected by Bridges and Carey (1973) from January 1971 through the end of 1972 in the areas show that between the two great differences exist in temperature, rainfall and humidity; third, they were isolated by a lava flow 2000 years ago (Smathers, quoted in Rockwood 1969) and by another recent flow, and both of which evidently constituted effective barrier to the fly. Taken in conjunction with the stability of the inversion frequencies at each location over a considerable periods of time, the information above suggest that the difference in the inversion frequencies have some adaptive significance. Similar explanation may also be applicable to the quantitative differences found between the two Oahu populations, 20 miles distant from each other. However, our interpretation in this case is less certain because we do not have conclusive information on their environments as well as on geographic barriers in terms of gene flow.

Table 5 is a compilation of some results thus far obtained by other workers on the inversion frequency at the heterozygous state. Of all comparisons the populations in Korea and Japan, which are in geographic proximity, are only ones which present a comparable picture. The Brazilian and Israeli populations are qualitatively identical in that Inversion A was the only aberrant chromosome in both populations, but they are distinguished by the amount of the inversion heterozygosity. The differences between the inversion frequencies in the widely separated local populations of South America have been ascribed by Brncic (1955) to racial differences resulting from local ecological differences.

These frequencies are much lower than those of the same polymorphic inversions reported for our own populations. A possible explanation of such quantitative differences may be that more ecological diversity exists on the islands of Hawaii compared

Table 5. Frequency of inversion heterozygotes and number of heterozygous inversions per larva in different populations of *D. immigrans*

Source reference	Locality	% Heterozygote inversions			Number per larva*
		A	B	C	
Brncic, 1955	Southern Chile	12.8	11.2	0.6	0.25
	Northern Chile	—	15.2	—	0.15
Freire-Maia <i>et al.</i> , 1953	Brazil	19.6	—	—	0.20
Gruber, 1958	Israel	33.9	—	—	0.34
Hirumi, 1961	Japan	7.2	11.8	0.4	0.19
Paik, 1973	Korea	7.7	10.4	0.7	0.18
Richmond and Dobzhansky, 1968	Maui, Hawaii	20.7	13.0	4.0	0.38

* Number of heterozygous inversions per larva.

to the continental areas (Dobzhansky *et al.* 1950; Powell 1971). But the problem remains open for future investigation. Further, Inversion C appears the most inferior in both its distribution and frequency in all the continental areas studied by other workers. The abundant occurrence of such a rare inversion on Hawaii and particularly on Kauai (20% at heterozygous state) is another difference of interest between the Hawaiian populations of *D. immigrans* and the continental areas. Such geographic differences may probably reflect differences in local ecological and bio-climatic conditions in the areas concerned, unless the practical importance of random drift in *D. immigrans* populations as a cause of differences in inversion frequencies has been validated. A study of long-range changes in the gene-arrangement frequencies in natural populations of this species would be of considerable interest.

SUMMARY

1. The natural populations of *D. immigrans* were sampled at various places on three main islands of Hawaii and the inversion polymorphism examined in 1532 "egg samples" and 103 "male samples". The samples at different places on each island were from dissimilar habitats.

2. Three inversions of Chromosomes II were widespread throughout the islands and always present in all the populations studied (except for a few inadequately sampled populations).

3. Within each island there is a general similarity from population to population in the relative frequencies of these inversions and in the amount of inversions per individual.

4. Nonetheless, significant differences in some inversion frequencies occurred between certain localities with dissimilar environmental conditions.

5. There were consistent results to indicate that the relative frequencies of the inversions remained fairly stable throughout the considerable periods of time in every population sampled more than twice. The general stability is considered to be maintained by selective differences on chromosomal types.

6. The relative distribution of the inversions were distinct from one island to another; the differences among islands are considered to be racial differences.

7. We are inclined to suggest that the differences shown in (4) and (6) are mostly due to adaptive response, perfected by natural selection, to differences in environmental conditions.

8. The magnitude of inversions per individual observed in the Hawaiian population of *D. immigrans* show a much higher range compared to that reported by other workers for continental areas.

9. One paracentric and one pericentric inversions were detected in the Hawaii populations, each at different local; they are probably endemic to the island.

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