PATTERNS OF GENE VARIATION IN CENTRAL AND MARGINAL POPULATIONS OF *DROSOPHILA ROBUSTA**

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ABSTRACT

The central and marginal populations of *D. robusta* differ greatly in the level of inversion polymorphism; the marginal populations are monomorphic or nearly so and the central populations are highly polymorphic. This paper presents the frequencies of alleles at forty gene loci in various populations of *D. robusta*, studied by electrophoresis of proteins and enzymes. Population samples were obtained from eight widely separated populations of *D. robusta* which included the central, the extreme marginal and the intervening populations between the center and the margins. We find that the proportion of polymorphic loci and average heterozygosity per individual is slightly higher in the marginal populations than the central populations. In *D. robusta* on an average, 39% of the loci are polymorphic and the average proportion of loci heterozygous per individual is 11%. A breakdown of loci in three categories, viz, hydrolytic enzymes and some other enzymes, larval proteins and glycolytic and Kreb's cycle enzymes, shows that in all populations the level of polymorphism is highest in the hydrolytic enzymes, intermediate in larval proteins and least in the glycolytic and Kreb's cycle enzymes. On the average, the proportion of loci heterozygous per individual for three groups of loci is: hydrolytic enzymes and others (.164), larval proteins (.115) and glycolytic and Kreb's cycle enzymes (.037). We also observe that in all populations the level of polymorphism on the *X* chromosome is far less than the expected 38%; in salivary gland cells the euchromatic length of the *X* chromosome is 38% of the entire genome. Lower levels of polymorphism for the *X* chromosome loci are explained due to low probability of balanced polymorphisms for the *X*-linked loci since the conditions for establishment of balanced polymorphism for *X*-linked loci are more restrictive than for the autosomal loci.—The polymorphic loci can be grouped according to pattern of allele frequencies in different populations as follows: (1) The allele frequencies are similar in all populations at the *XDH*, *Pep-1* and *Hex-1* loci. (2) The alleles at the *Est-1*, *Est-2*, *Amy* loci and the $AP\text{-}4^{1.0}$ and the $LAP\text{-}1^{.90}$ alleles show north south clinal change in frequency. (3) There is north south and east west differentiation at the *Pt-5*, *Pt-8* and *Pt-9* loci and the allele $AP\text{-}4^{.81}$. (4) Polymorphism at loci such as *Fum*, *B.Ox*, *Hex-8*, *Pep-2* and *Pep-3* are restricted to only one or two of the populations. (5) Allele frequencies at the *MDH* and *ODH* loci fluctuate between populations. (6) Allele frequencies at many polymorphic loci such as *Est-1*, *Est-2*, *LAP-1*, *AP-4*, *Pt-5*, *Pt-8*, *Pt-9*, *Pt-16*, *MDH*, *Fum* change clinally within a gene arrangement. The pattern of gene variation in *D. robusta* is very complex and cannot be easily explained due to migration of

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neutral alleles between once-isolated populations or to semi-isolation of neutral alleles. The observations of the pattern of allele variation in different populations, high levels of polymorphism in the marginal populations which have small population size and low levels of polymorphism of the *X* chromosome loci all support the argument in favor of balancing selection as the main mechanism for the maintenance of these polymorphisms. Environmental factors must play a role in the maintenance of a great deal of these polymorphisms, since we observe clinal allele frequency changes even within a given inversion type.

IT has been assumed by evolutionary biologists that marginal populations of a species, which exist at the ecological margins of the species range and have low population density due to unfavorable ecological conditions, have lower genetic variation than the populations near the center of the species range where high population density of the species is supported due to favorable ecological conditions. MAYR states (1970, p. 232) "Reduction of gene flow and increased selection pressure combined deplete the genetic variability of the peripheral populations. Lowered variability permits, if it does not favor, a shift into different ecological niches; for the selection pressure at the periphery is not only more severe, but also different." CARSON (1959) proposed that homo-selection, selection of homozygous genotypes, predominates in ecologically marginal populations which tend to be inbred, semi-isolated and have more crossing over than the central populations. According to CARSON, selection of homozygous genotypes which occurs in response to limited ecological niches at the margins leads to the formation of specialized genotypes, adapted to the marginal conditions which might then lead to sub-species and species formation. These statements of MAYR and CARSON are based on information obtained mainly from data on inversion polymorphism in Drosophila. In various species of Drosophila, such as *D. robusta*, *D. willistoni* and *D. nebulosa*, the central populations are highly polymorphic for chromosome inversions and the marginal populations have either greatly reduced or no inversion polymorphism (CARSON 1965).

An examination of gene variation at 24 loci by electrophoresis of proteins and enzymes of the central and marginal populations of *D. pseudoobscura* showed that gene variation, the proportion of polymorphic loci and the proportion of the genome heterozygous per individual, is about the same in the central and the marginal populations, if we disregard the polymorphic loci associated with the third chromosome inversions (PRAKASH, LEWONTIN, and HUBBY 1969); PRAKASH and LEWONTIN 1968, 1971). *D. pseudoobscura* however, does not show the classical picture of reduction in inversion polymorphism at the margins as is seen in *D. robusta*. In *D. robusta*, the central populations are polymorphic for gene arrangements in all or most of the chromosome arms XL XR, 2L 2R and 3L 3R. On the other hand, extreme marginal populations from Chadron, Nebraska and Astor, Florida are completely monomorphic (CARSON 1958; CARSON and HEED 1964). The intermediate populations between the center and the margins show a gradual decline in inversion polymorphism as they approach the margins. We have examined freshly collected samples of *D. robusta* from eight widely sepa-

rated localities which included two marginal, two intermediate, and four central populations. We have studied 40 different loci by electrophoresis in order to learn about the following: (1) Do the marginal populations have reduced genic polymorphism compared to those in the center? (2) Does the pattern of allele variation in different populations of this species provide any evidence for the role of natural selection *vs.* random drift in determining the genetic makeup of the species?

MATERIALS AND METHODS

Acrylamide gel electrophoresis: Larval proteins (Pt), larval acid phosphatase (AP), Octanol dehydrogenase (ODH), Glucose-6- phosphate dehydrogenase (G6PD) were studied in acrylamide gels according to the methods described by PRAKASH, LEWONTIN and HUBBY (1969) and HUBBY and LEWONTIN (1966). Esterase-1 (Est-1), Malic dehydrogenase (MDH), Malic enzyme (ME) and Amylase (Amy) were studied in acrylamide gels but with some modifications in the methods of PRAKASH, LEWONTIN and HUBBY (1969) and HUBBY and LEWONTIN (1966) as follows: Gels for *Est*-1 were made in 0.1 M tris-borate Na_2 EDTA pH 8.3 buffer. MDH was studied in split gels which had a 4% spacer and 6% running gel. TPN (Coenzyme II) was substituted for DPN (Coenzyme I) in the staining solution for the assay for malic enzyme. For the Amylase assay gels were made in 0.1 M trisborate pH 8.3 with 0.02 M $CaCl_2$. This buffer without $CaCl_2$ was used as bath buffer.

Agar gel electrophoresis: Hexokinases (Hex): Gels were made using 1.2 g agarose (Seakem brand) + 80 mg glucose + 150 ml of 0.1 *M* Trisborate-EDTA (TBE), pH 8.9 buffer, according to the following procedure: agarose was heated in 75 ml of distilled water, stirred constantly, and brought to a boil. The agarose solution was cooled slightly; 15 ml of 1 M TBE buffer was added along with enough distilled water to bring the total volume to 150 ml. After the agarose buffer solution had cooled to 110°F, it was poured into the vertical gel electrophoretic apparatus. Electrophoresis was carried out at 50 volts for 5 minutes and then at 450 volts for 1 hour and 10 minutes. Staining solution consisted of 75 ml of 0.1 M Tris-HCl, pH 7.5, 3.75 ml of 0.1 M $MgCl_2$, 60 mg TPN, 230 mg ATP (adenosine triphosphate), 45 mg NBT (nitro blue tetrazolium), 120 mg glucose, 100 units glucose-6-phosphate dehydrogenase, and 0.75 ml of 1% PMS (phenazine methosulfate).

Starch gel electrophoresis: Esterase-2 (Est-2) and Xanthine dehydrogenase (XDH): 10% gels were made in electrostarch using 0.1 M TBE pH 9 buffer. This buffer was also used as bath buffer and for grinding. Electrophoresis was carried out at 250 volts for 1 hour, 300 volts for 1 and 1/2 hours and 350 volts for 1 hour. The two anodal slices were stained for Esterase and XDH according to the methods described in HUBBY and LEWONTIN (1966) and PRAKASH, LEWONTIN and HUBBY (1969).

α-Glycerophosphate dehydrogenase (α-GPD), Fumarase (Fum), Adenylate kinase (AK), Aldolase (Ald) and Alcohol dehydrogenase (Adh): 12% gels were made in electrostarch using 0.1 M TBE pH 9 buffer. This buffer was also used as bath buffer and for grinding. Electrophoresis was performed at 360 volts for 1/2 hour and 400 volts for 3 and 1/2 hours. The cathodal strip was stained for Adh. One anodal strip was stained for α-GPD, Fum, and AK and the other anodal strip was stained for Aldolase. Staining solution for α-GPD is described in HUBBY and LEWONTIN (1966). Staining solutions for the rest of these assays are as follows: Fumarae: 100 ml 0.1 M Tris-HCl pH 8.5, 20 mg fumaric acid, 15 mg DPN, 20 mg NBT, 50 units malic dehydrogenase and 1 ml of 1% PMS. Adenylate kinase: add to the fumarase staining solution 50 mg ADP (adenosine diphosphate), 100 mg glucose, 20 mg TPN, 150 units hexokinase and 100 units glucose-6-phosphate dehydrogenase. Aldolase: 100 ml 0.1 M Tris-HCl pH 8.5, 15 mg DPN, 20 mg NBT, 50 mg fructose 1, 6 diphosphate: 100 units glyceraldehyde-3-phosphate dehydrogenase and 1 ml of 1% PMS. Alcohol dehydrogenase: 100 ml 0.1 M Tris-HCl pH 8.5, 40 mg DPN, 25 mg NBT, 75 ml isopropanol and 1 ml of 1% PMS.

Leucine aminopeptidase (LAP) and Benzaldehyde oxidase (B.Ox): 13% gels were run in Pouliks tris-citrate discontinuous buffer system at 300 volts for 1 hour, 360 volts for 1 hour and 400 volts for 1 hour. Flies were ground on 0.1 M tris-borate pH 9 containing 5 mM $MgCl_2$. Staining solutions are described in HUBBY and LEWONTIN (1966) and PRAKASH, LEWONTIN and HUBBY (1969).

Peptidases: were examined in 13% electrostarch gels according to the method of LEWIS and HARRIS (1967). .005 M tris-maleate gel buffer and 0.1 M tris maleate bath buffer were used. Flies were ground in the gel buffer. Electrophoresis was carried at 340 volts for 3 hours. The anodal strip was stained in the following solution: 75 ml, 0.2 M phosphate HCl pH 7.5 buffer, 30 mg leucyl alanine dipeptide, 15 mg *Crotalus adamanteus* venom (Eastern U. S. Diamondback Rattlesnake)20 mg horseradish peroxidase POD II and 1.5 ml of 0.1 M $MnCl_2$.

Populations studied: Figure 1 gives the distribution map of the species and the eight popula-

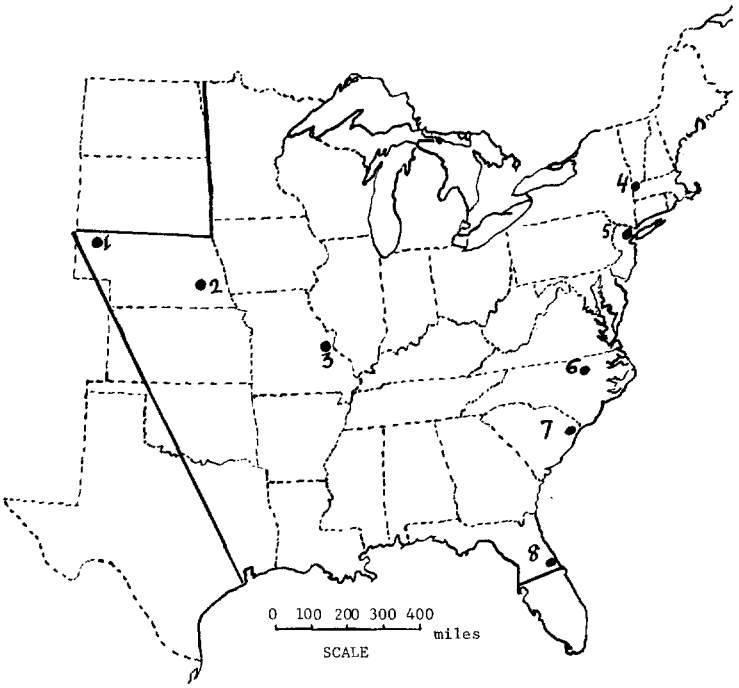

FIGURE 1.—Geographical distribution of *Drosophila robusta*. The northern and eastern limits of species distribution are the northern and the eastern boundaries of the United States. The southern boundary of the U. S. marks the southern limit of the species distribution except in Florida where a heavy line indicates the limit of the species distributions. A heavy line also shows the western limit of the species distribution. (Adapted from CARSON 1958).
Populations studied are marked with closed circles.

1 Chadron, Dawes County, Nebraska
2 Lincoln, Lancaster County, Nebraska
3 Olivette, St. Louis County, Missouri
4 Williamstown, Berkshire County, Massachusetts
5 Englewood, Bergen County, New Jersey
6 Raleigh, Johnston County, North Carolina
7 Myrtle Beach, Horry County, South Carolina
8 Astor, Volusia County, Florida

tions included in this study. Chadron (Nebraska) and Astor (Florida) are the extreme northwestern and southeastern marginal populations. Lincoln (Nebraska), Olivette (Missouri), Englewood (New Jersey) and Raleigh (North Carolina) are the central populations; and Williamstown (Massachusetts) and Myrtle Beach (South Carolina) are the intermediate populations between the center and the margin of the species. The numbers of freshly collected strains available for these studies are Chadron (60), Astor (25), Williamstown (10), Myrtle Beach (20), Lincoln (50), Olivette (100), Englewood (80), and Raleigh (100). In addition to the flies from these strains, wild flies were also examined from Englewood and Williamstown for some assays, The number of genomes studied for each locus is given in the Tables of gene frequencies for different loci.

Electrophoretic analysis: Homozygous strains for different alleles of polymorphic loci were derived in order to ascertain that the observed electrophoretic variation was actually genetic and not due to extraneous reasons such as instability of the enzyme in the gel conditions employed, or to the presence of different enzymes in different development stages which might appear as a polymorphic system. Flies from several appropriate homozygous control strains of different electrophoretic mobilities were put in a gel for the purpose of comparison.

RESULTS

Chromosome location of polymorphic genes: A majority of polymorphic loci have been associated to the chromosomes by using inversions as chromosome markers. For associating genes with various autosomes we used Chadron strains which are homokaryotypic for *XL1 XR*, *2L3 2R* and *3R* arrangements and Astor strains which are homokaryotypic for *XL XR2*, *2L1 2R1* and *3R1* arrangements. There is considerable recombination, due to interchromosomal effects of inversions on crossing over, in the second chromosome of F_1 ♀♀'s which are heterokaryotypic in the *XL*, *XR*, *2L*, *2R* and *3R* chromosome arms (CARSON 1953; LEVITAN 1958); these F_1 ♀♀'s were obtained from a cross of Chadron and Astor flies.

Sex-linked genes: AP-4 is a sex-linked locus since a cross of *.93/.93* ♀♀ × *1.0* ♂♂ gave hemizygous *.93* and heterozygous *.93/1.0* progeny. The reciprocal cross *1.0/1.0* ♀♀ × *.93* ♂♂ gave hemizygous *1.0* and heterozygous *.93/1.0* progeny.

Fumarase is a sex-linked locus since a cross of *1.35/1.35* ♀♀ × *1.0* ♂♂ gave hemizygous *1.35* male and *1.0/1.35* female offspring.

Pt-5 is a sex-linked locus. A cross of *Pt-5 1.0/1.0* ♀♀ × *Pt-5 .97* ♂♂ gave all F_1 *Pt-5 1.0/1.0* larvae. The reciprocal cross *.97/.97* ♀♀ and *1.0* ♂♂ gave both *.97/.97* and *1.0/1.0* offspring. $Pt\text{-}5^{1.0}$ is therefore dominant over $Pt\text{-}5^{.97}$.

Pep-2 is a sex-linked locus since heterozygotes were observed only in the females and not in the males.

Autosomal genes: Pep-1, *Pep-3*, *Hex-1*, *Hex-8*, *α-GPD*, *Adh-2*, *B.Ox* and *Pt-11* are autosomal loci since heterozygotes were observed in both males and females. These loci still remain to be associated with particular chromosomes.

Est-1 is located on the left arm of the second chromosome (PRAKASH and LEVITAN 1973).

Est-2 is located on the second chromosome since of 30 Backcross 1 progeny examined from a cross of *2L1 2R1* $Est\text{-}2^{.97}$*/2L3 2R* $Est\text{-}2^{1.0}$ F_1 ♂♂ × *2L3 2R* $Est\text{-}2^{1.0}$*/2L3 2R* $Est\text{-}2^{1.0}$ ♀♀ 16 were *2L1 2R1* $Est\text{-}2^{.97}$*/2L3 2R* $Est\text{-}2^{1.0}$ and 14 were *2L3 2R* $Est\text{-}2^{1.0}$*/2L3 2R* $Est\text{-}2^{1.0}$.

LAP-1 is located on the second chromosome since of 30 BC_1 progeny examined from a cross of *2L1 2R1 LAP-1$^{.90}$/2L3 2R LAP-1$^{1.0}$* F_1♂♂ × *2L1 2R1 LAP-1$^{.90}$/2L1 2R1 LAP-1$^{.90}$*♀♀ twenty were *2L1 2R1 LAP-1$^{.90}$/2L3 2R LAP-1$^{1.0}$* and ten were *2L1 2R1 LAP-1$^{.90}$/2L1 2R1 LAP-1$^{.90}$*. This locus is most likely on the 2L chromosome since a cross of *2L1 2R1 LAP-1$^{.90}$/2L3 2R LAP-1$^{1.0}$* F_1♀♀ × *2L1 2R1 LAP-1$^{.90}$/2L1 2R1 LAP-1$^{.90}$*♂♂ gave five recombinant *2L1 2R1 LAP-1$^{.90}$/2L1 2R LAP-1$^{.90}$* F_2 progeny in a total of thirteen F_2's examined.

The *XDH* locus is located on the second chromosome since a cross of *2L1 2R1 XDH$^{.94}$/2L3 2R XDH$^{1.0}$* F_1♂♂ × *2L1 2R1 XDH$^{.94}$/2L1 2R1 XDH$^{.94}$*♀♀ gave thirteen *2L1 2R1 XDH$^{.94}$/2L3 2R XDH$^{1.0}$* and seven *2L1 2R1 XDH$^{.94}$/2L1 2R1 XDH$^{.94}$* BC_1 progeny. A cross of *2L1 2R1 XDH$^{.94}$/2L3 2R XDH$^{1.0}$* F_1 ♀♀ × *2L1*

TABLE 1

Karyotype and Amylase genotype of BC_1 progeny

Cross	Second chromosome karyotype	*Amy* genotype .85/.85	*Amy* genotype .85/1.0
A. $\frac{2L1\ 2R1\ Amy^{.85}}{2L3\ 2R\ Amy^{1.0}}$ F_1♂♂ ×	$\frac{2L1\ 2R1}{2L3\ 2R}$	—	5
$\frac{2L1\ 2R1\ Amy^{.85}}{2L1\ 2R1\ Amy^{.85}}$♀♀	$\frac{2L1\ 2R1}{2L1\ 2R1}$	10	—
B. $\frac{2L1\ 2R1\ Amy^{.85}}{2L3\ 2R\ Amy^{1.0}}$ F_1♀♀ ×	$\frac{2L1\ 2R1}{2L3\ 2R}$	1	5
$\frac{2L1\ 2R1\ Amy^{.85}}{2L1\ 2R1\ Amy^{.85}}$♂♂	$\frac{2L1\ 2R1}{2L1\ 2R1}$	9	—
	$\frac{2L1\ 2R1}{2L3\ 2R1}$	3	—
	$\frac{2L1\ 2R1}{2L1\ 2R}$	—	2
		Amy genotype 1.0/1.0	*Amy* genotype .85/1.0
C. $\frac{2L1\ 2R1\ Amy^{.85}}{2L3\ 2R\ Amy^{1.0}}$ F_1♀♀ ×	$\frac{2L1\ 2R1}{2L3\ 2R}$	—	13
$\frac{2L3\ 2R\ Amy^{1.0}}{2L3\ 2R\ Amy^{1.0}}$♂♂	$\frac{2L3\ 2R}{2L3\ 2R}$	1	—
	$\frac{2L3\ 2R}{2L3\ 2R1}$	—	6
	$\frac{2L3\ 2R}{2L1\ 2R}$	1	—

2R1 XDH$^{.94}$/2L1 2R1 XDH$^{.94}$ ♂ ♂ gave twelve *2L1 2R1 XDH$^{.94}$/2L3 2R XDH$^{1.0}$* and seven *2L1 2R1 XDH$^{.94}$/2L1 2R1 XDH$^{.94}$* BC_1 parental progeny and six *2L1 2R1 XDH$^{.94}$/2L3 2R1 XDH$^{1.0}$* and one *2L1 2R1 XDH$^{.94}$/2L1 2R XDH$^{.94}$* BC_1 recombinant progeny. All seven BC_1 recombinant progeny show associations of the *XDH* alleles with the *2L* chromosome.

The *Amylase* locus is located on the *2R* chromosome since the recombinant chromosomes between *2L* and *2R* gene arrangements show associations of *Amy* alleles with the gene arrangements of the *2R* chromosome and not with those of the *2L* chromosome (Table 1B, C).

The *Pt-16* locus is located on the second chromosome since all *2L1 2R1/2L1 2R1* F_2 progeny were *Pt-16 .86/.86* (Table 2). The recombinant karyotypes show associations of the locus with the *2L* and not with the *2R* chromosome (Table 2).

The *Pt-9* locus is located on the second chromosome since of forty BC_1 progeny examined, from a cross of *2L3 2R Pt-9$^{1.0}$/2L1 2R1 Pt-9$^{1.04}$* F_1 ♂ ♂ × *2L3 2R Pt-9$^{1.0}$/2L3 2R Pt-9$^{1.0}$* ♀ ♀ twenty-two were *2L1 2R1 Pt-9$^{1.04}$/2L3 2R Pt-9$^{1.0}$* and eighteen were *2L3 2R Pt-9$^{1.0}$/2L3 2R Pt-9$^{1.0}$*. A cross of *2L3 2R Pt-9$^{1.0}$/2L1 2R1 Pt-9$^{1.04}$* F_1 ♀ ♀ × *2L3 2R Pt-9$^{1.0}$/2L3 2R Pt-9$^{1.0}$* ♂ ♂ gave the results shown in Table 3. Because of considerable recombination, the locus cannot be assigned to a particular arm of the second chromosome.

TABLE 2

Karyotype and Pt-16 *genotype of F_2 progeny from a cross of*

$$\frac{2L3\ 2R\ \text{Pt-16}^{1.0}}{2L1\ 2R1\ \text{Pt-16}^{.86}}\ F_1\ ♀\ ♀ \times \frac{2L3\ 2R\ \text{Pt-16}^{1.0}}{2L1\ 2R1\ \text{Pt-16}^{.86}}\ F_1\ ♂\ ♂$$

Second chromosome karyotype	*Pt-16* genotype .86/.86	.86/1.0	1.0/1.0
A. Parental karyotypes			
2L3 2R / 2L3 2R	—	2	—
2L3 2R / 2L1 2R1	1	8	2
2L1 2R1 / 2L1 2R1	12	—	—
B. Recombinant karyotypes			
2L1 2R1 / 2L3 2R1	—	1	—
2L1 2R / 2L3 2R	—	1	—
2L1 2R / 2L1 2R1	3	—	—
2L3 2R / 2L3 2R1	—	—	1

TABLE 3

Karyotype and Pt-9 *genotype of* BC_1 *progeny from a cross of*

$$\frac{2L3\ 2R\ \text{Pt-9}^{1.0}}{2L1\ 2R1\ \text{Pt-9}^{1.04}}\ F_1\ ♀♀ \times \frac{2L3\ 2R\ \text{Pt-9}^{1.0}}{2L3\ 2R\ \text{Pt-9}^{1.0}}\ ♂♂$$

Second chromosome karyotype	*Pt-9* genotype *1.0/1.0*	*1.0/1.04*
A. Parental karyotypes		
2L1 2R1 / 2L3 2R	3	13
2L3 2R / 2L3 2R	7	3
B. Recombinant karyotypes		
2L3 2R1 / 2L3 2R	3	4
2L1 2R / 2L3 2R	4	3

The *ODH* locus is present on the second chromosome but we do not know whether this locus is on the right or the left arm.

The *MDH* locus is present on the third chromosome. A cross of *3R* $MDH^{1.0}$/*3R1* $MDH^{1.20}$ F_1♂♂ × *3R1* $MDH^{1.20}$/*3R1* $MDH^{1.20}$♀♀ gave ten *3R* $MDH^{1.0}$/*3R1* $MDH^{1.20}$ and four *3R1* $MDH^{1.20}$/*3R1* $MDH^{1.20}$ offspring. A backcross of *3R* $MDH^{1.0}$/*3R1* $MDH^{1.20}$ F_1♀♀ × *3R1* $MDH^{1.20}$/*3R1* $MDH^{1.20}$♂♂ gave nine *3R* $MDH^{1.0}$/*3R1* $MDH^{1.20}$ and six *3R1* $MDH^{1.20}$/*3R1* $MDH^{1.20}$ progeny.

The *Pt-8* locus is also present on the third chromosome since a cross of *3R* $Pt\text{-}8^{1.0}$/*3R1* $Pt\text{-}8^{.98}$ F_1♂♂ × *3R* $Pt\text{-}8^{1.0}$/*3R* $Pt\text{-}8^{1.0}$♀♀ gave fourteen *3R* $Pt\text{-}8^{1.0}$/*3R* $Pt\text{-}8^{1.0}$ and sixteen *3R* $Pt\text{-}8^{1.0}$/*3R1* $Pt\text{-}8^{.98}$ progeny.

Inversion polymorphism: Table 4 gives the frequencies of gene arrangements and total inversion heterokaryosity in all of the populations studied. The populations are arranged in order of decreasing latitude from left to right. Chadron represents the northwestern and Astor the southeastern marginal population. Englewood, Lincoln, Olivette and Raleigh represent the central populations of this species. The Williamstown and Myrtle Beach populations are the intervening populations between the center and the margins. The two extreme marginal populations are monomorphic for gene arrangements and the central populations are highly polymorphic. North-south clinal changes are evident for the *XL*, *XL1*; the *2L1*, *2L3*; the *2R*, *2R*1 and the *3R*, *3R1* gene arrangements.

Gene polymorphism: Monomorphic loci: We did not observe any polymorphism at the following 18 loci. *Pt-1*, *-2*, *-6*, *-7*, *-10*, *-12*, *-13*, Malic enzyme, Tetrazolium oxidase, Leucine aminopeptidase -2, Alcohol dehydrogenase -1, Adenylate kinase, Aldolase -1 and -2, Hexokinase -2, -6 and -7 and Glucose-6-phosphate dehydrogenase.

TABLE 4

Frequencies of various gene arrangements in D. robusta *populations*

State:	Nebraska	Massachusetts	New Jersey	Nebraska	Missouri	North Carolina	South Carolina	Florida
Locality:	Chadron	Williamstown	Englewood	Lincoln	Olivette, St. Louis	Raleigh	Myrtle Beach	Astor
Latitude:	42°49.9'N	42°42.7'N	40°53.6'N	40°49.4'N	38°37.0'N	35°46.3'N	33°42.1'N	29°09.2'N
XL	—	—	.398	.79	.991	.72	1.0	1.0
XL1	1.0	1.0	.594	.21	.003	.02	—	—
XL2	—	—	.002	—	.006	.26	—	—
XR	1.0	.918	.880	.04	.398	—	.02	—
XR1	—	.012	.003	.96	.586	—	—	—
XR2	—	.069	.118	—	.016	1.0	.98	1.0
XR3	—	—	—	—	—	—	—	—
2L	—	.310	.487	.45	.483	.20	.02	—
2L1	—	.028	.338	.16	.431	.53	.98	1.0
2L2	—	.014	.012	.03	.08	.12	—	—
2L3	1.0	.648	.163	.36	.006	.16	—	—
2R	1.0	.99	.982	.92	.88	.59	.10	—
2R1	—	.01	.018	.08	.12	.41	.90	1.0
3R	1.0	.98	.976	.85	.73	.24	.02	—
3R1	—	.02	.024	.15	.27	.76	.98	1.0
nx	100	160	399	53	from CARSON 1958*	43	40	75
nA	200	210	545	103		74	40	100
Total expected gene arrangement heterozygosity in all 5 arms	0.000	.696	1.406	1.453	1.700	1.903	.300	0.000

*PROFESSOR CARSON informs me that gene arrangement frequencies in these Olivette, St. Louis samples were the same as those reported in CARSON 1958.

Polymorphic loci can be considered under the following categories according to their pattern of variation in different populations.

1. *Poylmorphisms with similar allele frequency among populations:* Three loci, the *XDH*, the *Pep-1* and the *Hex-1* (Tables 5A,B,C) show only little differentiation in allele frequencies in different populations. The alleles $XDH^{.94}$ and $XDH^{1.0}$ are present in intermediate frequencies in all the populations. On the other hand, the *Pep-1* and the *Hex-1* locus have one allele in very high frequency in all populations. Williamstown seems to differ from the rest of the populations in allele frequencies at the *Pep-1* and the *Hex-1* loci, but since the number of chromosomes examined from this population is so small not much reliance can be put in these allele frequencies.
2. *Polymorphisms with evidence of differentiation between populations:*
   A. *North-south clinal changes in allele frequencies:* Five of the polymorphic loci show changes in gene frequency along a north-south gradient. The magnitude and gradualness of clinal changes in allele frequencies at these five loci is different for each locus. At the *Est-1* (Table 6A) and the *AP-4*

TABLE 5

Allele frequencies at various loci in different populations of D. robusta. *Tables 5A, 5B, 5C show loci with similar allele frequencies in all populations.* n *refers to the number of genomes*

State: Locality:	Nebraska Chadron	Massachusetts Williamstown	New Jersey Englewood	Nebraska Lincoln	Missouri Olivette, St. Louis	North Carolina Raleigh	South Carolina Myrtle Beach	Florida Astor
A. Xanthine dehydrogenase (Chromosome 2L):								
Allele								
.85	.015	—	.014	.06	—	—	—	.03
.90	.045	—	—	—	—	—	—	—
.92	.045	—	.014	.06	.04	.03	.08	.065
.94	.43	.60	.495	.50	.48	.64	.57	.55
1.0	.46	.40	.457	.38	.42	.33	.35	.355
1.05	—	—	.02	—	.06	—	—	—
n	67	20	210	52	48	67	37	31
B. Peptidase-1 (Autosome):								
1.0	.885	.75	.95	.885	.90	.86	.97	.97
1.65	.115	.25	.05	.115	.10	.14	.03	.03
n	61	12	78	52	52	56	32	34
C. Hexokinase-1 (Autosome):								
.86	.03	.15	.08	.015	.025	.023	—	.02
1.0	.94	.70	.90	.94	.95	.954	.94	.96
1.12	.03	.15	.02	.045	.025	.023	.06	.02
n	125	26	50	65	42	88	29	42

(Table 6B) locus no allele is present in the highest frequency in all populations. The allele $Est\text{-}1^{.92}$ increases gradually from 14% in the northernmost Chadron population, to 70% in the southernmost Astor population; the allele $Est\text{-}1^{1.0}$ shows a gradual decline in its frequency from north to south; similarly the allele $Est\text{-}1^{.86}$ decreases from 23% in Chadron to 1% in Astor. PRAKASH and LEVITAN (1973) have described north-south clinal changes in allele frequencies at this locus in additional populations. The changes in allele frequencies at the *AP-4* locus are less gradual than those observed at the *Est-1* locus. The allele $AP\text{-}4^{.81}$ is 24% in Chadron; its frequency is 5% or less in all other populations. The allele $AP\text{-}4^{1.0}$ increases from 1% in Chadron to 63% in Astor. The three loci, *Est-2*, *LAP-1* and *Amy* (Tables 6C,D,E) have one allele in highest frequency in all the populations studied. The allele $Est\text{-}2^{.97}$ increases clinally in frequency from 4.4% in Chadron to 33% in Astor. At the *LAP-1* locus, we observe an increase in the frequency of the allele *.90* from 1.5% in Chadron to 30% in Astor. The frequencies of the alleles $Amy^{.85}$ and $Amy^{1.0}$ are very similar in all populations except in the Myrtle Beach and Astor populations where the allele *.85* increases to 87–93%.

B. *North-south and east-west clinal changes in allele frequencies:* Three loci, *Pt-8*, *Pt-5*, and *Pt-9* (Tables 7A,B,C) show clinal allele frequency changes

TABLE 6

Frequencies of alleles at loci showing north-south clinal change in allele frequencies. Populations are arranged from left to right according to their latitude (see Table 4 for latitudes) Chadron, Nebraska is the northernmost and Astor, Florida is the southernmost population

State: Locality:	Nebraska Chadron	Massachusetts Williamstown	New Jersey Englewood	Nebraska Lincoln	Missouri Olivette, St. Louis	North Carolina Raleigh	South Carolina Myrtle Beach	Florida Astor
			A. Esterase-1 (Chromosome 2L):					
Allele								
.86	.23	.096	.045	.11	.04	.03	.02	.01
.92	.14	.288	.524	.45	.65	.60	.78	.70
1.0	.59	.48	.396	.37	.27	.30	.15	.29
1.07	.03	.135	.035	.07	.04	.07	.05	—
n	91	104	802	81	188	182	41	66
			B. Acid phosphatase-4 (Chromosome X):					
—	.06	—	—	.02	.037	.07	—	.03
.81	.24	.05	.01	—	.01	—	—	—
.93	.68	.90	.93	.96	.89	.64	.80	.34
1.0	.01	.05	.06	.02	.065	.29	.20	.63
n	78	21	98	47	107	118	50	35
			C. Esterase-2 (Chromosome 2):					
—	.044	.05	—	—	—	.015	.026	.04
.90	.060	—	.05	—	—	.015	.077	—
.97	.044	.05	.15	.08	.23	.26	.33	.33
1.0	.85	.90	.80	.92	.77	.71	.565	.63
n	68	20	208	48	48	70	40	30
			D. Leucine aminopeptidase-1 (Chromosome 2L):					
.80	—	—	—	—	—	—	.03	—
.90	.015	—	.03	.02	—	.08	.11	.30
1.0	.985	1.0	.964	.98	.98	.92	.86	.70
1.10	—	—	.006	—	.02	—	—	—
n	67	20	166	50	42	86	36	46
			E. Amylase (Chromosome 2R):					
.85	.64	.75	.73	.76	.70	.65	.93	.87
1.0	.36	.25	.27	.24	.30	.35	.07	.13
n	100	20	70	29	44	80	44	45

in both north-south and east-west directions. At the *Pt-8* and the *Pt-5* loci there is no allele which is most frequent in all populations. A comparison of Chadron *vs.* Williamstown and Lincoln *vs.* Englewood shows that the frequency of the allele $Pt\text{-}8^{.98}$ is much higher in the eastern populations than in the western populations which are at about the same latitude. A clinal increase is observed in the allele $Pt\text{-}8^{.98}$ in the southwestern populations. The $Pt\text{-}8^{.98}$ allele occurs in about the same frequency in all eastern coastal plain populations except in Astor where this allele increases to 80%. At the *Pt-5* locus, we observe that the frequency of the allele *.97* is higher in the western populations of Lincoln and Olivette than the eastern populations which are at about the same latitude. An increase in the fre-

TABLE 7

Allele frequencies at loci with simultaneous east-west and north-south change in allele frequencies. The western and the eastern populations are arranged according to latitudes. Chadron, Nebraska is the northernmost and Olivette, St. Louis, Missouri the southernmost western population; Williamstown, Massachusetts is the northernmost and Astor, Florida the southernmost eastern population. Also see Figure 1 for latitudes and longitudes of these populations

	Western populations			Eastern Coastal plain populations				
State:	Nebraska	Nebraska	Missouri	Massachusetts	New Jersey	North Carolina	South Carolina	Florida
Locality:	Chadron	Lincoln	Olivette, St. Louis	Williamstown	Englewood	Raleigh	Myrtle Beach	Astor
Alleles								
			A. Larval protein-8 (Chromosome 3):					
.98	.20	.32	.43	.55	.62	.50	.50	.80
1.0	.80	.66	.57	.45	.38	.50	.50	.20
1.02	—	.03	—	—	—	—	—	—
n	84	38	37	20	47	42	30	47
			B. Larval protein-5 (Chromosome X):					
.97	—	.39	.39	.04	.06	—	.17	.59
1.0	1.0	.61	.61	.96	.94	1.0	.83	.41
n	112	30	54	24	82	98	42	56
			C. Larval protein-9 (Chromosome 2):					
.95	—	.05	—	—	—	—	—	—
1.0	.71	.59	.71	.60	.70	.70	.62	.83
1.02	.05	.18	.21	.40	.21	.23	.17	.17
1.04	.23	.17	.08	—	.09	.07	.21	—
n	112	54	72	24	107	98	42	54

quency of the allele *.97* is observed in the southern populations. The most conspicuous changes in allele frequency are observed in the two marginal populations from Chadron and Astor. The allele *1.0* at the *Pt-9* locus is the most frequent in all populations. The allele $Pt\text{-}9^{1.02}$ is quite frequent in Williamstown; this allele decreases in frequency in both the southern and the western populations.

C. *Allele frequency differentiation among populations:* The rest of the eleven polymorphic loci (Tables 8A–K) fall under this category. At all of these loci one allele is present in highest frequency in all populations, but different populations are polymorphic for different loci. Only the Chadron population is polymorphic at the *Fum* locus; both the Chadron and Astor populations are polymorphic at the *Benz oxidase* locus. Polymorphism was observed only in two populations at the *Pep-2* locus. At the *Pt-16* locus we observe a very sudden and large increase in the frequency of the allele *.86* which is either absent or present in low frequency in the other populations. At the *Pt-11* locus we did not observe any polymorphism in the two populations from Lincoln and Olivette. The allele $MDH^{1.20}$ is absent in Astor; the frequency of this allele is different in different populations. Similar differ-

TABLE 8

Loci which are polymorphic in some populations but not in others and in which the change in allele frequencies between populations does not show gradual clines

	Western populations			Eastern Coastal plain populations				
State:	Nebraska	Nebraska	Missouri	Massachusetts	New Jersey	North Carolina	South Carolina	Florida
Locality:	Chadron	Lincoln	Olivette, St. Louis	Williamstown	Englewood	Raleigh	Myrtle Beach	Astor
Alleles								
			A. Fumerase (Chromosome X):					
.70	.02	—	—	—	—	—	—	—
1.0	.87	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1.35	.11	—	—	—	—	—	—	—
n	95	72	70	20	70	80	30	40
			B. Benzaldehyde oxidase (Autosome):					
—	.015	—	—	—	—	—	—	.04
.80	.075	—	—	—	—	—	—	—
1.0	.91	1.0	1.0	1.0	1.0	1.0	1.0	.96
n	68	50	42	20	200	60	36	27
			C. Peptidase-2 (Chromosome X):					
.80	.22	—	—	—	—	—	.08	—
1.0	.78	1.0	1.0	1.0	1.0	1.0	.92	1.0
n	60	50	50	10	40	50	25	30
			D. Larval protein-16 (Chromosome 2L):					
.86	—	—	.01	—	.02	.08	—	.82
.92	—	—	.01	.03	—	.02	—	.04
1.0	1.0	.96	.96	.97	.98	.90	1.0	.14
1.08	—	.04	.02	—	—	—	—	—
n	110	30	75	31	98	100	40	66
			E. Larval protein-11 (Autosome):					
1.0	.86	1.0	1.0	.90	.82	.87	.96	.92
1.04	.14	—	—	.10	.18	.13	.04	.08
n	85	30	50	10	40	62	24	37
			F. Malic dehydrogenase (Chromosome 3):					
.80	—	—	—	—	—	—	—	.02
1.0	.89	.78	.86	.82	.85	.95	.87	.98
1.20	.055	.22	.14	.18	.15	.05	.13	—
1.50	.055	—	—	—	—	—	—	—
n	92	78	160	22	216	60	61	41
			G. Octanol dehydrogenase (Chromosome 2):					
.85	.07	—	—	.08	.04	—	—	.06
1.0	.93	.99	1.0	.92	.96	.98	1.0	.90
1.17	—	.01	—	—	—	.02	—	.04
n	71	72	130	12	110	100	40	48
			H. Hexokinase-8 (Autosome):					
.93	—	.08	—	—	—	.034	—	—
1.0	1.0	.92	1.0	1.0	1.0	.966	1.0	1.0
n	120	36	40	26	50	88	30	40
			I. Peptidase-3 (Autosome):					
.80	—	—	—	—	—	—	—	.03
1.0	.97	1.0	.98	1.0	1.0	1.0	1.0	.97

TABLE 8—Continued

	Western populations			Eastern Coastal plain populations				
State:	Nebraska	Nebraska	Missouri	Massachusetts	New Jersey	North Carolina	South Carolina	Florida
Locality:	Chadron	Lincoln	Olivette, St. Louis	Williamstown	Englewood	Raleigh	Myrtle Beach	Astor
Alleles								
1.07	.03	—	—	—	—	—	—	—
1.20	—	—	.02	—	—	—	—	—
n	60	50	52	15	92	52	32	40
J. α-glycerophosphate dehydrogenase (Autosome):								
.80	.03	—	.02	—	.01	—	—	—
1.0	.97	1.0	.98	1.0	.99	1.0	1.0	1.0
n	94	72	54	20	100	80	30	40
K. Alcohol dehydrogenase-2 (Autosome):								
—	—	—	.01	—	—	—	.13	—
1.0	1.0	1.0	.99	1.0	1.0	1.0	.87	1.0
n	90	72	90	20	100	80	30	40

entiation is observed at the *ODH* locus. At the four remaining loci (*Hex-8*, *Pep-3*, *α-GPD*, *Adh-2*) the rare alleles are observed only in some populations.

3. *Clinal changes in allele frequencies in gene arrangements:* The clinal changes in gene frequencies at most of the loci cannot be explained only by associations of genes with the inversions and changes in frequency of different inversions in different populations. A comparison of frequencies of gene arrangements (Table 4) and the allele frequencies at various loci in different populations (Tables 5–8) shows that the allele frequencies within an inversion type are different in different populations. For example, we have observed the alleles *.70* and *1.35* at the *Fum* locus only in the Chadron population. If this locus is present on the XL chromosome, then we expect the same frequencies of these alleles in Williamstown and a substantial frequency of these alleles in Lincoln. If the locus is present on the XR chromosome we expect these alleles in substantial frequency in the Williamstown and Englewood populations. The other loci on the *X* chromosome showing differentiation among populations are *AP-4*, *Pt-5* and *Pep-2*. In all these cases we observe changes in allele frequencies within a gene arrangement. The gene arrangements in the Myrtle Beach and Astor strains were almost identical, yet these two populations differ greatly in allele frequencies at the *AP-4*, the *Pt-5* and the *Pep-2* loci. A similar situation is observed at the *Est-1*, the *LAP-1* and the *Pt-16* loci on the left arm of the second chromosome, the *Amy* locus on the right arm of the second chromosome and the *Pt-8* and the *MDH* loci on the third chromosome. The associations of the *Est-1* alleles with various gene arrangements of the left arm of the second chromosome and clinal changes in the frequency of alleles within a gene arrangement have been discussed by PRAKASH and LEVITAN (1973). At the *LAP-1* and the *Pt-16* loci we observe differentiation between the Myrtle Beach and Astor populations. In both populations the *2L1* gene arrangement is 98% or higher. The allele frequency changes at the *Amy* locus also show

TABLE 9

Average heterozygosity at each of the polymorphic loci in various D. robusta *populations*

No.	locus	Chromosome location	Marginal				Central			
			Chadron (Nebraska)	Astor (Florida)	Williamstown (Massachusetts)	Myrtle Beach (So. Carolina)	Lincoln (Nebraska)	Englewood (New Jersey)	Olivette, St. Louis (Missouri)	Raleigh (No. Carolina)
1	*XDH*	2L	.600	.566	.480	.546	.598	.545	.588	.481
2	*Pep-1*	Autosome	.204	.058	.375	.058	.204	.095	.180	.241
3	*Hex-1*	Autosome	.115	.039	.465	.113	.114	.183	.096	.089
4	*Est-1*	2L	.578	.426	.659	.366	.614	.565	.501	.544
5	*Est-2*	2	.270	.492	.185	.565	.147	.335	.354	.428
6	*AP-4*	X	.476	.487	.185	.320	.078	.131	.202	.501
7	*LAP-1*	2L	.030	.420	0.000	.247	.040	.070	.040	.147
8	*Amy*	2R	.461	.226	.375	.130	.365	.394	.420	.455
9	*Pt-8*	3	.320	.320	.495	.500	.461	.471	.490	.500
10	*Pt-9*	2	.440	.282	.480	.543	.588	.458	.445	.452
11	*Pt-5*	X	0.000	.484	.077	.282	.476	.113	.476	0.000
12	*Fum*	X	.230	0.000	0.000	0.000	0.000	0.000	0.000	0.000
13	*Pep-2*	X	.343	0.000	0.000	.147	0.000	0.000	0.000	0.000
14	*Benz 0X*	Autosome	.166	.077	0.000	0.000	0.000	0.000	0.000	0.000
15	*Pt-16*	2L	0.000	.306	.058	0.000	.077	.040	.078	.183
16	*ODH*	2	.130	.185	.147	0.000	.020	.077	0.000	.040
17	*MDH*	3	.202	.040	.295	.226	.343	.365	.241	.095
18	*Hex-8*	Autosome	0.000	0.000	0.000	0.000	.147	0.000	0.000	.066
19	*Pt-11*	Autosome	.241	.147	.180	.077	0.000	.295	0.000	.226
20	*Pep-3*	Autosome	.058	.058	0.000	0.000	0.000	0.000	.040	0.000
21	*αGPD*	Autosome	.058	0.000	0.000	0.000	0.000	.020	.040	0.000
22	*Adh-2*	Autosome	0.000	0.000	0.000	.226	0.000	0.000	.020	0.000
		Total heterozygosity	4.922	4.613	4.456	4.346	4.302	4.157	4.211	4.448

different allele frequency within the *2R1* arrangements in different populations. The allele frequencies at this locus are similar in all populations except in Myrtle Beach and Astor even though the frequency of the *2R1* arrangement is different in different populations; this arrangement is 0% in Chadron and 41% in Raleigh, yet both populations have the same frequencies of alleles at the *Amy* locus. A comparison of allele frequencies at the *Pt-8* locus in Chadron *vs.* Williamstown and Myrtle Beach *vs.* Astor which have the same frequency of gene arrangements in the *3R* chromosome, shows that significant differences exist in allele frequencies in identical gene arrangements from different populations. The MDH locus also provides evidence of differentiation of the *3R* gene arrangement in different populations. Even though we do not know the location of the *Pt-9* locus on the second chromosome, for this locus also we have to postulate an existence of genetic differentiation of some arrangements in different populations.

DISCUSSION

Table 10 gives the proportion of polymorphic loci and the proportion of genome heterozygous per individual in eight populations of *D. robusta*. In *D. robusta*, on the average, the proportion of polymorphic loci is 39.4% and the proportion of the genome heterozygous per individual is 11%. We do not observe any reduction in polymorphism toward the margins: on the contrary, gene polymorphism is somewhat higher in the two marginal populations of Chadron and Astor than in the central and the other populations (Table 9). These results do not support MAYR's statement of reduced gene variation toward the margins due to reduction of gene flow and increased selection pressure or CARSON's idea of homoselection in the margins due to limited ecological niches. The available evidence shows that the marginal populations of *D. robusta* have a smaller population size than those in the center. The frequency of *D. robusta* relative to affinis group species in some

TABLE 10

Proportion of loci polymorphic (total loci = 40) and the proportion of loci estimated to be heterozygous in an average individual for each population studied

Population	Geographic location	Number of loci polymorphic	Proportion of loci polymorphic	Proportion of loci heterozygous per individual
Chadron, Neb.	Northwestern margin	18	.450	.123
Astor, Fla.	Southeastern margin	17	.425	.115
Williamstown, Mass.	Towards Northeastern margin	14	.350	.111
Myrtle Beach, S.C.	Towards Southeastern margin	15	.375	.109
Lincoln, Neb.	Central, Northwest	15	.375	.107
Englewood, N.J.	Central, Northeast	16	.400	.104
Olivette, Mo.	Central, Southwest	16	.400	.105
Raleigh, N.C.	Central, Southeast	15	.375	.111
Mean		15.75	.394	.110

of the populations is as follows: Astor, Fla. (1.7%) (CARSON and HEED 1964), Englewood, N. J. (6.4%), Olivette, St. Louis, Mo. (20.2%), Eastern Neb. (16%) (CARSON 1955a). Low frequency of *D. robusta* in the marginal populations suggests that the ecological and other conditions are poor at the margins and cannot sustain large populations of this species. The existence of somewhat higher or the same levels of genic polymorphism in the marginal populations despite much reduced population size can only be explained by stronger selection in favor of heterozygotes in the marginal populations in comparison to the central populations. Lack of inversion polymorphism in the margins provides an increased opportunity for recombination (CARSON 1955b). Marginal populations, then, have greater genetic variation than the central populations due to a high level of allozyme polymorphism and due to variation produced by free recombination.

These observations of gene polymorphism in *D. robusta* are similar to those reported in *D. pseudoobscura* (PRAKASH, LEWONTIN and HUBBY 1969). The magnitude of variation in the southeastern marginal population of Austin (Texas), which has a reduced population size, is about the same as in the large central populations. In *D. willistoni* as in *D. robusta* the central populations have a very high level of inversion polymorphism and the marginal populations have a much reduced level of inversion polymorphisms, yet the magnitude of allozyme polymorphism is about the same in all populations (AYALA *et al.* 1972). There is no evidence in support of reduced gene variation in marginal populations. The only example of reduced gene variation in populations of Drosophila is provided by the Bogotá, Colombia population of *D. pseudoobscura* which is isolated from the rest of the species by more than 1500 miles. The reduced variation in the Bogotá population is due to founder effect and inbreeding (PRAKASH, LEWONTIN and HUBBY 1969; PRAKASH 1972) and not due to homoselection.

Table 11 presents the level of polymorphism in different groups of loci which have been classified in three groups. In all populations the proportion of polymorphic loci and the average heterozygosity are highest in the hydrolytic enzymes, intermediate in the larval proteins and lowest in the enzymes of glucose metabolism and the Kreb's cycle. The average heterozygosity in the hydrolytic and other enzymes and the glycolytic and Kreb's cycle enzymes is 16.4% and 3.7%, respectively. These results confirm and extend similar observations made by KOJIMA, GILLESPIE and TOBARI (1970) in various Drosophila species, by RICHMOND (1972) in *D. paulistorum*, and by AYALA and POWELL (1972) in *Willistoni* group species and raise the question about the reliability of estimates of variation based on small sample of loci which are probably not a random sample of the genome (PRAKASH 1971).

Table 12 gives the proportion of polymorphic loci and the proportion of total heterozygosity on the *X* chromosome. The euchromatin in the two arms of the *X* chromosome is 38% of the total euchromatin. If the polymorphic loci are distributed randomly on the *X* chromosome and the autosomes, then we would expect 38% of the polymorphic loci to be located on the *X* chromosome and the remaining 62% on the autosomes. However, we find that in all populations of *D. robusta* much less than 38% of the polymorphic loci and much less than 38%

TABLE 11

Proportion of loci polymorphic and average heterozygosity in loci coding for enzymes of glycolytic pathway and Kreb's cycle (Hex-1,-2,-6,-7,-8,αGPD, Ald-1, Ald-2, G6PD, MDH, M.E., Fum) *hydrolytic and other enzymes* (AP-4, LAP-1, LAP-2, Est-1, Est-2, Amy, Pep-1,-2,-3 *and* Adh-1 *and* -2, Ak, ODH, B.0x XDH, *Oxidase*) *and larval hemolymph proteins*

Group	Populations:	Chadron (Neb.)	Astor (Fla.)	Williamstown (Mass.)	Myrtle Beach (S.C.)	Lincoln (Neb.)	Englewood (N.J.)	Olivette (Mo.)	Raleigh (N.C.)	Mean
I. Enzymes of Glycolytic and Kreb's cycle (12 loci)	Proportion of loci polymorphic	.333	.167	.167	.167	.250	.250	.250	.250	.229
	Average heterozygosity	.050	.006	.063	.028	.050	.047	.031	.021	.037
II. Hydrolytic and other enzymes (16 loci)	Proportion of loci polymorphic	.687	.625	.437	.562	.500	.500	.562	.500	.547
	Average heterozygosity	.207	.187	.150	.163	.131	.138	.146	.189	.164
III. Larval proteins (12 loci)	Proportion of loci polymorphic	.250	.417	.417	.333	.333	.417	.333	.333	.354
	Average heterozygosity	.083	.128	.107	.117	.133	.115	.124	.113	.115

TABLE 12

Proportion of polymorphic loci and proportion of total heterozygosity on the X *Chromosome*

Populations:	Chadron (Nebraska)	Astor (Florida)	Williamstown (Massachusetts)	Myrtle Beach (So. Carolina)	Lincoln (Nebraska)	Englewood (New Jersey)	Olivette (Missouri)	Raleigh (No. Carolina)	Mean
Proportion of polymorphic loci on the *X* Chromosome (Expected = .38)*	.167	.118	.143	.200	.133	.125	.125	.067	.135
Proportion of total heterozygosity on the *X* Chromosome (Expected = .38)*	.211	.210	.059	.172	.129	.059	.161	.113	.139

* Both arms of the *X* Chromosome make up 38% and the Autosomes the remaining 62% of the euchromatic length of the salivary gland chromosomes of *D. robusta* (CARSON 1955b). We thus expect 38% of the total polymorphism on the *X* Chromosome and the remaining 62% of the polymorphism on the autosomes.

of the total heterozygosity are present on the X chromosome. In Chadron only 16.7% of the polymorphic loci and 21.1% of the total heterozygosity are present on the X chromosome. In Astor the proportions of polymorphic loci and heterozygosity on the X chromosome are 11.8% and 21%, respectively. The proportion of total heterozygosity on the X chromosome in the four central populations from Lincoln, Englewood, Olivette, and Raleigh varies from 5.9% to 16.1% with a mean of 11.55%. The proportion of total heterozygosity on the X chromosome is higher in the two marginal populations than in the central populations. The mean proportions of polymorphic loci and of total heterozygosity on the X chromosome for all eight populations are 13.5% and 13.9%, respectively. These figures are much lower than the expected 38%. The possible explanations for this discrepancy are:

1. The structural genes for enzymes of different metabolic pathways may be distributed non-randomly on the X chromosome and the autosomes. For example, proportionately more loci which code for the enzymes involved in the glycolytic pathway and the Kreb's cycle, which characteristically show lower variation, may be located on the X chromosome and the autosomes may have proportionately more loci coding for hydrolytic enzymes which show greater variation. Of the four polymorphic loci which are located on the X chromosome of *D. robusta*, two are hydrolytic enzymes *AP-4* and *Pep-2* loci, one is a larval protein—*Pt-5* locus—and the fourth is a Kreb's cycle enzyme locus—*Fum.* Of the eighteen polymorphic loci on the autosomes, ten are loci for the hydrolytic enzymes and other loci such as *ODH, Adh*, etc., four are larval proteins loci and the remaining four loci are Kreb's cycle and glycolytic enzyme loci. These data suggest that the loci involved in different functions are randomly distributed in the X chromosome and the autosomes of *D. robusta.*

2. The number of polymorphic loci and the average heterozygosity on the X chromosome may be lower due to the effect of random drift. The population size of the X chromosome is 75% of the autosomes; therefore, the probability of random fixation of alleles at the polymorphic loci will be greater for the loci on the X chromosome. However, we observe that both the proportion of polymorphic loci and the average heterozygosity on the X chromosome are lower than $.38 \times .75 = .285$. Moreover, the marginal populations which have small population sizes have more heterozygosity on the X chromosome than the large central populations.

3. The low variation on the X chromosome may be due to the lower probability of balanced polymorphism on the X chromosome as compared to the autosomes. The conditions for the maintenance of balanced polymorphism on the X chromosome are more stringent than for the autosomal loci. For equilibrium to be stable for the X chromosome loci the following sets of inequalities have to be satisfied: $W12/W11 > 2W1/W1+W2$ and $W12/W22 > 2W2/W1+W2$ (Crow and Kimura 1970), where W1 and W2 are the fitnesses of the two genotypes A and A^1 in the males and W11, W12 and W22 are the fitnesses of the three genotypes in AA, AA^1 and A^1A^1 in the females.

Patterns of gene variation: A remarkable feature of variation in allele frequencies between different populations of *D. robusta* is the lack of random differentiation of allele frequencies among populations at most of the polymorphic loci. Some of the important observations are restated here: (1) The allele frequencies are essentially similar at the *XDH* locus, where the two major alleles are present in about equal frequencies and at the *Pep-1* and the *Hex-1* loci, where one allele is present in high frequency. (2) The alleles at the *Est-1*, *Est-2*, *Amy* loci and the $AP\text{-}4^{1.0}$ and the $LAP\text{-}1^{.90}$ alleles show a gradual north-south clinal change in frequency. (3) The alleles at the *Pt-5*, *Pt-8* and *Pt-9* loci and the allele $AP\text{-}4^{.81}$ show very considerable east-west and north-south differentiation. (4) The marginal populations of Chadron and Astor show a great deal of differentiation from other populations at several loci. The allele $Pt\text{-}16^{.86}$ is 82% in Astor; this allele is never more than 8% in any of the other populations. The alleles $Fum^{1.85}$, $Fum^{.70}$ and $B.Ox^{.80}$ have been observed only in the Chadron population. There is a fairly sudden differentiation at the *AP-4* locus in both marginal populations and at the *Amy*, the *Pt-8* and the *Pt-9* loci in the Astor population. (5) Allele frequencies at the *MDH* and the *ODH* loci fluctuate between populations while at some loci, such as *Hex-8* and *Pep-2*, only two widely separated populations are polymorphic. (6) The allele frequency changes in different populations cannot be explained merely by associations of alleles with inversions and changes in inversion frequencies in various populations. *There is enough evidence to show that for many loci allele frequencies within a particular gene arrangement change clinally.*

Two different but not mutually exclusive explanations can be offered for these observations. (1) *Selectively neutral polymorphisms and migration between once-isolated populations:* Gradual clines in allele frequencies between different populations may be a result of migration between populations which were once completely isolated and had differentiated in their gene pools mainly by random drift. CARSON (1959) supposes that during the glacial advance, northern and southern isolates of *D. robusta* may have existed in the Wisconsin driftless area and the Cumberland plateau, respectively; the present species distribution, according to CARSON, might represent an expansion of these once-isolated populations. But the complexity of the pattern of geographical variation cannot be explained merely by assuming migration between a few populations which were once isolated. The single-most important observation against this hypothesis is that there is no similarity in the pattern of geographic variation at most loci. The pattern of geographic variation is different for different loci. (2) *Most of the observed variation is of selective significance and is maintained by balancing selection.* All of the observations—(1) slightly higher gene variation in the marginal than the central populations even though the population sizes of the former are smaller, (2) the presence of a greater level of polymorphism on the autosomes than the *X* chromosome, and finally, (3) the complex geographic patterns of gene variation—can be explained by the hypothesis that most of the observed genic variation is of physiological significance and is maintained by some form of

balancing selection. Clinal changes of allele frequencies within particular gene arrangements show that environmental aspects play an important role in determining the allele frequencies in populations of *D. robusta*.

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