Reports and Letters

Marginal Homozygosity for Gene Arrangement in Drosophila robusta

Drosophila robusta inhabits the deciduous forest of the eastern United States. Like a number of other endemic species of the genus, most of its natural populations display chromosomal polymorphism due to the presence of inverted sections (*1*). These inversions cover long portions of the chromosomes and are widespread geographically. In some populations, especially those near the center of the species range (Virginia, Tennessee and southern Missouri), the amount of polymorphism is enormous; more than 95 per cent of the wild females are heterozygous for at least one sizable inversion, and some have as many as five inversions, one in each of the six major chromosome arms (*2*).

Recent studies of more or less marginally situated populations (that is, in Georgia-Alabama to the south and Wisconsin-Minnesota to the north) have shown that the degree of chromosomal polymorphism in populations at the edge is much less than it is in populations at the center of the range (*2*). The present note (*3*) reports the existence of a population at the extreme northwest margin of the species range that is essentially homozygous for gene arrangement. This finding is of particular interest because it provides evidence that marginal homozygosity in this species may be a more complete and striking phenomenon than has previously been suspected.

A collection of 281 specimens of *Drosophila robusta* was made 1–3 Aug. 1955 at Chadron State Park, Dawes County, in the northwestern corner of Nebraska. Such a sizable collection of this species in a marginal area is unprecedented in my experience—my attempts to obtain quantitative population samples in supposed marginal sites had been repeatedly unsuccessful in previous years.

Salivary gland chromosome analyses were carried out on F_1 individuals produced by the wild flies after they had been brought to the laboratory; the conventional acetoorcein technique was used. Table 1 gives the results for the five major chromosome arms that display variability in gene sequence in the populations of the central Missouri River Valley. It will be seen that the only chromosome arm that is not structurally homozygous in this population is the right arm of the X chromosome. In this case, moreover, only two instances, or 0.6 percent, of the alternative gene arrangement XR-1 were recorded. The left arm of chromosome 3 is also homozygous. It is not listed separately in Table 1, because no variability is known in it that does not also involve the right arm.

Study of a small sample of flies taken in the same locality in August 1950 (*4*) gave results that are compatible with those obtained in 1955. Of a total of 20 chromosomes tested for each of the chromosome arms listed in Table 1, only one instance of diversity of gene order (another XR-1 gene arrangement) was found.

This extraordinarily high degree of structural homozygosity is of particular interest in view of the special ecological and geographical conditions that exist in the Chadron area. Chadron Creek, along which the collections were made, arises in a series of springs not more than 4 mi above the state park on the northward-facing slope that is known locally as the "Pine Ridge." The flood plain of Chadron Creek in the vicinity of the park does not in most places exceed 100 yd in width. The American elm (*Ulmus americana*), the principal host tree for *Drosophila robusta,* grows abundantly in the narrow strip along the creek but is wholly absent from higher ground. The upland areas in this region support, in addition to a complex short-grass flora, only a moderate growth of western yellow pine (*Pinus ponderosa*). The existence of *Ulmus americana* and *Pinus ponderosa,* growing within a few feet of one another, typifies the meeting of eastern and western biota that occurs in the region. The *Drosophila* fauna likewise shows a mixture of eastern and western forms (*5*). The uplands of the pine ridge area and the treeless sandhills to the south, east, and west form a rigid ecological barrier for *Ulmus,* and the trees inhabiting the valley are clearly at the terminus of a riparian flora that is derived from the valley of the White River, into which Chadron Creek flows. This junction is about 10 mi north of the collecting site in the park and is approximately 5 mi west of the city of Chadron (elevation 3383 ft).

Although collections were not made in the surrounding sand hills, it is inferred that *D. robusta* could not exist there in permanent populations in the absence of a deciduous host tree. The distribution of the fly in this area, as elsewhere, would be expected to parallel that of the species of tree on which it breeds. The White River, after receiving Chadron Creek from the south, flows in a generally northeasterly direction across southern South Dakota and joins the Missouri River about 65 mi southeast of Pierre. Thus, the *D. robusta* populations of the northwestern corner of Nebraska along Chadron Creek and other tributaries at the headwaters of the White River, appear to be linear outposts derived from those of central South Dakota. They would be expected to have affinities with these northern populations, as yet unstudied, rather than with the populations of the more southeasterly drainage basins of Nebraska (for example, Niobrara and Loup rivers).

The suggestion has been made (*6*) that the amount of chromosomal polymorphism present in a particular population of a species is directly proportional to the number of ecological niches exploited by the members of that population. The data on chromosomal polymorphism in *Drosophila robusta* do not contradict this idea (*7*). Quite apart from this consideration, however, the very fact of marginal homozygosity for gene sequence carries important implications for the microevolutionary plasticity of the species. It is now well established that chromosome inversions act primarily as blockers of effective crossing over in the chromosome regions that they cover. From this, it follows that in a population that has very few or no inversions, the process of gene recombination may proceed unhampered, and novel genotypes may be readily synthe-

Table 1. Gene arrangements present in a population of *Drosophila robusta* from Chadron State Park, Neb., in August 1955; 1868 chromosome arms were tested; 674 X chromosome arms and 1194 autosomal arms.

Chromosome	Chromosome arm	Gene arrangements present	No. of each gene arrangement recovered
X	left	XL-1	337
X	right	XR	335
X	right	XR-1	2
2	left	2L-3	398
2	right	2R	398
3	right	3R	398

sized. Where polymorphism is extensive, on the other hand, the gene pool of the species is essentially broken up into a number of smaller gene pools, each of which corresponds to a chromosome arrangement. There is thus less opportunity for the hereditary variability present to undergo extensive free recombination. One may suggest, therefore, that marginal populations that are essentially homozygous for gene arrangement may be of special interest in evolutionary studies because they have a chromosomal system that allows maximum recombination by crossing over. They would appear to be peculiarly well suited for the attainment of future evolutionary advance.

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References and Notes

1. H. L. Carson and H. D. Stalker, *Evolution* 1, 113 (1947).
2. H. L. Carson, *J. Cellular Comp. Physiol.* 45, 221 (1955).
3. This work was supported by grants from the National Science Foundation and the Office of Naval Research.
4. This collection was made by D. D. Miller of the University of Nebraska, who is responsible for calling my attention to the unique features of the Chadron area.
5. D. D. Williams and D. D. Miller, *Bull. Nebraska State Museum* 3, 1 (1952).
6. A. B. da Cunha and T. Dobzhansky, *Evolution* 8, 119 (1954).
7. H. L. Carson, *Cold Spring Harbor Symposia Quant. Biol.*, in press.

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Influence of White Blood Cells on Lysis of Red Cells by Cobra Venom

It has been known since the early studies of Kyes (*1*) that snake venom hemolysis exhibits a species specificity. The difference in susceptibility of cells has been attributed by some to availability of intracellular lecithin for the action of venom lecithinase. On the other hand, it has not been possible to correlate lecithinase activity of venoms with their hemolytic activity (*2*). A recent discussion of the problem suggests that the facts are best reconciled in the idea that venoms contain, in addition to lecithinase, a lysin that has a direct hemolytic action (*3*). This view was apparently shared by Landsteiner, who compared the action of venoms on red cells with that of the phytoagglutinins and natural antibodies (*4*). The mode of the direct action of a native venom is still unexplained (*2*).

In the course of certain studies (*5*) on splenic cells, it has been found that washed red cells from the spleens of rabbit, swine, and ox are rapidly lyzed by traces of cobra venom although the erythrocytes of the peripheral blood are completely, or almost completely, unaffected by venom in comparatively high concentration. Evidence has been found that this apparently selective action on splenic cells is conditioned by the presence of leucocytes in the red-cell suspensions.

Table 1. Action of venom on splenic cells. Peripheral blood tested in same way showed no hemolysis whatever.

Time (min)	Venom dilution (× 10^3)							Control
	4	8	16	32	64	128	256	
20	++++	++++	++++	0	0	0	0	0
40	++++	++++	++++	++++	++++	+++	0	0
60	++++	++++	++++	++++	++++	++++	0	0

* ++++ = complete hemolysis.

Cobra (*Naja naja*) venom (*6*) stored as a dry powder was serially diluted for tests in 0.85-percent saline containing 0.02*M* phosphate buffer. Unless otherwise stated, *p*H was 7.4. To conserve material, the lowest venom dilution used was generally 1/4000 or 1/8000.

In some experiments, an animal lecithin (Eastman Kodak Co.) emulsion in buffer was added to the system in a volume of 0.1 ml containing 50 μg of lecithin.

Spleens and defibrinated blood of ox and swine were obtained from the slaughterhouse, and cell preparations were made within 1 or 2 hours of death. For studies of rabbit cells, freshly killed animals were used.

Cells of peripheral blood were washed three times in saline and made up to a 1- or 2-percent suspension. Fat and capsule were dissected from spleens, and cell suspensions were made by shaking teased-out pulp in saline. The cells were similarly washed.

Tests for hemolysis were made by adding 0.1 ml of cell suspension to 1 ml of venom dilution. The test tubes were placed at 37°C and read for hemolysis at intervals for 1 to 2 hours, and finally after an overnight interval at 4°C.

The selective action of venom on spleen cells is shown as a typical experiment with ox cells in Table 1. In eight such experiments with the ox, the peripheral blood cells showed hemolysis only once, in the 1/8000 dilution, and not until after 60 minutes of incubation. On the other hand, splenic cell suspensions were invariably hemolyzed rapidly in titers of venom ranging from 1/32,000 to 1/512,000.

Entirely similar findings were obtained with cell preparations from rabbit and swine, but since ox spleens were easier to work with, further studies were made on cell preparations from this animal alone.

It was found that hemolysis of splenic blood was maximum at *p*H 7.4 to 7.9 and that it decreased with lowering of *p*H, disappearing at *p*H 6.0. The lecithinase activity of the venom was tested by adding lecithin to venom and peripheral blood cells, which then hemolyzed rapidly. It was found that the optimum *p*H of this system is also in the range 7.5 to 7.9, and that activity decreases on acidification. Thus, the action of venom on splenic blood and on lecithin could not be distinguished in terms of *p*H optima.

White-cell counts were performed on many suspensions being tested, and random samplings were taken from those showing lysis and from controls. The number of leucocytes in the control tubes of splenic blood usually numbered 20,000 to 40,000/mm^3, while the corresponding preparations of peripheral blood had less than 200. When the content of white cells in spleen blood was reduced by repeated differential centrifugation, it could be shown that lysis of erythrocytes was slow and might fail altogether if the number of white cells fell below about 500/mm^3.

Conversely, it was found that leucocytes concentrated from peripheral whole blood could bring about lysis when they were added to a suspension of red cells that were otherwise insusceptible to the action of venom.

It was of some interest to note that lysis of the white blood cells did not appear to be essential for this effect. Table 2 shows that in the tubes containing the two highest dilutions of venom, the final white-cell counts were comparable to the controls even though hemolysis was ob-

Table 2. Final white counts on venom-splenic cell system.

Expt.	Venom dilution (× 10^3)						Controls	
	8	16	32	64	128	256	C_1	C_2
Lysis	++++	++++	++++	++++	++++	++++		
White count (× 10^3)	2.2	2.5	2.3	2.8	8.4	12.0	8.4	5.1