THE CHROMOSOMAL POLYMORPHISM OF DROSOPHILA BUSCKII IN NATURAL POPULATIONS[1]

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AS a result of recent intensive investigations of natural populations of cross-breeding organisms, the once dominant view of the population as a monomorphic structure, in both genetical and cytological respects, is being replaced by a concept of polymorphic, adaptively balanced structure. This concept holds that the high heterogeneity of natural populations is a condition of the existence and continuing persistence of the population itself, no less of the species as a whole (DOBZHANSKY 1951, 1955; PATTERSON and STONE 1952; STONE 1955, for review and references).

Many different organisms have served as objects of investigation of natural populations, and chief among them have been different species of Drosophila. Though quite a number of the culturable species of Drosophila have at least been scanned with respect to genic and sequential variability, a much larger basis is needed if general population laws are to be adduced by comparative genetics. This study now adds new and striking information on *Drosophila busckii* to the general pool.

D. busckii is a cosmopolitan species widely spread throughout all zoogeographical regions, almost equally often met with near human habitations as in remote, uninhabited regions. Taxonomically this species is isolated, being the only member of the subgenus Dorsilopha, evidently representing one of the more ancient species of the genus Drosophila (STURTEVANT 1921; THROCKMORTON 1962a, 1962b). In comparative genetical and cytological respects *D. busckii* is exceptionally interesting, and is now becoming reasonably well known. Despite this fact, little has yet been published on the cytogenetic structure of natural populations of this species. This fact can partly be explained by the relative difficulty of culturing this species under laboratory conditions.

In 1938–1940 I collected data on the genetic variability of the natural populations of *D. busckii* in the vicinity of Kiev, Ukraine. During those three years an enormous amount of material of exceptional value was collected. Unfortunately the results of this investigation were not published because of the war, although some of them were briefly mentioned in PROFESSOR GERSHENSON's work (1941) devoted to a study of natural populations of *D. fasciata* (*melanogaster*) that was carried out at the same time and under similar conditions.

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Without having the data obtained at that time before me, it is impossible to reconstruct from memory the results of those investigations in detail. However, it is possible to say that, in general, the natural populations of *D. busckii* represent a reservoir of very considerable genetic variability, and resemble in this respect other Drosophila species such as *D. pseudoobscura*, *D. melanogaster*, *D. willistoni*, and others.

In 1952, I collected *D. busckii* at Princeton, N. J. Of 22 females cytologically analyzed at that time, three possessed an identical X-chromosome inversion. Furthermore, a complex aberration of the second chromosome was detected in the progeny of one female. As will be shown, the structure of this complex aberration is of great interest. These findings stimulated me to continue anew my old investigation of the chromosomal structure of natural populations of *D. busckii*, and the results are now given in this report.

MATERIALS AND METHODS

D. busckii taken in the fruit and vegetable stores of Princeton, N.J., and in stores, parks and neighborhoods of Rochester, N.Y., served as the basic material for the present investigation. Flies were collected by means of traps baited with fermenting crushed potatoes which, in my experience, is the best medium for this purpose.

The bait was prepared in the following manner: raw, unwashed potatoes were crushed either by a grinder or other device. The crushed potato was then put into a large container and compressed. Thereafter the container was covered with four layers of cheese cloth, or with heavier cloth (as a towel), and left for two to three days in a warm place (30–32°C). By the end of this time, the crushed potato was usually in a state of active fermentation, having a strong, unpleasant odor. At this point the fermenting mixture was blended and put into traps. Pieces of paper towel were inserted in the traps to absorb excess liquid, and the traps were then set out and left for one to two days. After this period, they were covered tightly and brought to the laboratory where the flies were immediately removed. The species of Drosophila most often caught in these traps, in descending order of frequency, were: *busckii*, *funebris*, *hydei*, members of the *repleta group* (among them *mercatorum*), *affinis* group, *transversa* and, rarely, *melanogaster*. Of course the order of frequency is variable and depends upon the place where flies are collected.

The *D. busckii* females were, as a rule, already fertilized before capture. Each was put into a separate vial with fresh food, and its progeny studied. The captive males were usually discarded; sometimes, however, they were analyzed. In such a case, each was crossed with three or four females from the standard laboratory stock having normal chromosomal complements. The culture media for *D. busckii* used in this laboratory is prepared according to this recipe: 1000ml water, 8 to 10g agar, 80g corn meal, 50g dried brewer's yeast, 100g Karo syrup (crystal white), 6g linseed meal and 10ml of moldex or tegosept (10 percent solution in 95 percent alcohol).

Seven to ten larvae (chiefly female) were examined from the progeny of each captive fly that was analyzed, the salivary gland chromosomes being stained in aceto-orcein. Localization of the chromosomal breaks in rearrangements was made by means of maps of salivary gland chromosomes. The somewhat simplified map of the X chromosome given in one of my earlier publications (1955), though divided into 20 sections, is not sufficient for a precise localization of breakpoints. A more detailed map (not yet published) was thus used in which each of the 20 sections is in turn divided into four subsections, designated by letters A, B, C, and D. Breaks in the second chromosome were located by means of the maps of the two limbs published here for the first time; these correspond to chromosomal elements A and B of SIROTINA's work (1938). Each arm of this chromosome, in addition to its specific banding pattern, possesses distinct macro-morphological markers by means of which it can readily be recognized under the microscope. Thus 2L, in almost all stages of larval development, is characterized by a puff (of varying size) that is located at approximately one fifth of the length from the distal end. 2R has a constriction that is located not far from its proximal end, at the beginning of section 44 (see map). As I have earlier pointed out (KRIVSHENKO 1955), this constriction in 2R is also visible in the mitotic chromosomes of ganglion cells.

In the text of this paper, each type of detected aberration is designated by the number of the chromosome in which it is located (1, 2, and 3) followed by one or two letters indicating the place where the aberration was found for the first time. The inversions that are parts of aberrant complexes are designated by chromosomal arms and numerals.

In addition to the material collected in the places mentioned above, small samples or separate lines originating from geographically remote populations were received from correspondents. It is a most pleasant duty to express my deep gratitude to the following persons: DRS. WILSON STONE of the University of Texas, D. W. LINDSLEY of the Oak Ridge National Laboratory, H. D. STALKER and H. L. CARSON of Washington University, M. M. GREEN of the University of California at Davis, M. STRICKBERGER of the University of California at Berkeley, M. ARONSON of Dartmouth Medical School, O. BILANIUK of the University of Rochester, A. HUNTER of the University of the Andes, Bogota, Colombia, D. MORIWAKI of Tokyo Metropolitan University, Japan, J. A. BEARDMORE of the University of Groningen, Netherlands, S. B. PIPKIN of the Gorgas Memorial Laboratory, Panama, and MR. R. MAAS of the University of Rochester.

GENERAL DATA

The results of cytological analyses of samples of *D. busckii* from different populations are presented in Table 1.

In the first column of this table, the place and year of collection are shown. The second column gives the number of females and males subjected to progeny-analysis. In the following columns, the chromosomal aberrations are listed. The figures in these columns indicate the numbers of females and males (or lines) in the progeny of which aberrations were found. The X-chromosome aberrations in

TABLE 1

The data on chromosomal polymorphism of D. busckii *from natural populations*

Place and year of collecting flies	No. of flies	X-chromosome inversions	Aberrations of chromosome 2						Abberations of chromosome 3		Total aberrations	Percent aberrant	
		1-P	2-SP	2-P	2-Ro1	2-Ro2	2-Bo	2-T	3L-Br	3L-SP			
Princeton, N.J., 1952–1953	37♀♀	6	..	7	..	..	..	..	..	..	13	35.1	35.1
Rochester, N.Y., 1955–1962	300♀♀	36	..	28	4	11	..	..	..	..	90	30.0	29.1
	44♂♂	5	..	2	2	1	..	..	..	..	10	22.7	
Wilder, Vermont, 1961–1962	40♀♀	15	..	5	..	3	..	..	..	..	23	57.5	43.1
	18♂♂	1	..	1	..	..	..	..	..	..	2	11.1	
Oak Ridge, Tenn., 1961–1962	55♀♀	24	..	22	5	8	..	..	..	..	59	107.3	65.6
	76♂♂	8	..	12	..	6	..	1	..	..	27	35.5	
St. Louis, Mo., 1962	17♀♀	5	..	5	..	1	..	..	..	..	11	64.7	54.5
	27♂♂	4	..	5	2	2	..	..	..	..	13	48.2	
Sao Paulo, Brazil, 1961	7♀♀	2	1	..	..	4	..	..	1	1	9	128.6	76.5
	10♂♂	..	..	..	..	2	..	..	1	1	4	40.0	
Bogota, Colombia, 1960	7♀♀	..	..	..	..	..	1	..	..	..	1	14.3	14.3
	7♂♂	..	..	1	..	..	..	..	..	..	1	14.3	
Buenos Aires, Argentina, 1962	9♀♀	1	4	1	..	3	..	..	..	..	9	100.0	92.3
	4♂♂	..	2	..	..	1	..	..	..	..	3	75.0	
Groningen, Netherlands, 1962	4♀♀	..	..	..	..	1	..	..	..	..	1	25.0	14.3
	24♂♂	..	..	..	..	3	..	..	..	..	3	12.5	
Total	476♀♀	..	..	..	..	..	..	..	..	..	..	...	...
	210♂♂	..	..	..	..	..	..	..	..	..	..	...	...
	686♀♂	107	7	99	13	46	1	1	2	2	278	40.5	
Percent of analyzed flies		15.6	1.02	14.4	1.9	6.7	0.15	0.15	0.3	0.3	..	...	...
Percent of analyzed chromosomes		6.5	0.3	4.3	0.56	1.98	0.043	0.043	0.09	0.09	..	...	...

every case proved to be the same inversion; all are therefore placed in one column designated by the symbol 1-P.

(In the published abstract (KRIVSHENKO 1961) the aberrations 1-P were designated as P-X, 2-P as P-A, 2-Ro1 as P-A + R1, and 2-Ro2 as P-A + R2 + R1. The symbols have been changed because the previous system of designating aberrations was very complex and difficult to apply to aberrations derived from other line.)

The aberrations of the second chromosome represent complex paracentric inversions, and are subdivided into six subgroups corresponding to the cytological peculiarities of their structure. These subgroups are respectively designated by the symbols 2-SP, 2-P, 2-Ro1, 2-Ro2, 2-Bo, and 2-T. Two types of aberrations were found in the left limb of the third autosome, one representing a complex paracentric inversion (3L-Br), the other a simple paracentric inversion (3L-SP). The next to the last column of this table gives the percentages of the aberrant forms found among females and males in the samples from different populations; the last column gives the average percentages for both sexes. The three lower rows of this table present the frequencies of separate aberrations: in the first of these the absolute numbers of each of these aberrations are given, and in the second their percentages, based on the total number of analyzed flies (lines), as indicated in the table. Finally, the last row gives the percentages of the aberrant chromosomes based on the number of analyzed chromosomes.

In addition to the material summarized in this table, single strains or a few strains originating from different geographically remote populations were also analyzed, namely: from California (4 strains), Missouri (1), Montana (1), Nevada (1), Oregon (5), Texas (1), Australia (2), Canada (3), Costa Rica (1), Hawaii (1), Panama (3), Italy (1), Lebanon (1), Japan (11 strains—each from a different place), and Scotland (1). In one of the Californian lines, an X chromosomal inversion identical to inversion 1-P (see above) was found. The 1-P X-chromosomal inversion was found in the line originating from Texas, and, in addition, a complex inversion of the second chromosome (2-Ro2). The remaining strains yielded only normal gene sequences. Thus, as a result of the analysis of the progeny of 718 flies (or strains) collected in nature (namely, all of the material available to me), nine different types of chromosomal aberrations were found. Description of these now follows.

DESCRIPTION OF THE CHROMOSOMAL ABERRATIONS

Princeton inversion of the X chromosome (1-P) is a simple, paracentric inversion comprising the middle euchromatic part of the salivary gland X chromosome. The distal break of this inversion is located in the center of subsection 5D, the proximal in the right half of subsection 13D (Figure 3a). The inverted part is thus approximately equal to 50 percent of the length of the salivary gland chromosome. Both homo, and hemizygotes for this inversion are highly viable and fertile.

According to the data of Table 1, an average of 0.16 X-inversions were found

per analyzed fly taken from nature. Proceeding from the total number of analyzed X chromosomes (considering each female as representing three chromosomes and each male as representing one chromosome), the average frequency of inverted chromosomes is equal to 6.5 percent.

The aberration of the second chromosome 2-SP (Sao Paulo inversion-complex) represents a complex set consisting of eight small inversions, four of which are located in each arm of this chromosome. In Figure 1, top, a schematic drawing of this complex in the heterozygous condition is given. It is a remarkable fact that three of the four inversions in the left arm are located close to each other in the proximal half, as the diagram shows, only the fourth inversion lying separately in the distal half of 2L. Surprisingly, all four inversions of the right arm are also located close to each other and in the centromeric half of 2R. All inversions of the

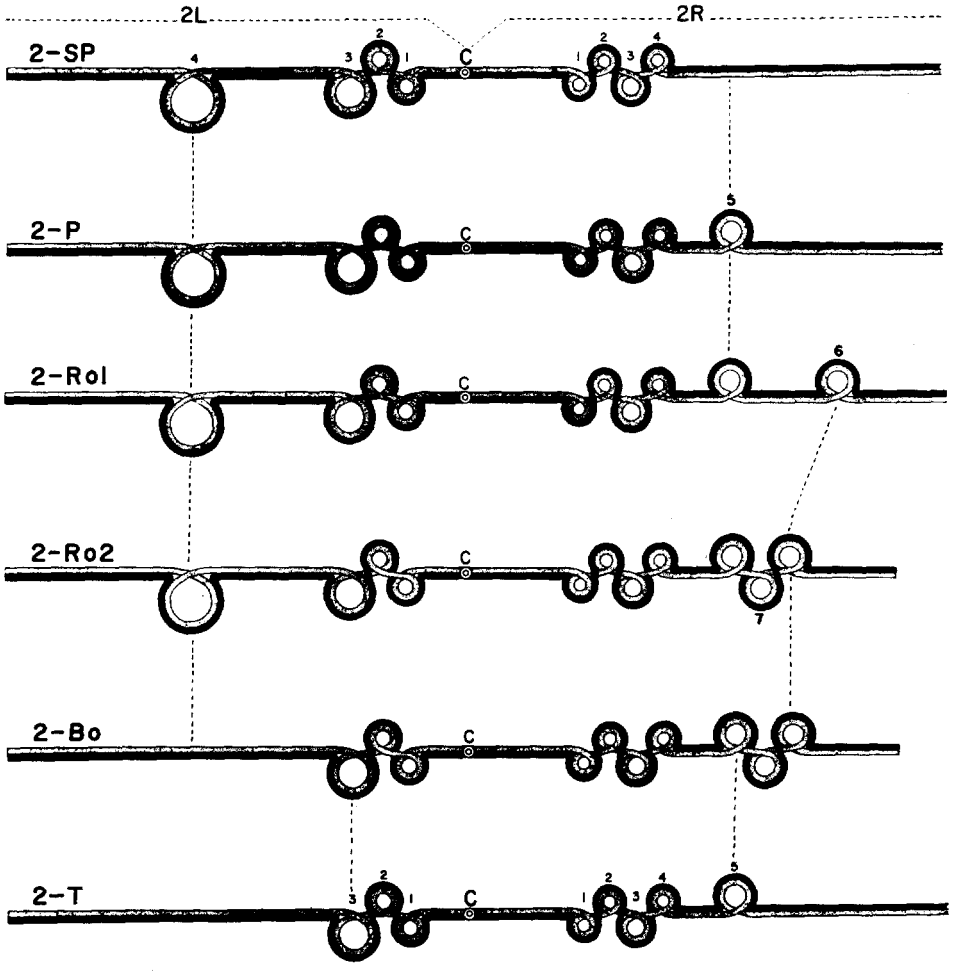

FIGURE 1.—Diagrammatic representation of different aberrations of the second chromosome of *Drosophila buscki* from natural populations (in heterozygous condition).

left and the right arms of this aberrant chromosome are indicated in the diagram by numerals ascending in order from the centromere to the distal end of each arm. The regions of inversions are also denoted by the same numbers on the map of the second salivary gland chromosome (Figure 2). The arrows to the left and right of each numeral indicate the locations of the breakpoints for each given inversion, and the broken arrows indicate those breakpoints not exactly located.

From five of the seven lines in which aberration 2-SP has been found, it was isolated and balanced in a laboratory stock carrying a dominant marker. In one of these balanced lines, the aberrant chromosome is lethal when homozygous, the viability of homozygotes of four other lines is slightly decreased.

Aberration 2-P (Princeton inversion complex of the second chromosome) represents a set consisting of nine inversions, of which eight are identical with 2-SP inversions of the preceding complex. One is an additional new inversion (2R-5) that is located in the center of the right arm, distal to inversion 2R-4 (see Figures 1, 2).

As seen from the table, the aberration 2-P has been found in 99 lines. From 35 lines it was isolated and balanced. In 12 of these balanced lines the aberrant chromosome is lethal when homozygous; in the rest it is semilethal or has a somewhat lower viability. The lethals in four of the lines (1099, 1137, 1139 and 1183) are allelic, but each of the remaining eight have lethals located at different loci. In one of the lethal lines (1100), and in five of the others (1102, 1140, 1185, 1197, and 1202), aberrant chromosomes carry an allelic recessive factor probably homologous to that which determines scarlet phenotype in *D. melanogaster*. Besides this, a semidominant factor is also present in the lethal line 1100 which determines the development of melanotic tumor in the gut of the heterozygote.

Approximately 0.15 heterozygous 2-P inversion complex was found per analyzed fly. Proceeding from the total number of analyzed second chromosomes (considering each female as representing four chromosomes, and each male as two) the frequency of 2-P is approximately 4.3 percent.

Aberration 2-Ro1 (first inversion-complex of the second chromosome from Rochester) includes the morphologically unchanged complex 2-P *plus* one new inversion (2R-6) that is located in approximately the middle of the distal half of the right arm (see Figures 1, 2).

Ten balanced stocks of 2-Ro1 were successfully made up from the 13 lines in which this aberration was found. In three of the lines the aberrant chromosome is semilethal when homozygous, and the viability is decreased in the remaining seven. In two of the latter lines (1201 and 1273) a recessive factor is also present which produces bright red eyes of the scarlet phenotype. This mutation is allelic to the morphologically similar mutations found in the six lines of complex 2-P mentioned above. The frequency of complex 2-Ro1 is much lower than that of 2-P (see Table 1).

Aberration 2-Ro2 (second inversion-complex of the second chromosome from Rochester) consists of 11 inversions, ten of which are the elements of the complex described as 2-Ro1, and one is a new inversion (2R-7) located between the independent inversions 2R-5 and 2R-6 in the distal half of the right arm (See Figures

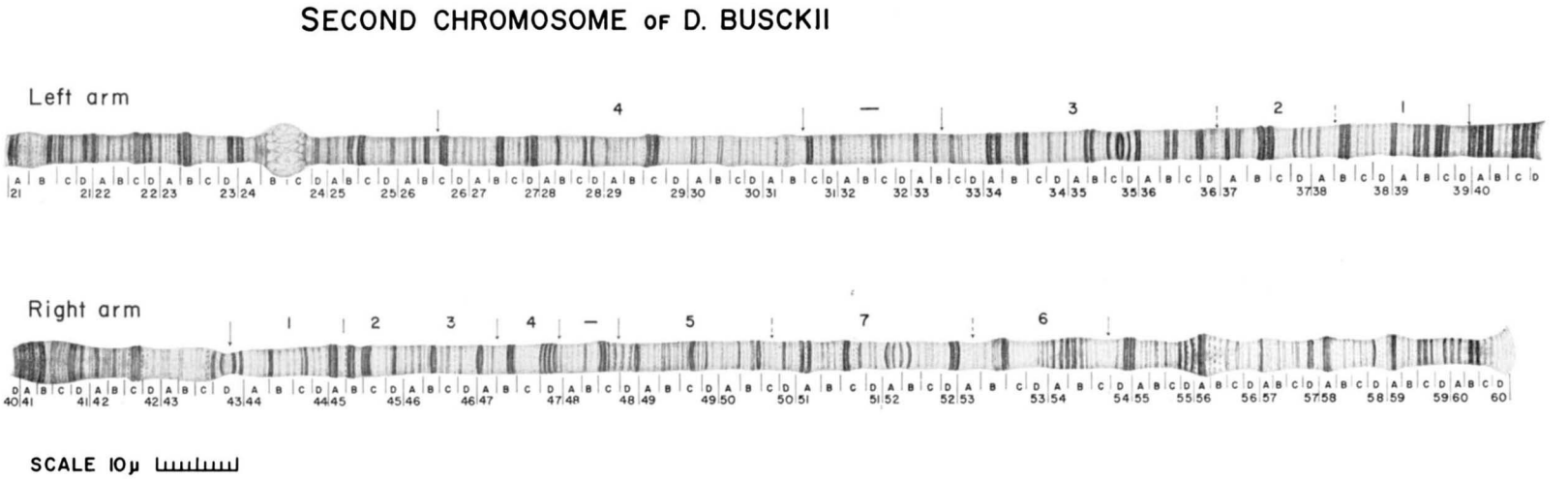

FIGURE 2.—Salivary map of the second chromosome of *Drosophila busckii*.

1, 2 and 3b). An average of 0.07 heterozygous 2-Ro2 were found per analyzed fly. The ratio of the 2-Ro2 inversion to the total number of analyzed second chromosomes is approximately 1.98 percent.

Of 22 isolated, balanced lines of 2-Ro2, seven are lethal when homozygous, and the rest are semilethal or have a decreased viability. Three of the included lethals are allelic to each other and to the four allelic lethals of aberrant complex 2-P.

Aberration 2-Bo (inversion complex from Bogota) represents a variant of the preceding complex 2-Ro2. The peculiarity of its structure consists in an absence of the distal independent inversion 2L-4 of the left arm, which is present in all of the earlier described complexes of the second chromosome (Figures 1, 2). Whether 2L-4 was lost by crossing over, or by reinversion, is not possible to determine. The first possibility seems to be the more plausible, despite the fact that under laboratory conditions crossing over in the region between inversions 2L-4 and 2L-3 has never been observed. The viability of the homozygote for 2-Bo is slightly decreased.

Aberration 2-T (inversion-complex from Tennessee) represents a variant of

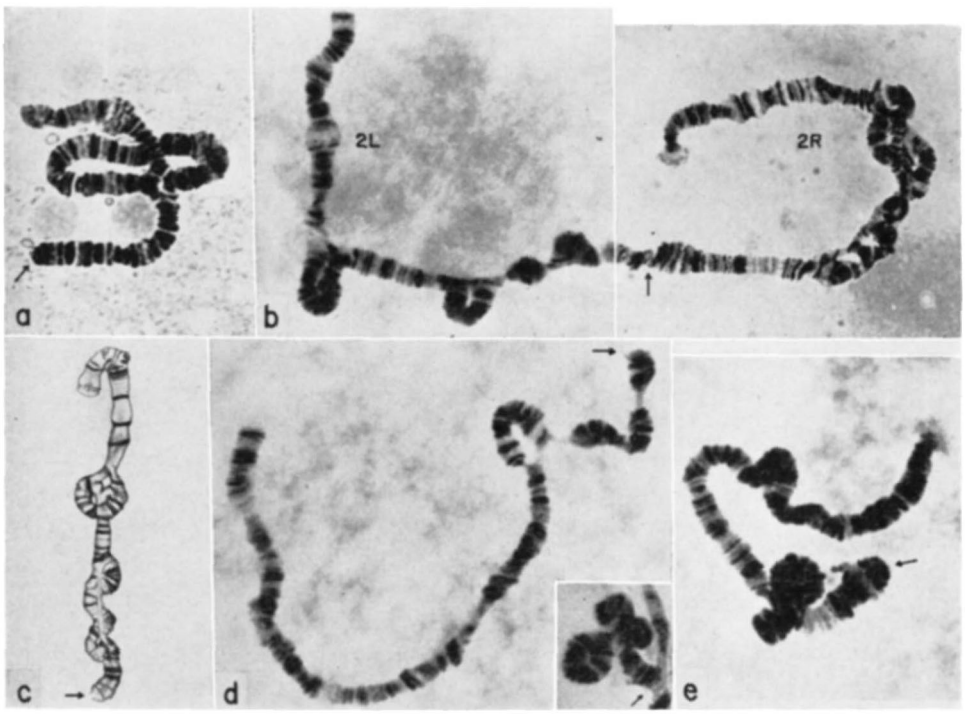

FIGURE 3.—Aberrant chromosomes of *D. busckii* from natural populations; (a) X-chromosome inversion 1-P; (b) Aberrant complex 2-Ro2 of the second chromosome; (c) Aberrant complex 3L-Br of the third chromosome; (d) and (e) Left and right arms of aberrant complex 2-Ro2 in homozygous condition. In the right corner of (d) a rosette consisting of three loops of left arm is inserted; here the distal, linearly stretched part of this arm is missing. In (e) a rosette consisting of two loops is present. The arrows show the centromeric ends of chromosomal elements.

the complex 2-P. In this complex, as in a complex 2-Bo, the distal independent inversion 2L-4 of the left arm is absent (Figures 1, 2). The origin of this complex, as in the previous case, is, evidently, due to the crossing over. The viability of the homozygote for this complex is decreased.

Aberration 3L-Br (inversion complex of the third chromosome from Brazil) consists of four or five small, adjacent, and probably, overlapping inversions in the proximal half, and one independent inversion located in the middle of the distal half of this arm (Figure 3c). This aberration was detected and isolated from two lines originating from the population at Sao Paulo, Brazil, but collected in different years (1956 and 1960). Homozygotes for 3L-Br are lethal in both lines, and the lethals are allelic.

The unusual sequence of small inversions in 3L-Br makes pairing of the homologous chromosomes irregular in heterozygotes. This, plus the inviability of the homozygote, rules out the possibility of precisely locating the breakpoints of the separate inversions and determining their interrelations.

3L-SP aberration (inversion of 3L from Sao Paulo) represents a small paracentric inversion located in the middle of the distal half of 3L. This inversion is homologous to the independent distal inversion of complex 3L-Br (see above). The origin of this inversion is evidently due to crossing over in some chromosomal region of normal gene sequence, thus separating this inversion from the group of adjacent inversions in the proximal half of the left arm of chromosome 3L-Br. The viability of the homozygote for this inversion is slightly decreased.

DISCUSSION AND CONCLUSIONS

The mitotic chromosomal complement of *D. busckii* consists of three pairs of chromosomes, one of which is rod-shaped and represents the sex chromosomes, two other pairs are V-shaped and are autosomes (SIROTINA 1938; KRIVSHENKO 1939, 1952, 1955; ELOFF 1940; WHARTON 1943). One of the autosomal pairs, which I earlier designated as the second (KRIVSHENKO 1939) proves to be homologous to the third chromosome of *D. melanogaster*; the other pair, which I have designated as the third, is homologous to the second chromosome of the same species. The homology in each case was deduced on the basis of both spontaneous and induced mutations, namely, on the locations of: aristapedia, cardinal, curled, Delta, Radius incompletus and scarlet which are located in the second chromosome of *D. busckii* and in the third chromosome of *D. melanogaster*, and of mutations cinnabar and Star which are located in the third chromosome of *D. busckii* but in the second chromosome in *D. melanogaster*. The fact that the mutation vestigial (dominant) found in the second chromosome of *D. busckii* is also characteristic of the second chromosome of *D. melanogaster*, gives reason to assume that during evolution an interautosomal exchange took place in the line of one of these species. The decrease in the number of chromosomal elements in the complement of *D. busckii*, as compared with *D. melanogaster*, is due to a translocation (or so called "centric fusion") between X and 4 ("microchromosome"), as well as to one between Y and 4 (KRIVSHENKO 1955, 1959).

Aside from the Y chromosome, chromosomal aberrations have been found by me in all three chromosomes. The total number of different types of aberrations is small. In the X chromosome only one inversion is present. In the second autosome, there are six inversion complexes known which differ from each other in their degrees of complexity. Finally, there are but two known aberrations in 3L, one of which is a complex of inversions, and the other is a simple inversion. These nine aberrations are, however, composed of 18 separate, inverted segments of which only one is in the X chromosome, 11 lie in the second autosome, and six in 3L.

The range in distribution of some of these aberrations is extremely wide. The X chromosomal inversion 1-P and the complex inversions 2-P and 2-Ro2 are, evidently, present in all populations inhabiting the vast territory from Rochester, N.Y., USA, to Buenos Aires, Argentina. The complex inversion 2-Ro1 is either not so widely spread or its frequency is low, because it has not yet been found in small samples. The aberrations 2-SP and 3-Br are, evidently, characteristic only (or mainly) of populations in South America. As to the aberrations of 2-Bo, 2-T and 3L-SP, which undoubtedly are derived from corresponding aberrant complexes (see above), they, probably, were found in or close to the places of their comparatively recent origin. These conclusions are of course based in most cases upon the analyses of relatively small samples from few populations, and must therefore be considered as tentative; the finding of the aberration 2-Ro2 in a small sample from Groningen, Netherlands, is good reason for this assumption. Nevertheless, peculiarities of the chromosomal polymorphism of geographically remote populations are clearly evident, and these are probably related to environmental conditions.

As seen from the table, and from the descriptions of separate aberrations, their average frequencies are low in the analyzed material. The frequencies of overall polymorphism are also comparatively low. Thus from the data of the table, 278 aberrant chromosomes were found in the progeny of 686 analyzed flies. This constitutes approximately 0.4 heterozygous aberrations per fly. In some populations, a considerable deviation from this average index was observed. For example, in the sample from the population of Rochester there are approximately 0.3 heterozygous aberrations per fly, whereas in the Oak Ridge, Saint Louis, Sao Paulo, and Buenos Aires populations there are at least twice as many aberrations per fly (see table). The data from the Rochester population are based upon an analysis of the progeny of 344 flies collected in different years from different localities but by the same methods, and undoubtedly reflect well the degree of chromosomal polymorphism in this population. Unfortunately, we can not say the same for the other populations. In them, in addition to the large sampling error inherent in analyses of small samples, other factors undoubtedly also interfere. Especially important among these is the mailing of captured females and males in the same containers. If transportation takes a long time, then repeated crosses occur, and when several aberrant males are present in a sample, such repeated matings quite naturally lead to an increase in the number of females which give progeny with aberrations. Evidently this is the reason why females in samples from some geo-

graphically remote populations gave progeny with aberrations more frequently than did the accompanying males.

The results of the analysis of the samples of flies from the Wilder, Oak Ridge, and Sao Paulo populations (see the table), in which the frequencies of X and 2 aberrations were seemingly much lower in analyzed males than females, stimulated me to undertake two small, very simple experiments to evaluate one possible reason for this difference, namely, whether the difference might not be due to differential mating effectiveness of males of different genotypes. These experiments also had the purpose of determining the frequency of repeated inseminations in *D. busckii.*

In the first of these experiments, one female and two males of known genetic structure were put into each vial with fresh food, thus giving the possibility of analyzing the results of the mating by analysis of the progeny. One of these males was of normal chromosomal structure, the other had the X chromosomal inversion 1-P. Altogether 172 such crosses were analyzed. In 128 of them, the males were discarded after 20 hours, and in the remainder after 48 hours. In the second of these experiments, consisting of 74 crosses, one female and three males of different chromosomal structure were placed in each vial: one male was normal, one had inversion 1-P, and the third was heterozygous for aberration 2-Ro2. In this experiment all males were discarded after 48 hours.

In the first experiment, 142 males of normal chromosomal structure and 140 males with the X chromosome inversion mated with the 172 females. In that part of the experiment in which males were discarded after 20 hours, 37 percent of the females were fertilized by two males; where the males were discarded after 48 hours, 82 percent of the females were inseminated by two males. In the second experiment 59 males of normal chromosomal structure, 45 males with inversion 1-P, and 40 males heterozygous for complex 2-Ro2 mated with 74 females for 48 hours. Here 29 percent of the females were fertilized by three males, 54 percent by two males, and 17 percent by one male.

Little if any difference thus exists in the sexual activity of males having the different chromosomal arrangements, and even in the second experiment the more frequent fertilization by males of normal chromosomal structure is of little significance ($\chi_2^2 = 4.04$, $P > 0.10$). As to repeated inseminations, the data show that they indeed take place and that their frequencies depend upon the length of time that females and males are together.

The data for determining the mating ability of males of different chromosomal structure were obtained from several series of 15 to 25 crosses. In these crosses several strains were chosen because of their high viability and fertility. They were: females from two strains, males with a normal chromosomal structure from three strains, males with 1-P X-chromosome inversion from two strains, and males with 2-Ro2 aberrant complex from two strains.

In the first experiment, where the mating ability of males of two different X-chromosome structures was tested, the males mainly originated from females heterozygous for both the tested chromosomes. Thus, the genetic background as well as the conditions under which they were raised were more or less equal. When males of three different chromosomal structures were tested, the males were taken directly from the corresponding strains. In this case the genetic background and the raising condiitons were different. It is possible that this was the cause of the ob-

served slight difference in the mating ability of males of different chromosomal structure in the second experiment, though this difference is statistically not significant. However, it is possible too, that in the presence of a larger number of males in the vial with one female, the mating ability of males of different chromosomal structure is better expressed (because of the stronger competition) and that the statistical insignificance of the difference in this experiment to due to comparatively small number of crosses. Undoubtedly, the work in this direction must be continued.

Of course all that is mentioned above does not exclude the possibility that there is a different frequency of chromosomal polymorphism in different populations, nor that sex differences may be connected with such different frequencies. Future investigations must throw light upon these as well as on other problems concerning the quantitative characteristics of the phenomenon under investigation. Nevertheless, the data do suggest that *D. busckii* occupies a middle position among the other species of Drosophila so far studied with regard to the frequency of chromosomal polymorphism. As to the few aberrant types and the wide distribution of some of them, *D. busckii* is similar to cosmopolitan species such as *melanogaster*, *funebris* and others (PATTERSON and STONE 1952; DA CUNHA 1955, 1960 for review and references).

In all cases in which I defined the frequencies of chromosomal polymorphism, I used the number of aberrant chromosomes independently of the complexity of their structure. I did not specify the separate inversions that are parts of these complexes as is usually done by some other authors. Taking into account the separate inversions, there is an average of 2.5 heterozygous inversions per analyzed fly. In the samples from different populations the number of heterozygous inversions per fly varied considerably. Thus in the Rochester, Princeton, and Wilder populations, 1.75, in Saint Louis, 3.5, in Oak Ridge, 4.2, in Sao Paulo, 5.2, and in Buenos Aires, 8.6 heterozygous inverted segments per fly were present. Judging from these indices, *D. busckii* must be considered to be a highly polymorphic species of Drosophila. Nevertheless, the number of separate inversions does not reflect the frequency of chromosomal polymorphism in the population; the unit of estimation must be the individual aberrant chromosome independent of the complexity of its structure. The number of inverted segments, however, is of considerable significance in connection with studies of comparative chromosomal variability and phylogeny among different species (STONE 1955, 1962; STONE, GUEST, and WILSON 1960).

As is well known, the genetic significance of inversions concerns chiefly the reduction of recovered crossover products from chromosomes heterozygous for them. Usually the degree of this reduction depends inversely upon the size of the inverted segments, and directly upon their number and position in the chromosome. All of the aberrations found by me when heterozygous, with the exception of the small inversion 3L-SP, suppress crossing over in long regions. Thus, in heterozygotes for the X-chromosome inversion 1-P, crossing over is "suppressed" in the region extending from the distal break of this inversion to the centromeric end of the chromosome, only very rare double crossovers being recovered. In half of the distal, noninverted part of the chromosome adjacent to this inversion, crossing

over is also suppressed, yet there is in fact, an increase in crossing over above that of the control in the telomeric half. In general, the frequency of crossing over in the X chromosome in heterozygotes for the 1-P inversion is effectively reduced by approximately 70 percent (that is, the relevant map length is reduced from 103 to 31). In heterozygotes for the aberrant complexes of the second autosome, despite the presence of portions of chromosomes inserted between inverted segments which have normal sequences of genes, crossing over has not been observed in the regions between the distal breaks of the distal inversions of the left and right arms. Thus in lines 2-SP and 2-Bo these regions are in each case approximately equal to 60 percent the length of the entire second chromosome. In line 2-P it is slightly higher and, finally, in lines 2-Ro1 and 2-Ro2 it is approximately 75–80 percent. In line 3L-Br, crossing over was not observed in that region of the chromosome running from the distal break of independent inversion up to the centromere, and this region of the chromosome is approximately equal to 75–80 percent of the left arm. Whether crossing over occurs in the distal, telomeric, noninverted regions of these chromosomes, and if so with what frequency, can not yet be determined because of the lack of necessary markers for these regions.

The above conclusions about the degree of the reduction of crossing over in heterozygotes for different aberrations are based upon observations made under laboratory conditions. However, the aberrations 2-T, 2-Bo, and 3L-SP, which we found and were assumed to represent the derivatives of the aberrant complexes 2-P, 2-Ro2 and 3L-Br, correspondingly, give reason to suppose that, at least in nature, crossing over does take place in portions of chromosome with normal gene sequences inserted between inverted segments. However, its frequency is probably low and depends upon the length of these portions and their position in the chromosome.

DOBZHANSKY and STURTEVANT (1938) discussing the results of their study of chromosomal polymorphism in natural populations of *D. pseudoobscura*, express the thought that "heterozygosis for inversions decreases the amount of crossing over, and this may be of selective value in connection with the heterosis effect." This idea was developed further in later investigations and has received wide recognition among geneticists. According to this idea in its current form, the heterotic effect of blocks of genes included within inverted segments, and the absence of recombination in heterozygotes in the regions of these blocks, are basic conditions to the existence and persistence of chromosomal polymorphism. The level of polymorphism in each population is then determined by the qualitative peculiarities of the gene composition within mutually inverted segments, and by their coadaptation to the rest of the genotype. Such coadaptation is taken to be the result of natural selection, the intensity of which is determined by the environmental conditions of the population (DORZHANSKY 1951, 1955, 1961; CARSON 1953). However, as shown by LEVENE (1953), LI (1955), LEWONTIN (1955), and BENNET (1958), polymorphism of a population can be maintained indefinitely, without a heterotic effect of heterozygotes, by different degrees of adaptation (selective value) of the polymorphic components to different environmental conditions.

Whether but one of these factors (that is, heterotic effect or adaptation), or their interaction determines the survival and frequencies of the aberrant forms in populations of *D. busckii* cannot be decided definitely now. The data I have accumulated concerning the viability of individuals with aberrant and normal chromosomes, in homo- and heterozygous conditions, permit only the supposition that the aberrant chromosomes are maintained in the populations of *D. busckii* by a hetorotic effect. However heterosis must evidently be manifested only under definite environmental and developmental conditions, of the heterozygous individuals. Full data and their discussion will be presented at a later date, but it is necessary now to point out here some striking peculiarities in the structure of the complex aberrations of *D. busckii*.

Complex inversions have been observed in many different species of Drosophila. As first postulated by STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY and STURTEVANT (1938), and DOBZHANSKY (1944), and thereafter confirmed by other investigators, all of the known complexes can be interpreted as the result of an accumulation within a chromosome, over a period of time, of simple two-break inversions of independent origin. On the basis of the location of the breakpoints of the inversions, complexes may be classified as consisting of (1) independent, (2) included, (3) overlapping, and (4) tandem or adjacent inversions. In different species of Drosophila, the first three types of complex inversion, or their combinations, are most common. As for tandem inversions, they were at one time considered to be improbable as spontaneous occurrences. The reasons for this opinion were (1) the absence of such aberrations in any of the studied populations of Drosophila, and (2) the existence of experimental data leading to the conclusion that a chromosome has an equal likelihood of breakage at any point, and therefore the probability of the juxtaposition of two independent breaks is negligible (DOBZHANSKY 1944). Complex, seemingly tandem inversions have now been found in populations of some species of Drosophila and, especially, in a number of species of Chironomus and *Tendipes decorus* (PHILIP 1942; HSU and LIU 1948; BAUER 1936; BEERMAN 1952, 1955; MAINX, KUNZE and KOSKE 1953; ACTON 1955; ROTHFELS and FAIRLIE 1957). Whether these complexes are in fact tandem, or only nearly tandem, in most cases is not exactly known.

The autosomal complexes of *D. busckii* are compound structurally; they consist of independent inversions as well as of groups of inversions which are morphologically tandem or near tandem. All inversions that are parts of these complexes are small, and those that are located in the centromeric halves of the chromosomal elements are especially small and form regular sets or groups. In 2L such a group consists of three, in 2R of four, and, finally, in 3L of four or five inversions (see Figures 1 and 3c). What are the true interrelations between the inverted segments composing the elements of these groups?

Since the aberrant complexes consist of small inversions only, conjugation in the heterozygote is disturbed as a rule, and it is therefore not possible to localize the breaks of the separate inversions or, consequently, to draw conclusions about the exact interrelations of the inversions in these groups. For this reason, the

homozygous rearrangements had to be analyzed. Though this proved to be possible in part for six of the seven complex autosomal rearrangements, the homozygote for the aberrant complex in 3L is unfortunately not viable, and the structure of this aberration accordingly remains poorly known. Although the good viability of both homozygotes and heterozygotes for the aberrant complexes of the second chromosome would seem to make the task of deciphering their structure straightforward, an unexpected difficulty prevented complete resolution of the task. The difficulty consists of the surprising fact that the homozygous structures form loops in the regions of the seemingly tandem inversions quite similar to those of the heterozygous complexes. Thus, in the centromeric region of the left arm of 2, three loops are usually observed (that is, just as many as in the corresponding heterozygote!), and sometimes as many as four loops! Furthermore, in the centromeric region of the homozygous, rearranged right arm of 2, two loops are usually observed, instead of the four loops characteristic of heterozygotes; occasionally there is only one loop, and then of variable size. This is also sometimes the case for the distal complex of right arm in homozygous 2-Ro2.

Often the loops of the centromeric halves of the left and right arms form rosettes. These rosettes are caused by the fusion (stickiness or conjugation?) of the bases of the loops. The adhesion is very strong, and it is therefore seldom possible to obtain a chromosome linearly stretched in these regions. If the rosette is successfully disrupted, the loops which formed it remain intact, or constrictions of varying size appear between the adjacent stretched, inverted segments. This is especially characteristic of 2L, and I therefore did not succeed in localizing exactly the breaks between the inversions 2L-1 and 2L-2, or between 2L-2 and 2L-3, or in defining the interrelations between them (Figures 3d,e).

Because of the reduction in the number of loops in the homozygote as compared with the heterozygote for 2R, and because the connections in the knot of rosette and at the ends of the loops seem not as strong, I succeeded in preparing this chromosomal element in a linearly stretched condition that permitted recognition of the sequence of bands. This analysis led directly to the conclusion that the group of inversions of the centromeric half of the right arm does indeed consist of tandem inversions. Each of the four inverted segments occupies exactly the same place as in the normal chromosome, each being rotated 180° in place, and no transposition or any insertion of chromosomal material having a banded structure can be detected between the inverted segments.

But what may the cause be of the formation of loops and rosettes in homozygotes for the aberrant complexes of the second chromosome? At least two explanations of this phenomenon are possible. The first considers the possibility of a redistribution of homologous material of euchromatic or heterochromatic nature, located at the breakpoints, and manifesting a specific or nonspecific pairing tendency that causes the formation of loops in the homozygote. The second holds that breaks of chromosomes may be accompanied by changes in the properties of the chromosomal material, manifested for example in nonspecific pairing, and in nature akin to "heterochromatization" (SCHULTZ 1939, 1947).

The consequences of both these mechanisms are equivalent. The first of them

is based mainly on the results of the study of the structure and rearrangements of polytene chromosomes in different organisms, and in my opinion it seems more plausible. However, the occasional appearance of the fourth loop in 2L, which in fact represents an uninverted segment of the chromosome located between the distal break of the inversion 2L-3 and the proximal break of the inversion 2L-4 (see Figures 1, 2), seems to show that the second mechanism is also quite possible. If this second mechanism occurs, then the production of inversions may not only give rise to the formation of blocks of genes isolated from recombination, but also to changes in the cytological and genetical properties of regions at the chromosomal breaks. Perhaps this change is the one of the factors that determines the survival of the aberrant forms in populations during the very first stage of their existence, and later, the levels of their frequencies in populations.

As mentioned above, all aberrant complexes of the second chromosome represent variations of the same type, each differing from the others in degree of complexity. The simplest of them is the 2-SP complex which consists of no less than eight inversions, then follows 2-P with nine, 2-Ro1 with ten, and, finally, 2-Ro2 with 11 inversions (complexes 2-Bo and 2-T, it will be recalled, represent the simple derivatives of 2-Ro2 and 2-P, correspondently, and are of no interest here). The most significant fact about these is that each succeeding complex differs from the preceding one by only one new inversion; thus the last complex of this series (2-Ro2) includes all of the inversions of the preceding complexes, and these presumably represent stages in its development.

Comparison of the structure of these complexes suggests that there was a succession in origin of three of these inversions corresponding to the numerals by which they are designated, namely 2R-5 → 2R-6 → 2R-7. Though we do not know in what succession the eight other inversions appeared, the tendency in formation of the aberrant complexes of the second chromosome implies a mono or unidirectional phylogeny of chromosomal variability. Evidently the same argument can be applied to the origin of aberrant complexes of 3L-Br, which consists of five or six inversions. Such a linear or unidirectional character of chromosomal variability in *D. busckii* would put this species in a special position compared with other species of Drosophila. In the latter, usually, different degrees of branched phylogeny are observed. How it comes to be that chromosomal variability of *D. busckii* is unidirected we do not know. It can of course be assumed that natural selection played a role in preserving these complexes, but the mechanism of their origins remains a mystery.

For the formation of a complex consisting of 11 inversions (as 2-Ro2), assuming that each arises independently, 22 breaks in the same chromosome are needed. Because the frequency of the spontaneous appearance of inversions is very low, some of the inversions of this complex are undoubtedly very ancient. Among the four complexes above mentioned, 2-SP is probably the oldest. What the destiny of the more primitive stages may have been, and whether they are now preserved in any populations of *D. busckii*, we do not know, but they make continued investigation of wild populations of *Drosophila busckii* an exciting prospect.

SUMMARY

As a result of cytological analyses of the progeny of 718 flies (lines) of *D. busckii* taken from geographically remote populations, nine different types of chromosomal aberrations have been found, of which one is a simple paracentric inversion of the X chromosome, six are complex paracentric inversions of the second chromosome and, finally, one is a complex and one is a simple paracentric inversion of 3L. These nine aberrations are composed of 18 separate inverted segments of which only one is in the X chromosome, 11 lie in the second autosome and six in 3L.

Some of these aberrations are present in all populations inhabiting the vast territory from Rochester, N. Y., USA, to Buenos Aires, Argentina; others have more limited range of distribution and these are undoubtedly related to specific adaptations to environmental conditions.

The average frequencies of different aberrant types vary from 0.0015 to 0.16 heterozygous aberrations per fly. The average overall frequency of polymorphism is approximately 0.4 heterozygous aberrations per fly. These data suggest that *D. busckii* occupies a middle position among the other species of Drosophila so far studied with regard to the frequency of chromosomal polymorphism. However, taking into account the separate inverted segments there is an average of 2.5 heterozygous inversions per analyzed fly. Judging from this index, *D. busckii* must then be considered to be a highly polymorphic species of Drosophila.

All of the aberrations when heterozygous, with exception of the small inversion in 3L, suppress crossing over in long regions. Thus, the X chromosome inversion effectively reduces crossing over by approximately 70 percent in this chromosome; the complex aberrations of the second chromosome and of 3L suppress crossing over along 60 to 80 percent of the length of corresponding chromosomal elements.

The autosomal complexes of this species consist of independent inversions and of groups of small inversions which are located in the centromeric halves of the chromosomal elements; in 2L such a group consists of three, in 2R of four and, finally, in 3L of four or five inversions. By analyzing the homozygous structure of these complexes it was found that group 2R consists of tandem inversions. The interrelations between inverted segments of 2L and 3L groups can not be explained as yet.

In the homozygotes for aberrant complexes of the second chromosome, in the regions where the groups of inversions are located, chromosomal loops are formed which are morphologically similar to the loops of heterozygotes. Often the loops of left and right arms form rosettes. These rosettes are caused by the fusion of the bases of the loops. At least two explanations of this phenomenon are possible. The first considers the possibility of redistribution of homologous material of euchromatic or heterochromatic nature located at the breakpoints, and manifesting a specific or nonspecific pairing tendency that causes the formation of loops and rosettes in the homozygotes. The second holds that breaks of chromosome may be accompanied by changes in the properties of chromosomal material

which are manifested in nonspecific pairing and in nature akin to "heterochromatization".

The simplest aberrant complex of the second chromosome consists of no less than eight inversions, then follow rearrangements with nine, with ten and, finally, with 11 inversions. The last complex of this series includes all of the inversions of the preceding complexes, and these presumably represent stages in the development of complexity. Comparison of the structure of these complexes implies a linear or unidirectional phylogeny of chromosomal variability. Evidently the same conclusion can be drawn for the aberrant complex of 3L, which consists of six inversions. This puts *D. busckii* in a special position compared with other species of Drosophila in which, usually, different degrees of branched phylogeny are observed. How it can be that chromosomal variability of *D. busckii* is unidirectional, and what the mechanism of the origin of its aberrant complexes may be, remain unknown.

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