CYTOLOGICAL AND GENETIC STUDIES ON DROSOPHILA ALGONQUIN AND RELATED SPECIES

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INTRODUCTION

Drosophila algonquin Sturt. and Dobz. (1936) is one of the American species of Drosophila included in the affinis group. That is, it is one of the six different species into which the old Drosophila affinis Sturt. (1916) has been divided (Sturtevant and Dobzhansky, 1936). The members of the affinis group appear to be close relatives of the western American Drosophila pseudoobscura Frol., which has within recent years proved to be of considerable value in cytological and genetic studies of wild populations and has given basis for speculation concerning the course of evolution within a Drosophila species and between species (review in Dobzhansky, 1939). The members of the affinis group as well promise to be useful in extending our knowledge of the nature of the relationships of forms of Drosophila found in nature, and it is with this in mind that a study of the cytology and genetics of one of these species --D. algonquin-- has been undertaken. In addition, the research has been extended to include an investigation of certain cases of species hybridization in the affinis group.

The known distribution of Drosophila algonquin is given on the map of figure 1. The distribution of this species overlaps the distributions of three of the other affinis group species: D. narragansett, which has been found within a rather narrow strip of territory from Woods Hole to Wooster; D. affinis, which occurs in addition in places south and east of the localities from which D. algonquin has been obtained; and D. athabasca subsp. mahican, which has been found southeast of the distribution area of D. algonquin (Great Smoky Mountains of North Caroline) but occurs mainly north and west of this region. In Texas the

FIGURE 1.—Distribution map for *Drosophila algonquin*. The black circles represent places from which strains of this fly were studied as to chromosome structure and variation. The circles with X's in them represent places from which *Drosophila algonquin* has been collected but from which no strains were available for this study.

distribution area of Drosophila algonquin overlaps that of D. pseudoobscura. The three remaining affinis group forms are apparently isolated geographically from D. algonquin: D. athabasca subsp. athabasca occurs in the northwestern United States, western Canada, and southern A laska; D. azteca has been found within a small area in northern California, but occurs principally in Mexico and Central America; D. seminole has been found in a rather restricted area in southern Alabama.

CHROMOSOMES OF DROSOPHILA ALGONQUIN*

Method

The study of the chromosomes of this species has been accomplished through the use of aceto-carmine smears of the ganglia and salivary glands of larvae. Both permanent and temporary preparations were used. The first salivary gland preparations, and most of those from which the Standard chromosome drawings (Plate I) were made, were permanent ones, prepared after the alcohol vapor method of Bridges (1937). In the investigation of the various strains of the species for chromosomal aberrations, where a large number of slides needed to be made, it was found sufficient to use temporary preparations, which, aside from being temporary, have seemed quite as satisfactory for general analysis as permanent ones. All drawings of these chromosomes have been made with the aid of a camera lucida.

Structure of the chromosomes

Drosophila algonquin has five pairs of chromosomes (Sturtevant and Dobzhansky, 1936). Seen at metaphase in the giant ganglion cells of the female larva these appear as a pair of large V-shaped X-chromosomes, a pair of V-shaped autosomes (except when sequence B- 3 is present, see below), two pairs of J-shaped autosomes --the smaller one with an almost terminal spindle attachment--, and a pair of dot-like autosomes (Plate I). In the male the Y-chromosome is J-shaped. In the salivary gland cells

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*Most of the subject matter of this section has already been presented in publication (Miller, 1939). Figures 1, 2, 3, 4, and 6 and Plate I are taken from the published paper.

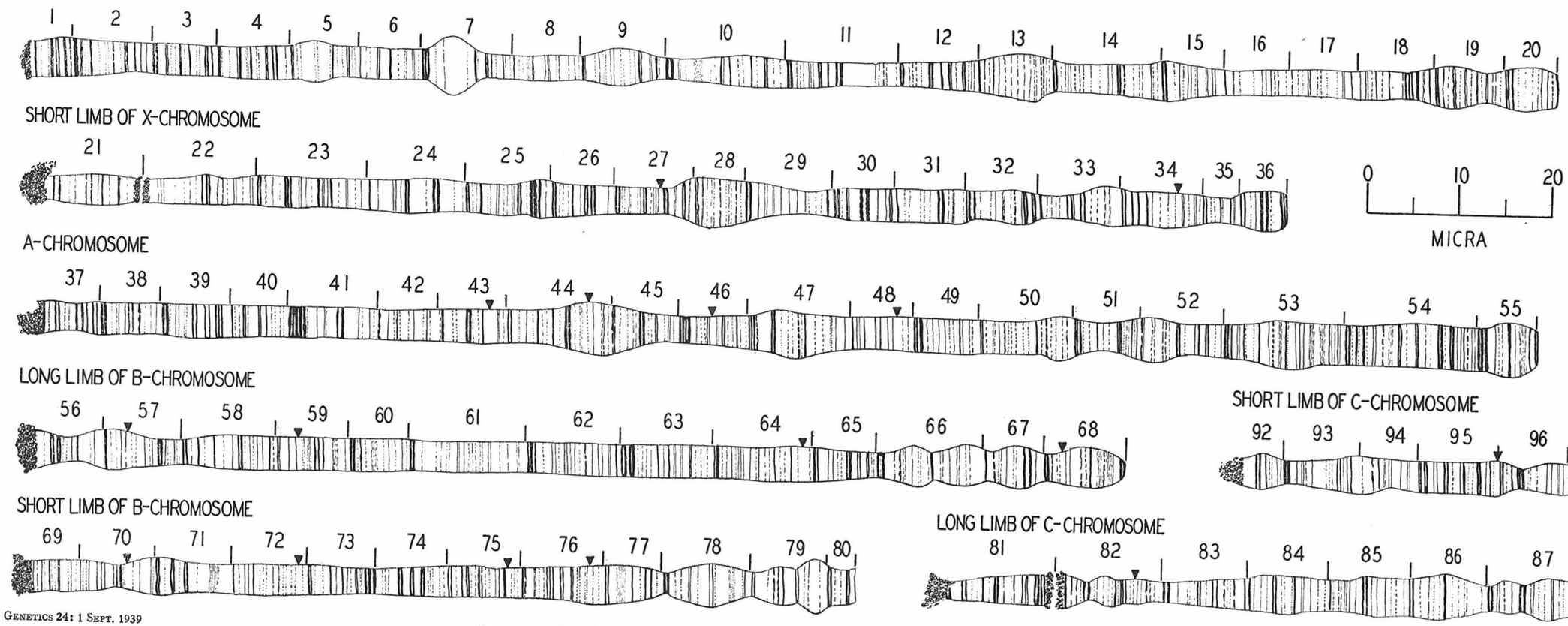


PLATE 1.—The salivary gland chromosomes of *Drosophila algonquin*. The sequence is the Standard used in this study. The black triangles indicate the breakage points of the inversions described in the text.

of the larva the nucleus contains seven long and one very short euchromatic strands extending outward from the chromocenter (Plate I).

The correspondence between the strands seen in the salivary gland cells and the metaphase chromosomes, although not securely established, is suggested by a number of facts. Two of the strands appear paler and thinner in preparations made from male larvae than in those from females. It is concluded that these represent the arms of the X-chromosome, which, since it is haploid in males and diploid in females, and since homologous chromosomes are generally paired very closely in salivary gland cells, might be expected to appear thinner in males than in females. According to Bauer (1936) the chromocenter of the salivary gland nucleus of Drosophila consists of the fused heterochromatic regions of all the chromosomes. If the chromocenter is broken, due to pressure applied on the coverslip of the salivary gland preparation, it seems to have a tendency to cleave along lines separating the different chromosomes. Assuming this to be true, through observation of a large number of salivary gland cells in which the chromocenter has been broken, one can get an impression of how the various euchromatic strands are attached to heterochromatic masses to form the chromosomes. In Drosophila algonquin the longest of the autosomal strands is very frequently seen entirely separate from the rest of the strands, with a small mass of rather compact heterochromatin adhering to its base. It is concluded that this association represents a chromosome, one arm of which is wholly heterochromatic, and this chromosome --designated the A-chromosome-- is identified with the short, almost telocentric chromosome of the metaphase plate. Two of the other strands are often seen attached to each other through

a small amount of heterochromatin. This association is called the B-chromosome. The two remaining long euchromatic strands are also frequently seen together. However, these arms are separated by considerably more heterochromatin than are the arms of the B-chromosome, and the mass of heterochromatin is often either broken in two or is incompletely detached from the rest of the chromocenter. This is called the C-chromosome. The identification of the B- and C-chromosomes with metaphase chromosomes is based on the presence in the B-chromosome of an inversion involving both arms, correlated with an altered shape of one of the metaphase chromosomes --the V-shaped autosome (see below). The metaphase chromosome undergoing this change of shape is called the B-chromosome. The remaining large metaphase chromosome is called the C-chromosome. The very small euchromatic mass of the salivary gland nucleus appears to be independent of the rest of the strands and most probably represents the dot-like chromosome seen at metaphase --called the D-chromosome.

The drawings of the salivary gland chromosomes represented in Plate I are composites of a number of camera lucida drawings which seemed to depict the chromosome strands reasonably well. No attempt, however, has been made to record all the fine, scarcely visible bands in these chromosomes, it being considered sufficient for the purposes at hand to note the relative positions of the most prominent ones. Consequently, one need not conclude that regions represented on these maps as devoid of bands are actually homogeneous and without special structure. For convenience in studying these euchromatic limbs the chromosome complement has been divided arbitrarily into 100 numbered sections, after the manner of Bridges (1935). Wherever it was possible without making two adjacent sections too different in

size a rather prominent band or group of bands was used as a section boundary. In the short limb of the X-chromosome and in the long limb of C there appears to be a region having a strong affinity for heterochromatin. The result is that in each of these strands the basal section --section 21 in the X, 81 in C-- usually is attached at both ends to the chromocenter. Each case is represented in the map of Plate I as a section separate from the rest of its arm with heterochromatin adhering to the separated ends. It would seem likely that these represent actual insertions of heterochromatin into euchromatic arms.

The nucleolus of the salivary gland nucleus appears in a number of figures in association with the short arm of the X-chromosome. In some especially clear figures the euchromatic part of the short arm of the X was separated quite completely from the chromocenter --very little if any heterochromatin adhered to it-- but was attached to the nucleolus by thin strands of rather lightly staining material. This association would seem to be similar to that described for the X-chromosome of Drosophila melanogaster by Kaufmann (1938).

Variation in the chromosomes

The study of the salivary gland chromosomes of a number of species of Drosophila has shown that the chromosome band pattern has a tendency to vary within the species. The nature of this variation is easily deduced from the configurations obtained in individuals heterozygous for pattern, and such differences as have been found appear to be due to inversions of sections of chromosomes. An investigation of the chromosomes of D. algonquin has shown a considerable degree of this sort of variation within this species.

Various strains of D. algonquin from places shown in the distribution map (fig. 1) were available for chromosome study. Each strain consisted of the descendants of a single female fly captured in the wild. An examination of the salivary gland chromosomes of individuals from the various strains has shown one of the gene arrangements to be present in all the localities from which collections were made --though not in all the strains. This arrangement has been arbitrarily chosen as the Standard Sequence. The nature of the arrangements present in each of the available strains --and in a few wild males-- was investigated by mating flies from it to individuals of the Standard type and then examining the salivary gland chromosomes of the offspring larvae. If a strain contained a sequence different from the Standard, the character of the difference was studied through the pairing configuration achieved by the homologous chromosomes in the salivary gland nuclei.

No sequence besides Standard was found for the long arm of the X-chromosome. In the short arm of the X a sequence differing from Standard by a single inversion was found. The limits of this inversion were determined to be in sections 27 and 34. This new sequence was called X-1, and the configuration foundin the salivary gland nucleus of a Standard/X-1 heterozygote is shown in figure 2A. Sequence X-1 occurred in strains from Belfast and Twin Mountain (near Franconia Notch).

Two sequences besides Standard occur in the A-chromosome. One of them --called A-1-- differs from Standard by a single inversion the limits of which are in sections 44 and 48 (figure 2B). Sequence A-1 has been found in strains from the following localities (named from northeast to southwest): Twin Mountain,

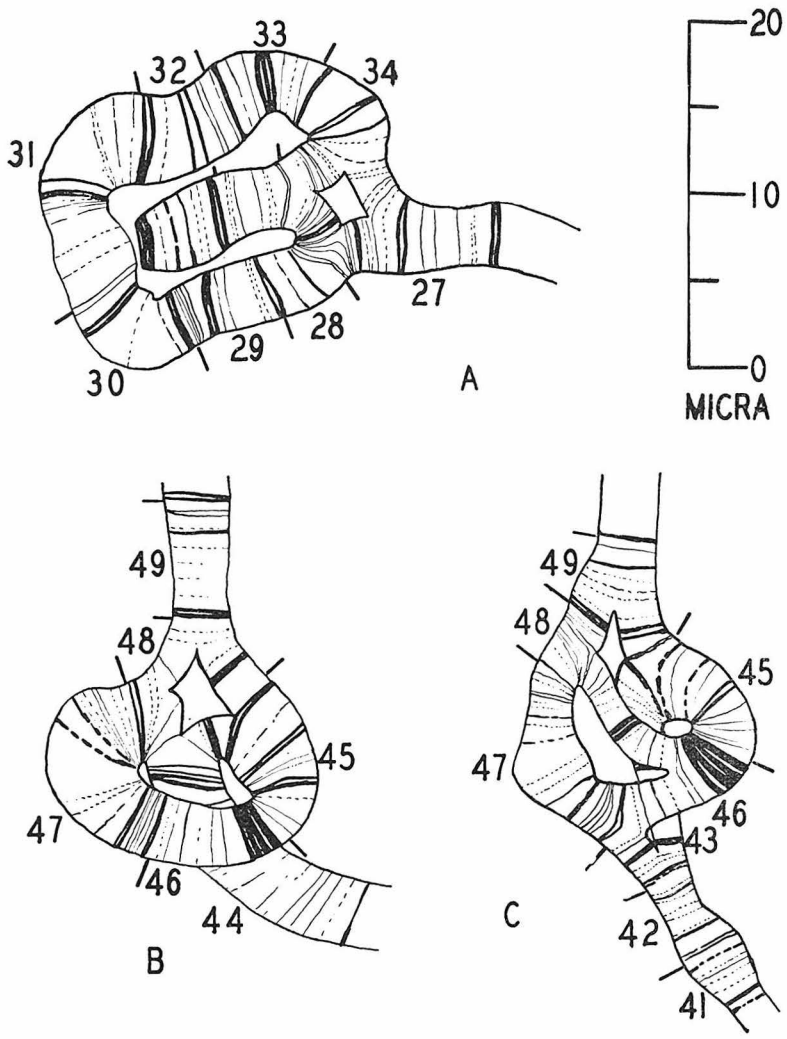


FIGURE 2.—Inversion configurations in the salivary chromosomes of individuals heterozygous for the Standard sequence and these inversions. A. Standard/X-1. B. Standard/A-1 C. Standard/A-2.

Woods Hole, South Amherst, Meriden, Derby (near Meriden), Varna (near Ithaca), Ithaca, Chautauqua, and Wooster. The other sequence --A-2-- differs from Standard by two overlapping inversions (figure 2C). A structural heterozygote of the constitution A-1/A-2 exhibits a single loop configuration, so that it seems that one of the inversions whereby A-2 differs from Standard is identical with the inversion differentiating A-1 from Standard. This suspicion is confirmed through an inspection of the limits of these inversions. The inversion overlapping the A-1 inversion in A-2 has breakage points in sections 43 and 46. Sequence A-2 has been found to occur in Belfast, Gray, Twin Mountain, Woods Hole and Derby.

It has been pointed out (Sturtevant and Dobzhansky, 1936a; Dobzhansky and Sturtevant, 1938) that sequences differing by overlapping inversions are of particular interest because of the insight they give into the probable phylogeny of the chromosome. It has been explained that the most probable relationship of two such sequences is through a third sequence differing from each of the former by a single inversion. That is, although one may not be able to tell which sequence arose first in the phylogeny of the chromosome, the conclusion seems most reasonable that one of the two arrangements did not arise directly from the other, but rather arose after the other had been changed through a single inversion; or, perhaps, the intermediate sequence came first and by a process of two inversions gave rise to the sequences in question. Now, given two chromosome arrangements differing by overlapping inversions, it is possible to hypothesize the intermediate sequence. In the case of the A-chromosome sequences Standard and A-2, however, sequence A-1 represents such an intermediate sequence. The probable relationship of

these three sequences is diagrammed in figure 3.

Three gene arrangements other than Standard have been found in the B-chromosome. One of them --B-1-- involves an inversion in the long arm of B with break points in sections 59 and 68 (figure 4A). Sequence B-1 is found at Gray and Woods Hole. Another is B-2, which differs from Standard by an inversion in the short arm of B (figure 4B). This sequence (inversion limits in sections 70 and 76) has been found only in one of the strains from Woods Hole. Since the inversions that differentiate sequences B-1 and B-2 from Standard are presumably quite independent, separable through crossing-over, a name has not been adopted for the B-chromosome arrangement differing from Standard by both the B-1 and B-2 inversions, such a sequence when encountered merely being considered to be a combination of sequences B-1 and B-2.

Sequence B-3 differs from Standard by two overlapping inversions one of which includes the spindle attachment --and all the heterochromatic region of the chromosome as well. The spindle attachment inversion has break points in section 57 of the long arm of B and in section 75 of the short arm, and the inversion that overlaps it has limits in sections 64 and 72. The hypothetical sequence intermediate between Standard and B-3 (as A-1 is intermediate between Standard and A -2, above) should differ from Standard by the spindle-attachment inversion alone. Such a sequence has not been found. The supposed relationship of sequences Standard, Hypothetical, and B-3 in the B-chromosome is diagrammed in figure 3. Sequence B-3 is quite widespread, occurring in the following localities: Belfast, Gray, Valmorin, Twin Mountain, Gale River (near Franconia Notch), Woods Hole, South Amherst, Meriden, Derby, Varna, and Wooster.

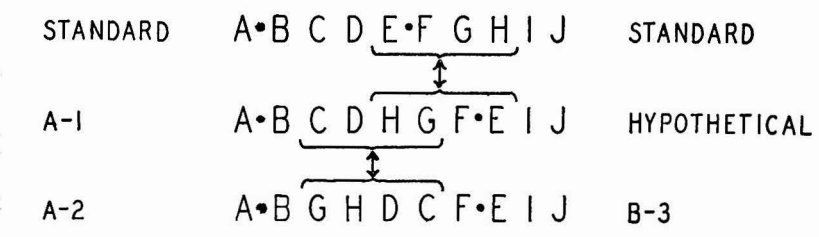


Figure 3. Diagram showing the most probable relationship of gene arrangements differing by overlapping inversions. Such a difference involves four breakage points. It seems likely that the rearrangement has occurred in two steps, two breakages resulting in one of the inversions at one time, two more breakages resulting in the other inversion at a later time. This process seems especially likely to have taken place if in addition to the two sequences in question the hypothetical intermediate arrangement is found in nature. This is the case with the sequences found in the D. algonquin A-chromosome, to which the labels on the left pertain. The spindle attachment of this chromosome is represented by the dot between A and B. The labels at the right pertain to the B-chromosome of algonquin. The spindle attachment of this chromosome is represented by the dot between E and F.

The B-chromosome of sequence B-3 has the length relationship of its two arms considerably different from that of the Standard B-chromosome. The long arm appears longer; the short arm shorter, by virtue of the fact that the long arm gains by the spindle attachment inversion more than half the length of the short arm (euchromatic) --the part proximal to the breakage point in section 75-- in return for the short section proximal to the break point in 57. Such a difference seems to be reflected in the metaphase chromosomes of the ganglion cells in that with the presence of sequence B-3 there appears in the metaphase group a J-shaped chromosome not otherwise present. Sturtevant and Dobzhansky (1936) describe the metaphase chromosome group of D. algonquin as containing no V-shaped autosome. It is most likely that theindividuals examined by these workers contained the B-chromosome sequence B-3 and not the Standard. Metaphase plate drawings for individuals homozygous and heterozygous for sequence B-3 are shown in figures 4D and 4E respectively.

An individual heterozygous for an inversion across the spindle attachment may be expected to give rise to a number of inviable offspring due to crossing-over in the region of the inversion, which should result in a duplication and deficiency in the cross-over product chromosomes (Sturtevant and Beadle, 1936). Thus, a sequence arising in a wild population through an inversion across the spindle attachment might be exptected to be at a disadvantage in the face of natural selection, since heterozygotes for it and the prevailing sequence are rendered partially sterile. However, if the spindle attachment inversion is overlapped by another inversion, heterozygotes for this new sequence and the original one might be expected to be at less of an evolutionary disadvantage than the above mentioned heterozygotes, since cross-

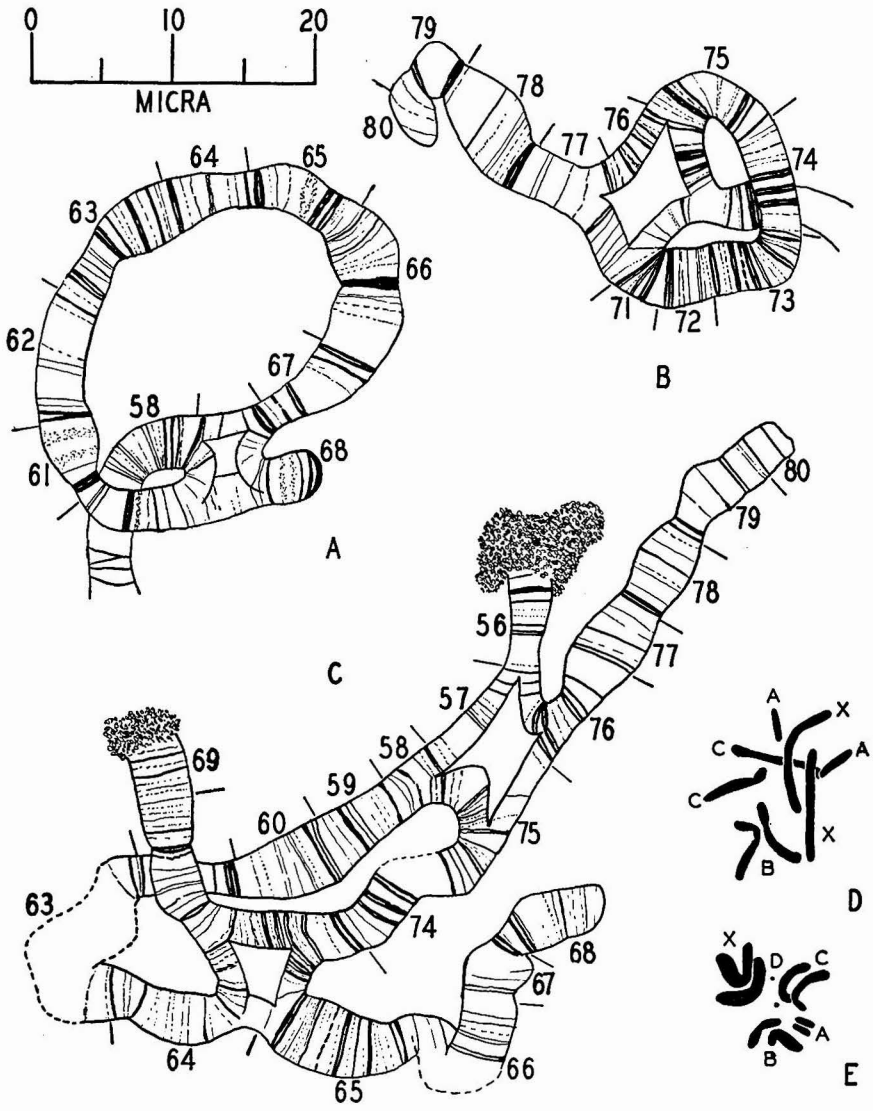
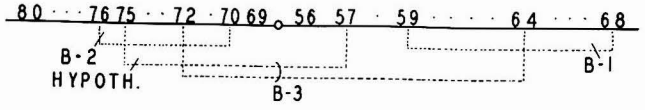


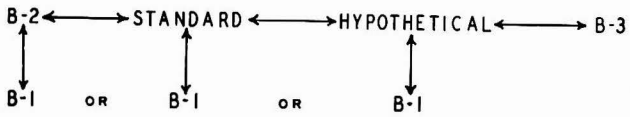
FIGURE 4—Inversion configurations. A. Standard/B-1 B. Standard/B-2 C. Standard/B-3. The chromosome may be considered to be continuous through heterochromatin between sections 69 and 56, although in this particular figure the heterochromatin connection is broken. D. Ganglion cell metaphase plate from an individual homozygous for sequence B-3. Compare the shape of the B chromosome here with that of the B chromosome in the metaphase plate shown in Plate 1. E. Ganglion cell metaphase plate from a Standard/B-3 individual, showing the two kinds of B chromosome. The scale of micra for this figure pertains only to the salivary chromosome drawings. The ganglion cell chromosomes are drawn on a slightly larger scale.

ing-over in the region overlapped by the two inversions (since one includes the spindle attachment) results in products that are not distributed to egg pronuclei (Sturtevant and Beadle, 1936); that is, crossing-over in the region of the spindle attachment inversion is virtually reduced. Such is the relationship of the Drosophila algonquin B-chromosome sequences Standard and B-3; each differs from the other by an inversion including the spindle attachment and an overlapping inversion. Apparently Standard and B-3 are at little disadvantage in the presence of each other, since this study shows that they exist together in a considerable number of places. According to the above reasoning, a combination of Standard and the B-chromosome Hypothetical sequence (differing from Standard by the spindle attachment inversion alone) might be expected to be attended with greater disadvantage. But according to the theory of overlapping inversions, Standard and Hypothetical must have existed together at one time. Thus, in spite of the apparent disadvantage, it seems that Standard arose from Hypothetical and persisted or that Hypothetical arose from Standard and remained long enough to give rise to B-3. The supposition that this evolutionary step is difficult is supported by the fact that until now, as far as the author is aware, no inversion across the spindle attachment had been found in wild populations of Drosophila, although such chromosomal aberrations have been derived by X-rays ("eucentric inversions" of Catcheside, 1938). (According to a personal communication of Professor Th. Dobzhansky, a spindle attachment inversion apparently exists within Drosophila duncani.)

The spindle attachment inversion (inversion of Hypothetical with respect to Standard) overlaps the inversion of sequence B-2. Thus, Standard forms a sequence intermediate between B-3



A



B

Figure 5. A. Diagram showing the inversions with respect to Standard in the B-chromosome of D. algonquin. The broken lines connect the limits of the inversions. B. Probable phylogeny of the B-chromosome, based on overlapping inversions.

and Hypothetical on the one hand and B-2 on the other. The inversion overlapping the spindle attachment inversion overlaps as well the inversion of sequence B-1. However, the spindle attachment inversion itself is independent of the B-1 inversion. Consequently B-1 may be considered to be separated from B-3 in point of time by Hypothetical at least, but possible by Standard, or even B-2 as well, since the inversions that differentiate each of these sequences from Hypothetical are quite independent of that by which B-1 differs from Hypothetical. The diagrams of figure 5 may serve to make the matter of probable phylogenies in the B-chromosome clear.

In the C-chromosome there have been found two arrangements besides Standard. One of them differs from Standard by an inversion in the long arm of C extending from section 82 to the boundary of sections 88 and 89 (figure 6A). This sequence -- called C-1-- has been found in Belfast, Gray, Twin Mountain, and South Amherst. The other non-Standard sequence differs from Standard by an inversion in the short arm with one breakage point at the 95-96 boundary and the other quite close to the tip in section 99. In structural heterozygotes of the Standard/C-2 type the short region distal to the inversion is generally not paired in the salivary gland cells, and the configuration has been illustrated from one of the usual figures (figures 6B and 6C).

The distribution of the gene sequences described above is given in Table I, as well as the total number of strains examined. Since the known distribution of the species follow roughly a line running northeast-southwest, the place names have been listed as they come from northeast to southwest. The impression may

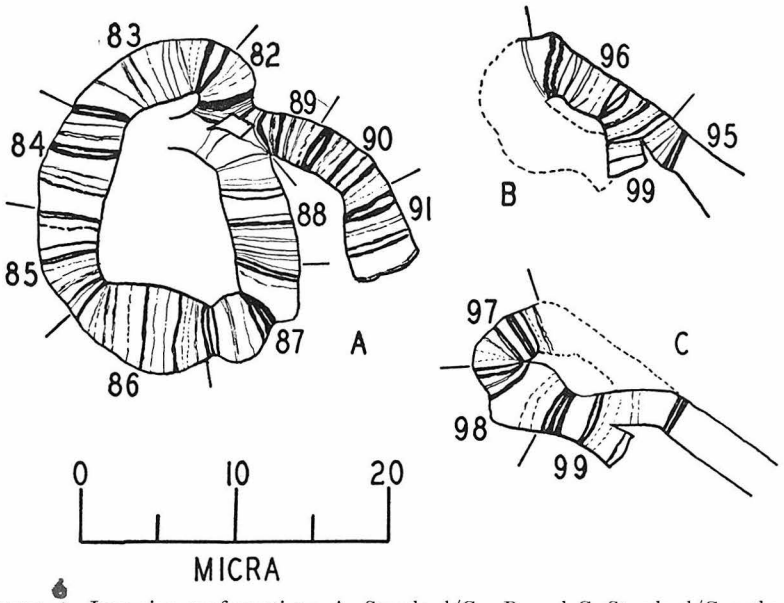


FIGURE 7.—Inversion configurations. A. Standard/C-1 B. and C. Standard/C-2, the same configuration at two different foci. This configuration is typical for this inversion heterozygote. Sometimes the last two discs of section 99 pair completely.

Table I

Locality	Strains	Sequences besides Standard
Belfast	1	X-1, A-2, B-3, C-1, C-2
Gray	2	A-2, B-1, B-3, C-1
Valmorin	1	B-3
Franconia Notch (Twin Mountain, Gale River)	5	X-1, A-1, A-2, B-3, C-1, C-2
Woods Hole	5	A-1, A-2, B-1, B-2, B-3
South Amherst	1	A-1, B-3, C-1
Meriden (Derby)	3	A-1, A-2, B-3
Ithaca (Varna)	5	A-1, B-3
Chautauqua	6	A-1
Wooster	2, 13 males	A-1, B-3
Wichita Falls	0, 1 male	none
Austin (Aldrich)	43	none
San Antonio	1	none
	75 14 males	

be gotten that chromosome arrangement in D. algonquin is more variable in the northeast than inthe southwest, although the numbers of wild individuals studied is rather too small to allow for generalizations of this sort. At any rate the uniformity of the Texas population of the species is striking. In the fall of 1938 there were examined 39 strains from Aldrich, and in none of these was a gene sequence besides Standard found. It was suggested (Miller, 1939) that the population from which these strains originated might have been the result of an introduction of a few D. algonquin individuals from the north. In the fall of 1939 there were received and studied four more Aldrich strains and flies from two new Texas localities --Wichita Falls and San Antonio. In none of these was a non-Standard sequence found . In view of the continued occurrence of Standard D. algonquin at Aldrich and the finding of this species at Wichita Falls and San Antonio, the suggestion that D. algonquin was artificially introduced into Texas seems now rather implausible. A study of D. algonquin from the territory between the Texas region and the remaining localities in which the species has been found should prove most interesting in view of the apparent homogeneity of the Texas population and the marked heterogeneity found in the species elsewhere.

Comparison of the chromosomes with those of other species

The salivary gland chromosomes of some of the other members of the affinis group have been studied: those of Drosophila azteca (Dobzhansky and Sokolov, 1939), D. athabasca (Novitski, unpub.), and D. affinis (Paul, unpub.). In addition, the existence of certain interspecific hybrids between affinis group members has made it possible to make direct comparisons between the chromo-

somes of some of these closely related species (Bauer and Dobzhansky, 1937; and see below).

In the salivary gland nucleus of each of the affinis group species studied there appear to be seven long euchromatic strands and one very short one. (This now seems to be true of D. azteca, since the recent discovery in this species of a chromosome arm not included in the maps of Dobzhansky and Sokolov, 1939). An examination of the drawings of these chromosomes, supplemented by some observations on the chromosomes themselves, shows that, although in general the gene arrangements chosen as Standard for each of these species have little recognizable similarity to each other, a few points of likeness suggest themselves. For example, in each of these species one of the arms of the X-chromosome is very long and contains a slightly bulging subterminal region devoid of darkly staining bands --depicted in the D. algonquin map in section 20, in the D. azteca map of Dobzhansky and Sokolov (1939) in section 23. Whether or not such apparently similar regions are actually homologous is difficult to determine in this way. However, such observations may serve to direct one's attention to possible homologies when methods better suited to testing homology are available --such as interspecific hybrid chromosome study (see below). In D. algonquin, D. athabasca, and in D. azteca, but apparently not in D. affinis, one of the autosome arms is very long, being of about the same order of length as the long arm of the X. In all four of these species two of the autosome arms are quite constantly attached to each other through a small amount of heterochromatin --the B-chromosome of D. algonquin, and of D. azteca. A rather constant feature of the short arm of this chromosome among these

species is a pattern of bulges and constrictions near the tip --shown in sections 78, 79, and 80 of the D. algonquin map, and in sections 89, 90, and 91 of the D. azteca map. The pattern of section 91 of the long arm of the C-chromosome of D. algonquin as well as the characteristic terminal flare of this limb is found in a chromosome arm of comparable length in each of the other species studied --for example, the unillustrated limb of D. azteca (Dobzhansky and Sokolov, 1939). Also, the short arm of the C-chromosome of D. algonquin has an apparent homologue in each of these species in that the pattern of its distal region appears to be nearly duplicated in a chromosome strand of each of them --compare section 99 of the D. algonquin map with section 99 of the map of the C-chromosome of D. azteca. Finally, the fifth chromosome seems quite similar in the different affinis group species studied, so that it might well be suspected of being homologous throughout.

Fortunately, it has been found possible to cross D. algonquin to another affinis group species, D. athabasca. (A general account of this hybrid is given below , see Interspecific Hybrids.) A number of observations have been made on the salivary gland chromosomes of hybrid larvae. Although hybrids are quite viable and apparently robust, the salivary glands of even the best of these larvae seem to be smaller than is generally the case for either species, and the chromosomes in turn are small and slender and not well fitted for examination. The salivary gland nucleus of hybrid larvae has the appearance of a mass of coiled strands, most of them, if not all, unpaired. However, there may be an association of what may be taken for homologous strands in cases even where no intimate synapsis has taken place, the homologues being coiled loosely about each other with their bases arising

from the chromocenter rather close together. In the salivary glands of both D. algonquin and D. athabasca it is rather a common occurrence for definitely homologous regions to fail to synapse, although pairing is the general rule. This tendency is evidently characteristic of hybrids in that certain similar regions of the chromosomes have been found both in the paired and unpaired conditions and still other regions that seem to be alike have never been seen paired.

The drawings of figure 7 have been made from preparations of salivary glands of larvae from the cross D. algonquin (Aldrich) females times D. athabasca (Grays River, Wyoming) males. These depict regions that were found paired or sufficiently apposed so that homology might be considered likely. It is not meant to be implied that these represent the only cases of such extensive homology between the chromosome sets, it being quite probable that further study will show more instances and will give a better idea of the nature of the differences between the chromosomes of the two species. No instances of synapsis have been observed between the X-chromosomes of these species. Neither has any been found between the A-chromosome of D. algonquin and its supposed homologue in D. athabasca. The long arm of the B-chromosome has been found to be paired with a strand of similar length only near the base in part of section 56 (figure 7A). The short arm, however, seems to be more completely similar in the two species in that pairing has been found near the base, in the middle, and towards the tip (figures 7B, -C, and -D). The basal section of the long arm of the C-chromosome pairs with a similar region in D. athabasca (figure 7E). Towards the tip of this chromosome arm there is also synapsis. However, a point of difference exists here in that the prominent dark terminal band

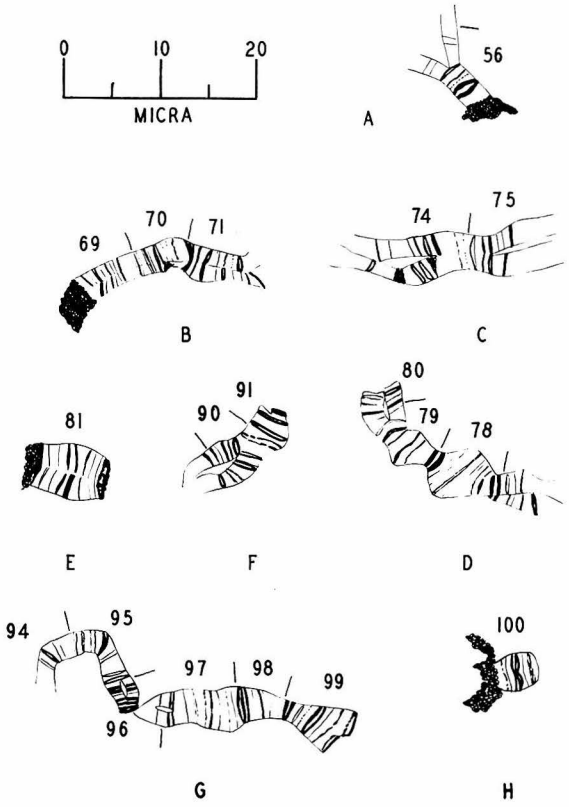


Figure 7. Examples of pairing or close approximation of the chromosomes of D. algonquin and D. athabasca as seen in the salivary glands of hybrids. A. Base of the long arm of the B-chromosome. B. Base of the short arm of the B-chromosome. C. Central region of the short limb of B. D. Tip of the short limb of B. E. Base of the long arm of the C-chromosome. F. Tip of the long arm of C. The athabasca homologue has a heavy terminal band apparently not present in the algonquin chromosome. G. Short limb of the C-chromosome. H. D-chromosome.

of the D. athabasca chromosome seems not to be present in the D. algonquin homologue (figure 7F). An example of such a terminal difference between otherwise homologous chromosomes has been pointed out by Kikkawa (1938) within the species D. ananassae. Also, Metz (1935) has shown that such terminal differences occur in the chromosomes of Sciara, where minute variations in chromosome structure are rather common. Whether the present case indicates a duplication or deficiency in one species with respect to the other or some otherkind of difference cannot be determined from the evidence at hand. The short arm of the C-chromosome of D. algonquin finds an almost entire homologue in D. athabasca. It is been seen paired from within section 94 to the distal end with but two small regions of incomplete synapsis intervening (figure 7G). The small D-chromosome has been seen paired throughout (figure 7H).

The analysis of the salivary gland chromosomes of hybrids as a method for determining the extent of chromosome difference between species has been made in a number of cases in Drosophila: in the D. pseudoobscura race hybrid (Tan, 1935; Koller, 1936), the pseudoobscura/miranda hybrid by Dobzhansky and Tan (1936), the melanogaster/simulans hybrid (Patau, 1935; Kerkis, 1936; Horton, 1939), the athabasca/ azteca hybrid by Bauer and Dobzhansky (1937), and the D. virilis subspecies hybrid (Hughes, 1939). Varying degrees of determinable difference have been found between the chromosomes entering into these combinations, from a few inversions to many inversions and small translocations. In the athabasca/ algonquin hybrid the available evidence allows for very little to be concluded as to the nature of the changes that have taken place between the chromosomes of these species. Probably due to the added difficulty of pairing that would be

expected to attend such a rearrangement, not even an example of homologous regions inverted with respect to each other has been found in this hybrid. However, that considerable change has taken place between the chromosome arrangements of the strains used in deriving the hybrid, probably involving many inversions, seems likely from the apparent differences in band pattern between the species, as well as from the high degree of failure of synapsis in hybrids. For the present it seems most profitable to concentrate attention on those regions that have remained similar to the extent of being able to pair in hybrids. Such regions are dmonstrable only in the shortest chromosome arms of these species.

Of much interest in connection with a comparison of the chromosomes of D. algonquin with those of closely related species is an analysis of the salivary gland chromosomes of another hybrid --that involving D. athabasca and D. azteca. A preliminary account of these chromosomes has been published by Bauer and Dobzhansky (1937), but the writer is indebted to Prof. Th. Dobzhansky for further information on chromosome pairing in this hybrid. The nature of the salivary chromosomes seems to be quite similar to that of the ones found in the above mentioned algonquin/athabasca hybrid. That is, the salivary gland nucleus generally consists of a mass of coiled, unpaired strands the relationship of which to each other is difficult to make out. However, a few instances of homology have been found. Here, as well as between D. athabasca and D. algonquin, homologies are most easily demonstrable between the shortest chromosome arms. Instances of pairing have been found in both arms of the B-chromosome, in the C-chromosome, and in the small, dot-like D-chromosome.

Particularly striking is the almost complete homology of the C-chromosome of D. azteca with the strand in D. athabasca which has been found to be largely homologous to the short arm of the C of D. algonquin. Also, the D-chromosome of D. azteca seems likely to be entirely homologous to the small chromosome of D. athabasca, which seems in turn homologous to the D-chromosome of D. algonquin.

On the basis of these observations a number of tentative conclusions as to the comparative structure of the chromosomes of D. algonquin, D. athabasca, and D. azteca may be made. The same number of euchromatic strands exists in all three species, and the relative lengths of these arms appear so similar in them that it may be concluded that no major interchange between arms, uncompensated for, has occurred between the species. However, the salivary gland chromosome patterns of these species seem to differ considerably --although possibly less than has been concluded on the basis of impressions of dissimilarity and failure of pairing in hybrids. That the occurrence of inversions in the chromosomes is largely responsible for the interspecific pattern differences seems very likely on the basis of the fact that intra-specific variation in chromosome sequence has been found to be due to this mechanism in D. algonquin , D. athabasca (Novitski, unpub.), and D. azteca (Dobzhansky and Sokolov, 1939). The possibility that translocations have also taken place is not to be excluded, though the amount of rearrangement accomplished in this way is probably small. Dobzhansky and Tan (1936) have noted a few instances of interchanges between chromosomes in the pseudoobscura/miranda hybrid, though these have been called into question by MacKnight (1939).

One might suppose that in the evolution of these species

the patterns of those regions found alike in each species were the original ones --at least for all but the most primitive species in case two of them are descended from the other one. Consequently, any intraspecific variation on this pattern could be considered likely to be a recent development. In D. algonquin the Standard sequence of the short arm of the C-chromosome is one that is largely identical with a chromosome arrangement in D. athabasca and in D. azteca. However, the C-2 inversion apparently effects a rearrangement within this chromosome that has not occurred interspecifically, and for this reason the C-2 sequence might be concluded to be a more recent development in the species than Standard.

The salivary gland chromosomes of Drosophila algonquin bear little resemblance to those of species outside the affinis group, judging from the published drawings of the chromosomes of D. melanogaster (Bridges, 1935), D. pseudoobscura and D. miranda (Dobzhansky and Tan, 1936; Tan, 1937), and D. virilis (Hughes, 1939). Aside from the absence of special similarity in band pattern, these chromosomes differ from those of D. algonquin in that in each of these sets there are but six euchromatic arms --five long ones and one very short-- instead of the eight found in each of the affinis group species so far investigated. Indeed, six salivary gland chromosome arms seems to be characteristic of every Drosophila speciesin which these chromosomes have been studied (except D. duncani, according to Prof. Th. Dobzhansky), aside from the members of the affinis group.

It is of interest to speculate as to how a change in the number of euchromatic chromosome limbs could be achieved in nature. Assuming that euchromatin is rearranged, not created or lost, two methods suggest themselves. A chromosome may be thought

of as acquiring, or losing, euchromatic material in an arm otherwise heterochromatic --neglecting the possibility of its being a telocentric chromosome-- through (1) a translocation or (2) an inversion across the spindle attachment. That is, a chromosome may undergo a change in the amount of euchromatin in one of its arms either through this arm's entering into an exchange with another chromosome or with the other arm of the same chromosome. In either case, unless the translocation were nonreciprocal or the inversion terminal, one would expect a terminal mass of heterochromatin to accompany the production of a new arm. Moreover, the loss of a euchromatic arm would require a nonreciprocal translocation or a terminal inversion, and unless the nonterminal breakage point occurred just at the boundary of heterochromatin and euchromatin, an interstitial mass of heterochromatin should result. (See figure 8.) It is not possible at present to say in which direction the process would be the more difficult. Chromosomal changes that seem terminal have been reported in Drosophila (for example, the terminal inversion of D. ananassae, Kaufmann, 1936). Interstitial chromosome arm segments that appear to have heterochromatin properties have been reported in Drosophila in a number of cases, of which the D. algonquin instance --short arm of X, long arm of C-- is one (also, the Y-chromosome of D. miranda, MacKnight, 1939; suggestions that such segments occur in the X of D. melanogaster, Kaufmann, 1939). The the ends of chromosomes consist of heterochromatin has seemed likely to a number of workers on the basis of the non-specific attractions of the tips of the salivary gland arms --resulting in occasional attachments of ends to each other and to the chromocenter (for example, the allegations of Prokofyeva-Belgovskaya, 1939). However, no large terminal mass of

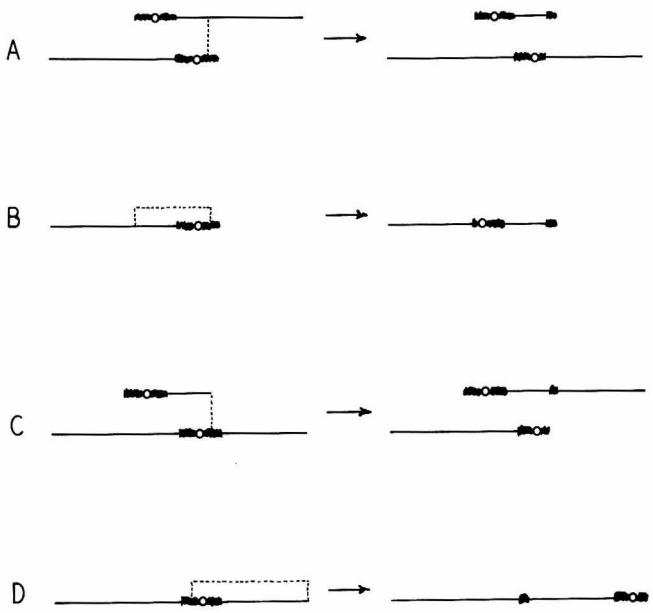


Figure 8. Schemes whereby a chromosome could either acquire a euchromatic arm or lose one, thereby increasing or decreasing the number of salivary strands of the species. In each case euchromatin is designated by a narrow line, heterochromatin by a thick, irregular one, the spindle attachment by a circle. The dotted lines connect points of transfer. A. Acquisition of a new euchromatic arm through translocation. B. Acquisition of such an arm through a spindle attachment inversion. C. Loss of a euchromatic arm through translocation. D. Loss of a euchromatic arm through a spindle attachment inversion.

heterochromatin has been observed in Drosophila. What the fate of such an arrangement would be --for instance, whether or not the terminal inert material would be lost readily-- is difficult to say.

Both mechanisms suggested --translocation and spindle attachment inversion-- might be expected to be attended with some disadvantage in establishment in a population (Sturtevant and Beadle, 1936). However, the successful establishment of a spindle attachment inversion in nature has evidently taken place within D. algonquin, as described above. An example of an intraspecific translocation has been reported by White (1940) in a species of grasshopper. As mentioned above, Dobzhansky and Tan (1936) have noted the chromosomes of D. pseudoobscura and D. miranda to differ by several small translocations. Sturtevant (in press) has made a comparison of the mutants of D. affinis with those of a number of species outside the affinis group, particularly D. pseudoobscura and D. melanogaster. A study of the linkage relationships in these species has led to the conclusion that no major translocation of genetic material from one chromosome to another --as would be evidenced by a difference of linkage relationships of apparently homologous genes-- has taken place between the eight-euchromatin-armed species D. affinis and some of those having only six --that D. pseudoobscura and D. melanogaster were not differentiated by interarm exchanges had already been concluded by Sturtevant and Tan (1937) in a similar study. This evidence would seem to favor the theory of transformation by spindle attachment inversions rather than by translocations.

MUTANTS OF DROSOPHILA ALGONQUIN

Descriptions of the mutants

All mutations so far discovered in this species are recessive characters. Each of those that has been retained for genetic study has very good expression --very little if any overlapping of the normal phenotype-- and reasonably good viability and fertility.

Two sex-linked mutants are known in D. algonquin --dusky (dy) and swollen tarsi (swt). The effect of dusky (The mutants dusky and rough -see below- were discovered in this species by Dr. A. H. Sturtevant) is to reduce the size of the wings so that they scarcely cover the abdomen and to give them a decidedly dark texture. This is, the phenotype is very similar to that of the sex-linked mutant dusky of D. melanogaster and of other species (Morgan, Bridges, and Sturtevant, 1925). The effect of swollen tarsi, as the name implies, is principally a distortion of the legs. The leg segments have an inflated appearance, particularly noticeable in the tarsus. This mutant also affects the wings, making them somewhat shorter and broader than in the wild type fly. There seems to be a considerable lethal effect accompanying swollen tarsi under certain conditions, judging from the proportion of such flies gotten in the linkage experiments (Table V). There also seems to be some reduction of the fertility of females showing this character.

In D. algonquin there are four autosomal mutants --rough (ro), cinnabar (cn), droop (dr), and brown (bw). Flies of the rough type have compound eyes in which an irregular arrangement of the facets and hairs produces a decidedly uneven surface texture. In addition, eyes of this type occasionally contain dark

areas of various sizes, apparently due to erupted ommatidia. These spots are particularly noticeable when the character occurs in combination with the bright eye color cinnabar. The presence of black erupted areas on the eye has been noted in the case of the D. melanogaster character roughoid (Morgan, Bridges, and Sturtevant, 1925).

In Drosophila algonquin one bright red eye mutant has been found. This character is particularly striking in flies that have just emerged. As the adults become old, the eye color darkens somewhat, so that in flies several days after hatching the mutant is not so easy to distinguish from wild type. In addition to the compound eye character flies of this kind have colorless ocelli. Within Drosophila species there occur four bright red eye mutants which are often indistinguishable in appearance --vermilion, cinnabar, scarlet, and cardinal. However, it has been discovered that the first two differ from each other and from the others in the reactions they give in transplantations of imaginal eye discs (Beadle and Ephrussi, 1936). Vermilion and cinnabar eye discs are both altered in the direction of wild type pigmentation when transplanted into hosts that are neither vermilion nor cinnabar. Moreover, vermilion eye discs become wild type when transplanted into cinnabar hosts. It has been concluded that each of these two mutant types is due to the lack of a diffusible substance which hosts of the proper type can furnish --in the case of vermilion the vermilion plus hormone, in the case of cinnabar the cinnabar plus hormone. A technique for determining whether or not flies of a given mutant type produce either or both of these hormones has been developed by Beadle and Law (1938). This consists of feeding crushed pupae of the type to be tested to D. melanogaster vermilion brown and cinna-

bar brown larvae. If the type in question is neither vermilion nor cinnabar -- by virture of producing both the v plus and cn plus substances --, both the vermilion brown and cinnabar brown flies will develop eyes altered from the usual pale pink color to a brownish or brown pigmentation. If the type is cinnabar, the change will be effected in the vermilion brown flies, but not in the cinnabar brown ones; if vermilion, the transformation will take p lace in neither kind of test flies. Thus, a bright red eye mutant of any species of Drosophila can be identified as vermilion, cinnabar, or something else on the basis of what kinds of substances are produced in its development. In D. algonquin the mutant of this type has been identified as cinnabar on the grounds that pupae of this character --chosen at a time when eye pigmentation was just beginning to become apparent-- when fed to D. melanogaster larvae of the v bw and cn bw kinds, caused v bw eyes to be altered to brown but did not effect cn bw eyes. Wild type D. algonquin pupae of the same age caused brown pigmentation to appear in the eyes of both kinds of test flies. That is, the D. algonquin mutant differs from wild type in not producing the cinnabar plus substance, though the vermilion plus hormone is produced.

A third autosomal mutant in D. algonquin is droop. Flies of this type have wings that are somewhat shorter and broader than normal. In addition, the texture of the wings has a thin, shiny appearance. Usually, though not always, the wings are concave underneath so that the edges hang below the normal level of the wings at rest. A similar effect has been described for the mutant curved of D. melanogaster (Morgan, Bridges, and Sturtevant, 1925). However, in the D. algonquin mutant the wings are not divergent and uplifted, as they are in curved.

The mutant brown causes the eyes to have a dull, dark brownish appearance. The dark movable fleck on the compound eye characteristic of wild type is missing, and the eye seems to be rather translucent. A microscopic examination of the ommatidia show less of an aggregation of the dark pigment granules in the distal regions than is true of wild type, and one might conclude that the translucent appearance is due to the fact that light may thus enter the ommatidia laterally as well as through the distal ends. The ocelli of this type are colorless, and the testes, which are bright orange in wild type, are unpigmented here. A combination of brown with the above described cinnabar causes the eyes to be of a pale yellowish color which darkens to brown as the flies mature --just as the cinnabar eye itself darkens with age to nearly wild type. The ocelli and testes here are also colorless. Examination of the ommatidia shows the distal regions to contain a number of yellow pigment granules which seem to be soluble in hot water. The presence of such granules is not characteristic of the brown mutant of D. melanogaster. (The writer is indebted to Dr. J. Schultz for this information.) In addition, the D. algonquin brown mutant seems to be associated with a semi-lethal effect, as became evident in tests made to determine its linkage relations (see Tables II, III, and IV).

Linkage relationships

The linkage relationships of the autosomal mutants of D. algonquin have been studied through making matings of all the possible combinations of these mutants and examining the F_2 generation flies for double homozygous individuals. Due to the oc-

currence of no crossing-over in the male in Drosophila one expects that two genes that are linked will not be found together in the homozygous conditions in the F_2 of a cross into which they entered separately. On the basis of this expectation the presence of a double homozygote in the F_2 of such a cross is evidence that the genes concerned are not linked. From this sort of evidence it has been concluded that the D. algonquin mutants rough, cinnabar, and droop are not linked to each other --that is, they form three separate linkage "groups". Similarly it was discovered that brown is linked to neither rough nor cinnabar. However, the F_2 generation of the cross between brown and droop contained no brown droop individuals (Table II) (the absence of this class is not in itself strong evidence for linkage in this case, since it might be supposed that the double homozygote would be inviable, judging from the apparent deleterious effect of brown alone). Since D. algonquin has but four autosomes --one of them very small, and since two of these -- containing the linkage groups of rough and cinnabar-- were shown not to contain brown, it was thought likely that brown was linked to droop, assuming it unlikely that any of the genes were located in the very small chromosome. Another test on this possibility was made. A brown droop stock was derived from individuals of subsequent generations and a cross was made between brown droop and wild type. F_1 males were then backcrossed to brown droop females. If the genes responsible for these characters were linked, such a backcross should yield only individuals of the parental types, no recombinations. The results of this experiment --the absence of the recombination classes (Table III)-- confirm the suspicion that brown and droop are linked. Thus, the known D. algonquin mutants arrange themselves into four lin-

Table II. F_2 of a cross between droop and brown.

wild type	dr	bw	dr bw
122	49	14	0

total = 185

Table III. Progeny of the cross bw dr ♀ × bw dr / + + ♂

wild type	bw dr	dr	bw
134	67	0	0

total = 201

Table IV. Degree of linkage of droop and brown.

A. Progeny of the cross bw/dr ♀ × bw dr ♂

bw	dr	bw dr	wild type
50	186	34	88

total = 358
recombinations = 122

% recombinations = 34.08

B. Progeny of the cross bw dr/++ ♀ × bw dr ♂

bw dr	wild type	bw	dr
8 7	152	33	42

total = 314
recombinations = 75

% recombinations = 23.09

Table V. Degree of linkage of dusky and swollen tarsi.
A. F_2 males of the cross dy ♀ x swt ♂

swt	dy	dy swt	wild type
78	200	97	215

total = 590
recombinations = 312

% recombinations = 52.88

B. F_2 males of the cross wild type ♀ x dy swt ♂

dy swt	wild type	dy	swt
118	222	171	84

total = 595
recombinations = 255

% recombinations = 42.86

kage systems: (1) the sex linked mutants dusky and swollen tarsi, (2) rough, (3) cinnabar, and (4) droop and brown.

Degrees of linkage between droop and brown and between dusky and swollen tarsi were determined as percentages of recombinations from both coupling and repulsion data (tables IV and V). In both instances the values for recombination gotten by the two methods were appreciably different. This has been interpreted as being due to the disadvantage with respect to wild type exhibited by the mutants concerned, since in each case the higher recombination value was gotten when one of the recombination classes was wild type. Swollen tarsi and brown seemed to carry rather definite lethal effects.

Allocation of the mutants to the chromosomes

It has been desired to find out to which chromosomes of D. algonquin --as observed in the salivary gland-- the known genes should be assigned. A method for determining this was made available through the presence in this species of cytologically observable landmarks the inheritance of which could be studied --namely, the inversions whereby the chromosome sequences differ.

A stock of D. algonquin homozygous for rough, cinnabar, and droop was made up --each of the three autosomal linkage "groups" being thus represented. Cytologically, the stock seemed to carry throughout the Standard sequence in the A-chromosome and the B-3 sequence in the B-chromosome. Individuals of this stock were mated to wild type flies of the Woods Hole strain 7.8b, which was determined to contain the A-2 sequence in the A-chromosome and the B-1, B-2, and Standard sequences in the B-chromosome, but not the B-3 arrangement. Consequently, the

progeny of this cross were expected to be heterozygous for gene sequence in the A- and B-chromosomes and to be heterozygous for mutants in three different autosomes. Males of this generation were then backcrossed to rough, cinnabar, droop females. In the absence of crossing-over in the males, it would be expected that the mutant type progeny of this cross would be homozygous with respect to strain for the entire chromosomes carrying the genes expressed. Consequently, since two of the autosomes were marked by inversions, homozygosis or heterozygosis could be determined for them cytologically and the presence or absence of genes in these chromosomes detected. Thus, for example, if such a mutant individual could be found to be heterozygous for chromosome sequence in the A-chromosome, then it could be concluded that the gene responsible for the mutant was not carried in that chromosome. Similarly, a gene could be excluded from the B-chromosome, or from both the A- and B-chromosomes. However, if a mutant individual is found to be homozygous for sequence in the A-chromosome, one might conclude either that the gene expressed is contained in the A-chromosome or that the A-chromosome carries none of the genes represented in the multiple mutant stock. The latter condition seems unlikely on the basis of the small likelihood that any of the three linkage "groups" is to be allocated to the small D-chromosome. Similarly, thus, one should be able to assigne a gene to the B-chromosome, or two genes to the A- and B-chromosomes together. Determinations of homozygosis or heterozygosis for chromosome sequence in the A- and B-chromosomes were made for a number of mutant backcross individuals by mating them to flies of the wild type strain and examining the salivary glands of their offspring, heterozygosis --and consequently exclusion of the gene, or genes, of the mutant type

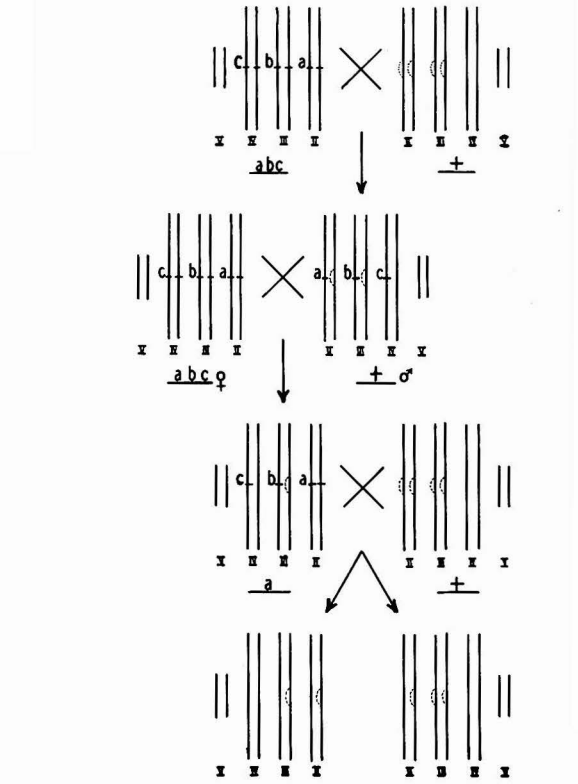


Figure 9. Demonstration that a gene "a" is located in chromosome II, not in chromosomes III nor IV, of a Drosophila species in which each of the autosomes, except the very small one, is assumed to contain a gene --designated by the small letters "a", "b", and "c", represented on the chromosomes as cross lines. The first cross is made between a multiple mutant stock a,b,c and a wild type stock differing from it by inversions in chromosomes II and III --shown as curved broken lines. A male offspring is backcrossed to the mutant stock. From the progeny of this cross an individual exhibiting only gene "a" is mated to an individual of the wild type stock. Examination of the salivary glands of the offspring show all of them to be heterozygous for sequence in II, half heterozygous and half homozygous in III.

from the chromosome so determined-- being assumed if part of the larvae were heterozygous and part homozygous in either or both chromosomes, homozygosis being considered likely --and consequently a tentative assignment of the gene or genes to the chromosome or chromosomes so determined-- if seven or more of the larvae showed heterozygosis for chromosome sequence (the chance that a heterozygous individual would have seven offspring of one kind in a backcross being 1 in 128). An outline of the method is given in figure 9.

Conclusions as to the allocation of the genes of D. algonquin could be drawn from two mutant individuals. One rough droop male proved to be homozygous for both the A- and B-chromosomes. Thus, it seemed likely that rough and droop were located in the A- and B-chromosomes. A droop male showed himself to be homozygous for the A-chromosome, heterozygous for the B. Consequently, droop seemed to be contained in the A-chromosome, not in the B, and from the constitution of the preceding individual, rough may be concluded to be carried in the B-chromosome. By exclusion, cinnabar should be assigned to the C-chromosome. In confirmation of these conclusions, a cinnabar female was found heterozygous for A and B, thus excluding cinnabar from A and B; a rough cinnabar female was determined to be heterozygous for the A-chromosome, thus excluding both rough and cinnabar from A; a droop female was found heterozygous for B, thus excluding droop from B. In summary, the following gene-chromosome relations seem to exist in D. algonquin: A-chromosome --Droop and brown, B-chromosome --rough, C-chromosome --cinnabar (excluding, of course, the possibility that one of these "groups" should be assigned to D.

The mutant cinnabar, as described above, is one that can

be identified in other species. Consequently, from the allocation of this mutant to the C-chromosome of D. algonquin it can be concluded that the C-chromosome of this species is at least in part homologous to chromosome III of D. pseudoobscura and to the right limb of chromosome II of D. melanogaster, since a gene with the properties of cinnabar has been located in these positions in these species (orange of D. pseudoobscura has been determined to be cinnabar through the transplantation experiments of Tan and Poulson -1937-).

INTERSPECIFIC HYBRIDS

The algonquin/athabasca hybrid

Certain matings between Drosophila algonquin females and D. athabasca males have been found to yield progeny (unpublished data of Dr. A. H. Sturtevant and results of the writer). These offspring, though quite normal in general appearance, have seemed sufficiently different from D. algonquin to raise the suspicion that they were hybrids and not the result of non-virginity. The supposition that hybrids could be gotten between these two species has been confirmed in that a cross between rough D. algonquin females and D. athabasca (Grays River, Wyoming) males gave offspring that were wild type with respect to eye character.

Continued interspecific matings have shown that hybridization between D. algonquin and D. athabasca is rather difficult to obtain. Indeed, the cross athabasca females times algonquin males has failed so far to yield hybrids at all. An investigation of interspecific insemination frequency has been made. In this experiment it has been the practice to mate at a time about ten females of one species to as many males of the other. These flies were then kept together for ten or eleven days. After this time the females were dissected and whether or not any of them had been inseminated determined by observing microscopically the presence or absence of sperms in the seminal receptacles. As a check on possible non-virginity only mutant type females have been used in these crosses, the mutants being ones that have been determined to be recessive in hybrids as well as in the pure species. Insemination data from matings yielding matroclinous mutant offspring have been discarded. A summary of interspecific insemination data derived from crosses

Table VI. Interspecific insemination, between D. algonquin and D. athabasca.

D. algonquin ♀ × D. athabasca ♂		D. athabasca ♀ × D. algonquin ♂	
droop × vermilion	3/53	vermilion × droop	0/104
droop × cinnabar*	0/54	vermilion × cinn.	0/36
cinn. × vermilion	0/25	vermilion × Aldrich	0/19
cinn. × Sundance, Wy.	0/12		0/159
	3/144		
	= 2.08%		

* mahican

between algonquin and athabasca is presented in Table VI. Apparently not all cases in which insemination occurs yield progeny. None of the three instances of insemination of droop females by vermilion males was accompanied by the occurrence of hybrids. The nature of this failure of inseminated females to produce offspring is not known, whether due to inhibition of egg-laying or to high mortality of hybrids at early stages or to some other cause. A case in which a much higher frequency of interspecific insemination is attended with low hybrid production is thatinvolving D. affinis females and D. athabasca males. This has been investigated in greater detail, as described below.

Algonquin/athabasca hybrids have been derived principally from a series of group matings. In each of these crosses five D. algonquin females were kept with five D. athabasca males for about a month, and longer than this if hybrids were being produced. The D. algonquin females contained various mutants and combinations of mutants, and a number of strains of D. athabasca were employed. Out of 342 such crosses 13 yielded hybrids. A record of these successful matings is given in Table VII (the crosses to which cross-numbers have been given). In the case of each of the D. algonquin mutants --cinnabar, droop, rough, and dusky, and the D. athabasca mutant vermilion the character was suppressed completely in the hybrids, thus indicating the presence of the wild type alelomorphs of these genes in both species.

The algonquin/athabasca hybrids appear to be quite viable and robust. However, the size of families gotten from interspecific crosses is considerably smaller than those generally gotten within D. algonquin, even from pair matings. Neither sex seems

Table VII. D. algonquin ♀ × D. athabasca ♂. Crosses yielding hybrids.

cross	cross number	progeny males	progeny females	total
rough × Grays River 71.1		4	3	7
Aldrich × Sundance		0	1	1
cinn. × vermilion		1	0	1
cinn. × vermilion		3	4	7
cinn. × Grays River 71.1	# 6	41	36	77
cinn. × Grays River 71.1	# 16	21	27	48
cinn. × Grays River 71.2	# 13	32	33	65
cinn. × vermilion	# 4	1	0	1
cinn. × vermilion	# 20	3	8	11
cinn. × vermilion	# 21	14	9	23
droop × Grays River 71.2	# 10	16	8	24
droop × Grays River 71.2	# 23	13	11	24
rough × Grays River 71.1	# 12	6	34	40
rough × Grays River 71.1	# 105	0	1	1
rough × Grays River 71.2	# 19	18	32	50
rough × Gravina	# 71	1	1	2
dusky × Gravina	# 141	4	9	13
		178	217	385

to have any obvious abnormalities. The hybrid totals given in Table VII show that there have been gotten altogether fewer males than females --178 to 217. However, this aberration of the sex ratio seems to depend on the data derived from two of the crosses involving rough D. algonquin females --Table VII, crosses #12 and #19, from which there were gotten a total of 24 males and 67 females. Disregarding these figures, the hybrid totals are 154 males and 150 females. It is possible that the disparity of male and female hybrids derived from the D. algonquin rough females is due to an otherwise undetected sex-linked lethal for which each of these females was heterozygous.

In general appearance the algonquin/athabasca hybrids are very similar to D. algonquin, though in a few respects they show the influence of D. athabasca. D. athabasca is a considerably darker form than D. algonquin and hybrids between these two species have a tendency to darken with age more completely than algonquin, though probably not as much so as athabasca. In athabasca the wings are slightly longer and narrower relative to the body size than in algonquin, and a tendency in this direction has been observed in hybrids.

D. athabasca appears to differ clearly from D. algonquin in the size of the sex comb. D. athabasca males have small sex combs usually consisting of four teeth, while the D. algonquin sex combs are quite large, consisting of from six to ten teeth. (According to Sturtevant and Dobzhansky -1936b- D. athabasca has four sex comb teeth, D. algonquin from eight to ten. The writer has observed a few D. athabasca sex combs with but three teeth -Gravina- and some with five -vermilion-, and counts made on D. algonquin males have shown there to be individuals with six and seven teeth per sex comb -cinnabar (figure 10A) and rough-).

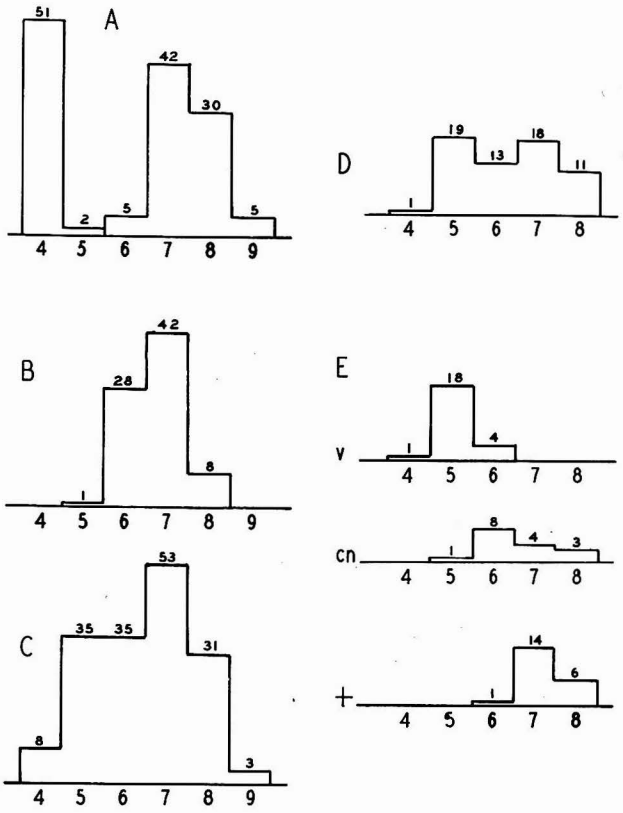


Figure 10. Histograms representing sex comb teeth counts in D. algonquin, D. athabasca, hybrids, and males of the backcross of hybrid females to D. algonquin. The abscissa classes represent numbers of teeth in the sex comb on the right prothoracic leg. The number at the top of each column is the number of individuals in the class. A. The histogram at the left applies to athabasca vermilion males, the one on the right to algonquin cinnabar males. B. Hybrids involving the algonquin cn strain. C. Backcross males, the algonquin strain being cn. D. Part of the males of "C" derived from hybrids containing athabasca v. E. The types constituting the backcross males of "D".

Some counts of sex comb teeth are presented as histograms in figure 10. Figure 10A shows the existence in the athabasca vermilion strain of males having four or five teeth per sex comb (right prothoracic leg) and of individuals varying in sex comb teeth number from 6 through 9 in the algonquin cinnabar strain. Figure 10B shows sex comb teeth number to vary from 5 through 8 in a number of hybrid males in which the D. algonquin parent was of the cinnabar strain. According to Castle et al (1906) the number of teeth in the sex comb in Drosophila melanogaster is quite variable --ranging from 7 to 14--, depending to a considerable extent on the culture conditions. That there are genetic factors concerned in the variability has been shown by Hoge (1915) who succeeded in isolating lines of D. melanogaster with high and low mean sex comb teeth numbers. It is likely that in D. algonquin and in D. athabasca the number of teeth of the sex comb depends in its variability on factors of the sort that operate in D. melanogaster, although a study of this matter has not been made in these species. In view of this possibility, it is difficult at present to evaluate the significance of the difference in sex comb teeth number between D. algonquin and the algonquin/athabasca hybrids.

The algonquin/athabasca males are quite sterile. An examination of hybrid testes has shown them to be somewhat smaller than those of either species, though the spiral shape common to the testes of these species is retained as much as possible. The rest of the genitalia seem to be normal. An investigation of hybrid testis structure has been made through a study of acetocarmine smears. Spermatogenesis seems to be impaired to the extent that no spermatozoa are produced. Scattered through the smears there were seen groups of rather small cells with large

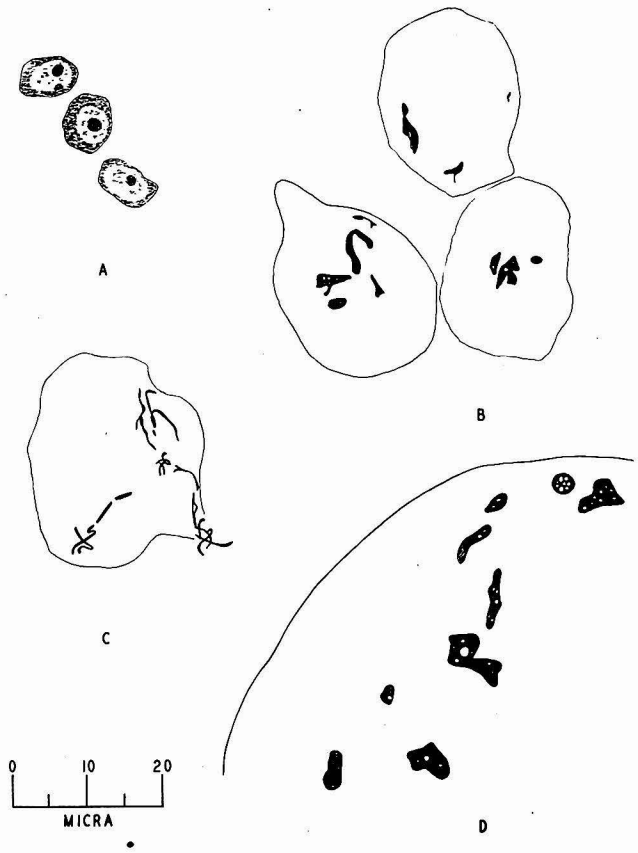


Figure 11. Structures found in the testes of hybrids. A. Spermatogonia in algonquin/athabasca testes. The one at the top has two nucleoli. B. "Spermatocytes" in algonquin/athabasca testes. C. "Spermatocyte" in an affinis/athabasca testis. D. Part of a giant syncitial mass found in the affinis/athabasca testes. This body measured about 100 micra across and contained 37 "nuclei".

nuclei, some of which contained two nucleoli. These were interpreted to be the spermatogonia (figure 11A). In addition, the testes contained groups of considerably larger cells, of the order of size of the primary spermatocytes of normal testes. These cells contained a variable number of irregular, darkly staining masses, usually quite vacuolated (figure 11B). It seems likely that these cells are spermatocytes in which the normal maturation divisions have been obstructed and that the dark masses are chromosomes or groups of chromosomes which have formed pyknotic pseudo-nuclei. Dobzhansky (1934) has described as the final stage in spermatogenesis in Drosophila pseudoobscura race hybrids of the weak B female times weak A male cross the formation of irregular clumps of chromosomes within the spermatocyte body.

The algonquin/athabasca females are fertile and have been backcrossed successfully to D. algonquin males. These matings --made as pair matings, each lasting about a month-- have succeeded in somewhat less than half the attempts --46 out of 128. Some attempts at backcrossing hybrid females to D. athabasca males have been made, but none has been successful so far. In Table VIII are presented the results of backcrosses of algonquin/athabasca females to D. algonquin males. In each case the backcross was made to the algonquin mutant strain involved in the interspecific cross; consequently the progeny were both mutant and wild type. In cross #21 the athabasca sex-linked mutant vermilion was used, so among the backcross offspring there were some vermilion males. In this instance it was relatively easy to distinguish vermilion and cinnabar individuals since vermilion was much more extreme in expression than cinnabar. The number of progeny per backcross was appreciably less than the usual number gotten from interspecific matings, though, of course,

Table VIII. Progeny of algonquin/athabasca females backcrossed to D. algonquin males.

cross		number fams.	progeny: males ±	males mut.	females ±	females mut.	totals
cn/G.R.71.1 #6	x cn	11	52^{3}	38^{4}	54^{2}	50	194
#16	x cn	3	1^{1}	7^{1}	9	2	19
cn/G.R.71.2 #13	x cn	10	20^{3}	22^{2}	26	23^{3}	91
dr/G.R.71.2 #10	x dr	1	1?		1	1	3
ro/G.R.71.1 #12	x ro	2	1		2		3
ro/G.R.71.2 #19	x ro	16	46^{7}	32	61^{3}	55^{4}	194
totals			120^{14}1?	99^{7}	153^{5}	131^{7}	504
			±	v cn			
cn/v #21	x cn	3	27^{1}	24^{1} 25	47^{1}	47	170
			males		females		
totals			295^{23}		378^{13}		674^{36}

since the interspecific crosses were made as group matings, it was never certain whether more than one female was participating in a single cross. The mean family size in interspecific matings was 25.1, that gotten in backcrosses 14.4. There was a general tendency towards the production of fewer backcross males than females. Lancefield (1929) observed deviations from the 1:1 sex ratio in backcross progenies of the D. pseudoobscura race hybrids. In addition, there were a number of backcross individuals, both males and females, that were definitely abnormal in general appearance --having such abnormalities as rough eyes, missing or reduced bristles, wings held out or up, abnormal abdomen, though none of these has been found to occur in the hybrids themselves. Some of the abnormal males were found to have small misshapen testes as well. An estimate of the frequency of these aberrant individuals has been included in Table VIII (the numbers written as underlined exponents being the numbers of abnormal individuals found in the various classes). Presumably these abnormalities occur as the result of a state, or states of "unbalance" accompanying certain combinations of the algonquin and athabasca chromosomes. However, in these crosses the chromosomes were not sufficiently marked to make it possible to determine the nature of the chromosomal conditions on which the peculiarities depended.

Among backcross males there was even greater variation in the number of sex comb teeth than was found in hybrids. A few backcross males had sex combs consisting of only four teeth, and individuals with 5 or 6 teeth seemed relatively more frequent than among the hybrids (fig. 10C). In the cross involving athabasca vermilion, where it was possible to distinguish in the backcross those males bearing the athabasca X-chromosome, there

was a marked tendency for backcross males with the athabasca X to have sex combs with few teeth (fig. 10E), and the total sex comb teeth frequency distribution among backcross males of this combination seemed to be somewhat bimodal on this account (fig. 10D). It is suggested, thus, that the D. athabasca X-chromosome has an influence in reducing the number of sex comb teeth in backcross males, thus causing there to appear in the backcross (to algonquin) individuals with smaller sex combs than were found among the hybrids --where the males carried the algonquin X. An inspection of Table VIII shows that in the case where such individuals could be detected, males carrying the athabasca X-chromosome were less frequent than those having the algonquin X --24 to 52 (cn/v #21 times cn). It would seem from this that the D. athabasca X-chromosome has a deleterious effect in combination with algonquin cytoplasm and chromosomes. However, a possible unfavorable influence of the vermilion gene in this case must not be discounted. Dobzhansky (1937) has shown that certain D. pseudoobscura mutants of ordinarily good viability have semi-lethal effects in backcross individuals of race hybrids. It may well be that reduction of sex comb teeth number among certain backcross males is symptomatic of general decrease of viability. At any rate, a better knowledge of the influence of "physical condition" on sex comb teeth number needs to be gotten before the genetic importance of data such as these can be evaluated properly.

The affinis/athabasca hybrid

As was the case between D. algonquin and D. athabasca a number of crosses between wild type D. affinis females and D. athabasca males yielded offspring. To investigate the possibi-

Table IX. Hybrids derived from the cross D. affinis ♀♀ × D. athabasca ♂♂

cross			progeny			
affinis		athabasca	males		females	totals
cut veinlet	×	Sundance, Wy.	1 ct vnlt*		2	3
white	×	Sundance, Wy.			1	1
pinkish, tiny bristle	×	Sundance, Wy.			1	1
rugose	×	vermilion	1			1
net	×	Sundance, Wy.		1 gynandromorph		1
rugose	×	vermilion	1		1	2
			3	1	5	9

* sex-linked in affinis

lity that this combination occasionally forms hybrids further interspecific matings were made using mutant D. affinis females. These crosses have given wild type offspring --though in very small numbers-- indicating the existence of affinis/athabasca hybrids (Table IX).

An investigation of interspecific insemination between D. affinis and D. athabasca has been made. The method employed in this experiment was the same as that described above for the algonquin/athabasca interspecific insemination study. The results are presented in Table X. In view of the rareness of species hybrids in this instance it is surprising that interspecific insemination was found to be so frequent, at any rate in the cross D. affinis females times D. athabasca males. A further discussion of this phenomenon is presented below in the next section. Hybrids have not been gotten at all from the cross D. athabasca females times D. affinis males.

The affinis/athabasca hybrids seem to be quite viable, although they have been obtained only in small numbers. These hybrids appear to be more darkly pigmented than D. affinis, which is a lighter form than D. athabasca. Neither sex has been demonstrated to be fertile. The ovaries of the females, however, appear to be quite normal, and eggs are deposited. An examination of the testes of one of the males has been made in an aceto-carmine smear. No spermatozoa were found. A few spermatogonia-like cells were seen. In one region of the smear there were found a few large cells of the size of spermatocytes. In oneof them there occurred an irregular array of rather distinct chromosomes, apparently more than the diploid number, though it was not possible to count them accurately (fig. 11C). However, the most striking feature of these testes was the presence of a num-

Table X. Interspecific insemination, between D. affinis and D. athabasca.

D. affinis ♀ × D. athabasca ♂		D. athabasca ♀ × D. affinis ♂	
rugose × vermilion	56/82	vermilion × rugose	0/102
rugose × cinnabar*	34/54	cinnabar* × rugose	0/24
rugose × Sund., Wy.	8/10	vermilion × Vict.B, Texas	1/15
	98/146	vermilion × Gtlnbg., Tenn.	11/19
	= 67.12%	vermilion × Balt.B, Md.	2/19
			14/179
			= 7.82%

* mahican

ber of very large round bodies containing irregular, vacuolated chromatin masses quite similar to those found in the "spermatocytes" of the algonquin/athabasca hybrids, as described above. Most of these giant structures were apparently broken in the process of smearing and the "nuclei" from them scattered about, but one such body was found more or less intact (fig. 11D). This was found to measure about 100 micra in diameter and to contain 37 chromatin masses. Prof. Th. Dobzhansky informs me that similar giant structures are to be found in the testes of athabasca/azteca hybrids. This phenomenon might be compared as well to the abnormal multiplication of nuclei taking place in the "spermatocytes" of the D. pseudoobscura race hybrids of the cross strong B females times strong A males (Dobzhansky, 1934).

The affinis/athabasca isolation mechanism

The data of Table X show that insemination takes place between Drosophila affinis and D. athabasca in both directions. Between affinis rugose females and athabasca males mating was rather frequent in the ten day period allowed --taking place in more than two-thirds of the cases. Yet hybrids between these species have been but rarely produced, and then in only small numbers (Table IX). Several explanations of this apparent failure of inseminated females to produce offspring may be offered: (1) egg-laying of these females is for some reason inhibited, (2) mortality of hybrids is very high at early stages --in the embryonic period or in early larvae, (3) spermatozoa of one species are inactivated to the extent that they usually cannot effect fertilization of the eggs of the other species. The latter alternative may be divided into two parts: (a) inactivation of

sperms takes place in the genital tract of the female --after they arrive in the seminal receptacles-- or (b) the sperms are able to enter the eggs but inactivation generally takes place soon after penetration so that embryonic development isn't initiated.

A number of D. affinis females that had been kept with D. athabasca males for ten days were isolated individually into vials containing fly food. After three days these females were dissected and it was determined which of them had been inseminated. From the food of females found to be inseminated it was possible to collect a number of eggs --130 altogether. None of the eggs had hatched. The eggs were kept two days longer and observed again. Still none of the eggs had hatched, and microscopic examination of some of them after the chorion had been removed showed disintegration to be taking place --aggregation of the yolk into large globular masses and concentration of the opaque material of the egg towards the center, leaving the ends relatively transparent. It was concluded from this that interspecifically inseminated females of this sort do lay eggs, but that the eggs in general do not hatch.

It was next desired to know the extent of embryonic development in these eggs. After a suggestion of Dr. D. F. Poulson it was decided to stain eggs of interspecifically inseminated females with fuchsin-sulfurous acid according to the Feulgen technique and examine them whole for embryonic structures. D. affinis rugose females that had been kept with D. athabasca vermilion males for ten days were isolated and allowed to lay eggs over a three hour period at 25 degrees. At the end of this time eggs laid by females determined to have been inseminated were fixed in formol-alcohol-acetic acid (5:15:1), the chorions hav-

ing been removed and the vitelline membranes punctured with a very fine needle after the manner of Poulson (1937). As a control eggs laid by D. affinis rugose females known to have been inseminated by males of their own strain were prepared in the same way. According to a schedule suggested by Dr. Poulson both kinds of eggs were stained and mounted whole for examination. The eggs laid by the affinis females inseminated by males of their own species were found to contain many nuclei. In most of these the blastoderm stage had been reached --that is, in these cases the eggsurface was lined by many nuclei between which cell membranes had formed or were forming, and in the interior a number of nuclei were suspended in the yolk in protoplasmic islands, as described in the case of the D. melanogaster embryo by Poulson (1937). In the eggs of the interspecifically inseminated females, however, no nuclei were seen at all. Although eggs obviously spoiling had been rejected before fixation, some of these eggs seemed to show the beginnings of disintegration. Moreover, barring disintegration, it should have been possible to find in these eggs at least the oocyte nucleus, even though fertilization might not have taken place. It was concluded that these eggs had probably been laid towards the beginning of the egg laying period and, since development in them had proceeded either not at all or only a little, they had had time to spoil.

Eggs laid by D. affinis rugose females inseminated by D. athabasca vermilion males were next collected and fixed within an hour after laying. Since there seemed to be the possibility that these eggs were being laid without fertilization, it was planned to pay particular attention to the region in which maturation is supposed to take place in Drosophila. Huettner (1924) has described maturation in the Drosophila melanogaster egg.

According to him the oocyte nucleus comes to lie in a relatively clear protoplasmic region at the dorsal surface of the egg about a third of the length of the egg from the micropylar end. In eggs ready for fertilization this nucleus has advanced to the first metaphase of meiosis, and presumably the remainder of the maturation process does not take place unless stimulated to do so by the act of fertilization. Thus, unfertilized Drosophila eggs might be expected to contain the first maturation spindle with the chromosomes at metaphase and no other nuclear structures. An examination of the eggs of the interspecifically inseminated D. affinis females showed there to be a number of kinds of configurations in the region of maturation, though no sperms nor evidence of cleavage were seen in these eggs. Although a spindle with chromosomes more or less at metaphase was seen in one instance, there were found cases in which there were two, and even three, nuclei or groups of chromosomes. The number of chromosomes in these groups could not be determined with certainty, but it seemed to be variable. It appeared from this that the eggs of interspecifically inseminated females were not fertilized, but that somehow maturation had proceeded beyond the first metaphase. It was realized, however, that sperm heads, being very small, could easily have been overlooked in these eggs.

A number of eggs laid by D. affinis rugose virgin females were gotten during the first hour after laying, and preparations of these eggs were examined. What appeared to be the same sorts of phenomena found in the "interspecific" eggs described above were observed here --irregular groups of chromosomes and nuclei. In some instances two spindles were observed --in one of these one of the spindles was tripolar. It was concluded that in D. affinis eggs maturation proceeds without the influence of ferti-

lization, though probably attended with some abnormalities. On the basis of these observations eggs laid by D. affinis females inseminated by D. athabasca males have seemed to be not essentially different from eggs known to be unfertilized. In figure 12A is presented a drawing of the region of maturation in an egg laid by a virgin D. affinis female, showing two spindles. In 12B is shown a comparable region in an egg of an athabasca inseminated affinis female, containing three irregular bodies. The orientation of these structures in these eggs has probably been altered somewhat in the process of mounting the eggs.

Of the explanations offered above for the phenomenon of failure of interspecific insemination to result in offspring the third seems to be the most reasonable --that the D. athabasca sperms are inactivated. Some evidence seems to favor the suggestion that the sperms are inactivated in the seminal receptacles of the D. affinis females rather than within the eggs. Observations of D. athabasca sperms in D. affinis seminal receptacles have suggested a sort of inhibition of these sperms. In determining whether or not a Drosophila female has been inseminated the vagina and associated structures are dissected out in fly Ringer's Solution and the preparation examined microscopically as quickly as possible, the presence or absence of sperms in the receptacles being observed directly. If the insemination has been intraspecific, the ventral receptacle is usually found to contain a number of rapidly moving sperms, although the mobility of these sperms is subject to some variation. In cases where D. affinis females have been inseminated by D. athabasca males the impression has been gotten that there is less activity of sperms in the receptacle than is characteristic

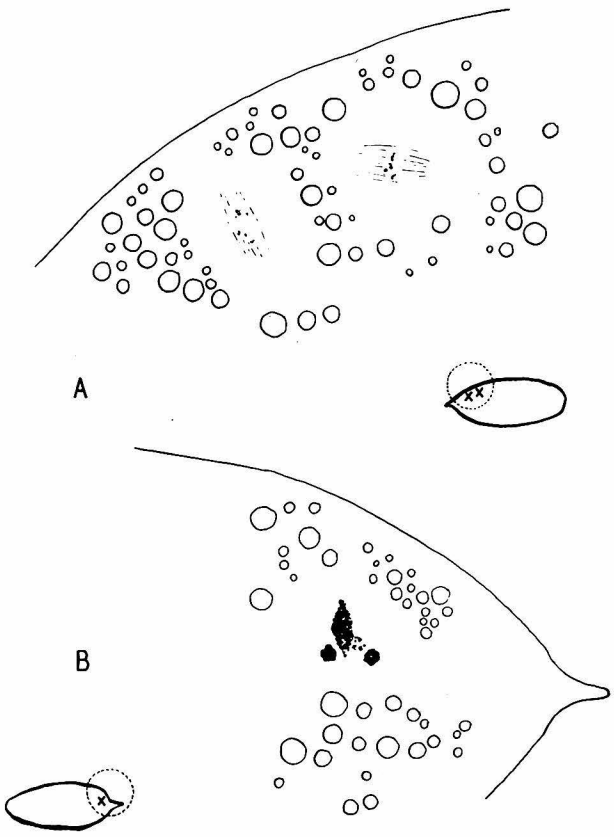


Figure 12. Structures found in D. affinis eggs stained with fuchsin-sulfurous acid (whole mounts). A. Egg deposited by a virgin female, showing two spindles in the region of maturation. B. Egg laid by a female inseminated by a D. athabasca male, showing three darkly staining bodies in the maturation region, probably the products of meiotic divisions. The small figures show the positions of the objects with respect to the whole eggs.

when the insemination has been intraspecific. Occasionally the sperms appear to be quite motionless. That the inactivation of D. athabasca sperm in the genital tract of D. affinis females is not always complete would have to be conceded on the basis of the fact that some interspecific hybrids have been produced. The inhibition of spermatozoa of one species in the genital tract of another species has been reported in birds and in mammals, although not, so far as the writer is aware, in insects. Serebrovsky (1935) has reported the inactivation of sperm of the domestic fowl and of the goose in the genital tract of the duck. Yochem (1929) observed that in the case of the rat/guinea pig combination the inactivation and removal of sperms was much more rapid after an insemination that was interspecific than after an intraspecific one.

Dobzhansky (1937) has discussed the various means by which the free interbreeding of separate forms in nature appears to be prevented. To these phenomena has been applied the general term "isolating mechanisms". Examples of such mechanisms are: geographical isolation and ecological isolation, in either of which circumstances hybridization between two forms is prevented due to the fact that individuals of the different kinds do not meet; sexual isolation, which depends on the "disinclination" of individuals of separate kinds to interbreed; mechanical isolation, which consists of a block to interspecific mating due to incongruence of the genitalia of the two species; inviability of hybrids; and hybrid sterility. Between D. affinis and D. athabasca sexual isolation is incomplete; especially between affinis females and athabasca males mating has been found to take place with an appreciable frequency. However, hybridization --the production of adult offspring from this cross-- takes

place but rarely under these conditions. The reason for this phenomenan seems to lie in an inactivation of D. athabasca sperms to the extent that fertilization of D. affinis eggs is generally not effected. Thus, in the case of these two species a further isolating mechanism --which might be termed "physiological"-- appears to operate to prevent the production of hybrids even when interspecific insemination becomes possible.

SUMMARY

A study has been made of the structure and variation of the salivary gland chromosomes of Drosophila algonquin. Descriptions and drawings of these chromosomes are included. The chromosomes of this species are compared with those of other species of Drosophila. A direct comparison of the chromosomes of D. algonquin with those of D. athabasca has been possible in hybrids.

Descriptions are given of the mutants found in D. algonquin. Association of these mutants into linkage groups has been determined, and a tentative allocation of these linkage groups to the chromosomes has been made.

An account of the hybrid between D. algonquin and D. athabasca is presented. Hybrid males are sterile, and a brief note on spermatogenesis in these males is included. Hybrid females are fertile and have been backcrossed successfully to D. algonquin. An account of hybridization between D. affinis and D. athabasca is given, including notes on spermatogenesis in this hybrid. An investigation has been made of the failure to produce offspring of D. affinis females inseminated by D. athabasca males. Since the eggs laid by these females seem not to be fertilized, the reason for lack of hybridization has been attributed to inactivation of sperms, constituting a "physiological" isolation mechanism between these species.

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