GENETICAL AND CYTOLOGICAL STUDIES OF A DEFICIENCY (NOTOPLEURAL) IN THE SECOND CHROMOSOME OF DROSOPHILA MELANOGASTER

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THE conclusion that two dominant venation mutants (Plexate and Plexate[2]) and two minutes (Minute-1 and Minute-4) are due to losses from the gene-string—that is, to deficiency—was drawn several years ago on the basis of genetic data and was later confirmed by examination of the salivary chromosomes. Several other genetic deficiencies, notably the Notches, have been similarly located through salivary analysis, by PAINTER, MACKENSEN, MULLER, PROKOFYEVA, DEMEREC and other workers. All of the dominants, especially those which are lethal when homozygous, require checking for chromosomal rearrangements, such as deficiency, duplication, translocation and inversion. An important goal in this work is the establishment of close correspondences between the loci on the genetic maps, deduced from linkage studies, and the particular chromosome localities, deduced from study of the transverse bands which form a diversified series along the salivary chromosomes.

ORIGIN AND CHARACTERISTICS OF NOTOPLEURAL

In the balanced stock of Stubble/*C3,l3a* it was observed by SKOOG (Feb. 20, 1933) that a small percentage of both males and females had shorter, wider, blunter wings, with venation irregularly thickened or branched in a few places. In some individuals there was a break in the posterior crossvein. A Stubble male showing this spontaneous mutant character was outcrossed to Curly (Exp. 610). In F_1 the mutant wing type appeared in about half the progeny of both sexes, hence is an autosomal dominant. Upon inbreeding the F_1 ("Mutant"/Cy; $Sb/3^+$) flies, it was found that all F_2 flies showed both Curly and the "Mutant"; hence "Mutant" has its locus in Chromosome II, and is completely lethal when homozygous. A balanced stock of "Mutant"/Cy, freed of Stubble, was obtained in F_3 and kept. When the "Mutant" was thus separated from Stubble it was found to have itself somewhat shortened bristles, especially the humerals, notopleurals and pretarsals (see fig. 1, hetero-

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zygous female). The shortened bristles proved more reliable for classification than the venation and other wing characters; hence the "Mutant" was renamed Notopleural, with symbol *Np*. There are several other alterations characteristic of the Notopleural type, of which the most obvious is the straggly arrangement of hairs on the thorax. All the Notopleural characteristics are somewhat more extreme in females, and at tempera-

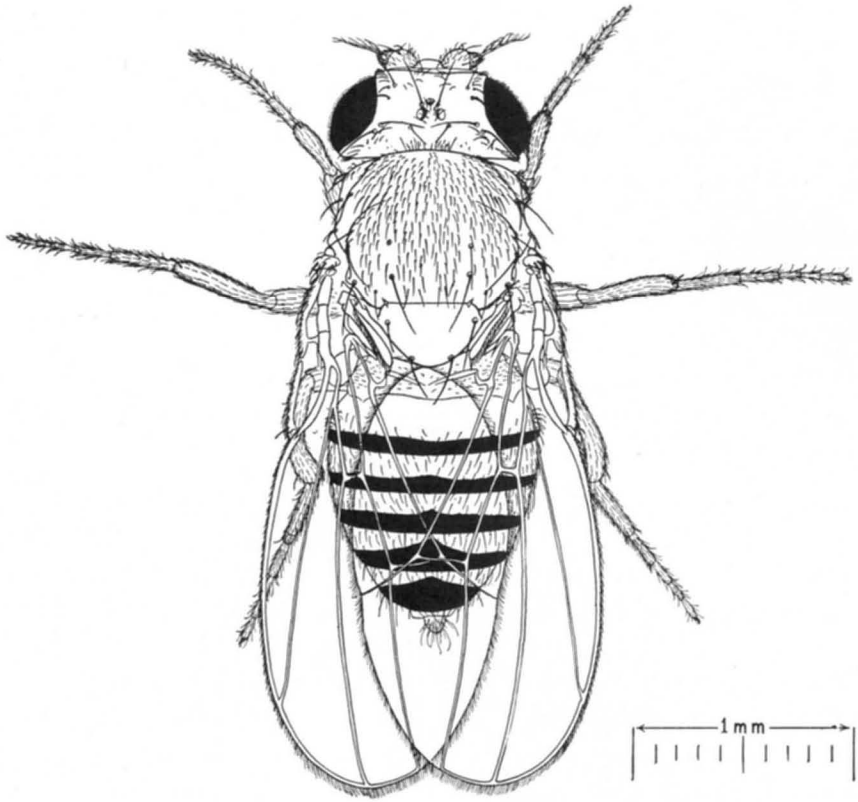

FIGURE 1. Notopleural female heterozygote. (E. M. Wallace, Del.)

tures above 25°C. A majority of *Np* females are completely sterile, while the fertile ones give progenies reduced to half or even to a small fraction of the normal number. The males are fertile and productive. Viability of *Np* is erratic, from practically normal to half-normal. Emergence is delayed somewhat by slow development, but the resulting flies are large and strong in appearance. (In relative valuation the mutant is classed in Rank-4.)

LINKAGE RELATIONS AND LOCUS OF NOTOPLEURAL

Female testcrosses of Notopleural with brown (104.5) and speck (107.0)

gave free recombinations, showing that the locus of *Np* is far to the left of those of *bw* and *sp* (Exp. 610A; 3 fertile, 25 sterile cultures; SKOOG; 117–19; June 7, 1933):

Np	*bw sp*	*Np bw sp*	+	*Np sp*	*bw*	n	*Np-bw R*	*bw-sp R*
105	119	57	66	2	3	352	35.0	1.4%

Next, it was found that the *Np* locus is to the right of Sternopleural (22.0) and of black (48.5) (Exp. 610B; 6 fertile cultures; BRIDGES; 20,370–76; July 19, 1933):

Sp b	*Np*	*Sp Np*	*b*	*Sp Np b*	+	n	*Sp-b R*	*b-Np R*
40	33	9	4	3	3	92	14.1%	6.5%

Further *b* +/+ *Np* linkage data were obtained (Exp. 610C; 3 fertile cultures; BRIDGES; 20,383–85; Aug. 1, 1933):

b	*Np*	*b Np*	+	n	*b-Np R*
106	89	7	12	214	8.9%

Testcrosses of the type *b Np* +/+ + L^2 (Exp. 610D; 14 fertile; BRIDGES; 20,440 ff; Aug. 29, 1933) gave:

b Np	L^2	*b* L^2	*Np*	*b Np* L^2	+	*b*	*Np* L^2	n	*R-1*	*R-2*
468	753	94	76	68	165	19	11	1654	12.1	15.9

From the three foregoing tests in which black and Notopleural were involved, 1986 flies gave 11.4 as the mean percentage of *b-Np* recombinations, with the locus of *Np* between *b* and L^2, at about 60 (48.5+11.5).

More precise localization, as well as more accurate determination of possible crossing over reduction, was made by use of the nearer loci purple (54.5) and engrailed (62.0) in female testcrosses with Notopleural (Exp. 610G; 10 fertile cultures, 3 ♀ ♀ each; SKOOG; 216 ff; Oct. 5, 1933):

pr en	*Np*	*pr Np*	*en*	*pr*	*Np en*	+	n	*R-1*	*R-2*
392	342	13	31	9	2	1	790	5.7%	1.5%

From this testcross the *pr-en* crossing over was 7.2, as compared with the standard 7.5; evidently no marked reduction of crossing over was produced by the Notopleural mutation. The locus of Notopleural was indicated as 1.5 to the left of engrailed (62.0), hence at 60.5, in agreement with the position as deduced from the *b-Np* data.

At this stage cytological examination of the salivary chromosomes (see below) confirmed the hypothesis that Notopleural is due to a deficiency. Since the deficiency is nearly 5 per cent of the length of the right arm of chromosome II, a local reduction in crossing over is expected, hence more precise tests of the linkages were carried out by LI to check this relation.

There are two good characters with loci between purple and engrailed,

namely, cinnabar eye-color (57.8) and bloated wing (59.0). The character bloated (found by P. T. IVES, June 26, 1933), although fairly good (RK2), has been little utilized. For closer study two new stocks were required, namely, *cn en* and *blo en*. To secure *cn en*, *pr en* was crossed to *cn*, and the F_1+ ♀ ♀ backcrossed to *pr en* ♂ ♂ (Exp. 610J; 7 cultures; LI; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
758	851	103	140	1852	13.1%

Some of the not-*pr en* flies (140) were crossed together in mass cultures. In the next generation they produced *cn en* flies, which were bred together for the required stock.

Similarly, the female testcrosses of *pr*+*en*/+*blo*+ ♀ × *pr*+*en* ♂ ♂ gave *pr-en* recombination data and in due course a stock of *blo en* (Exp. 610K; 9 cultures; LI; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
1365	1511	125	148	3149	8.8%

The above two control experiments gave diverse values of 8.8 and 13.1 per cent of recombination for *pr-en*, as compared with standard 7.5. Also, great fluctuation occurred from culture to culture within both tests. Such high variability is rather characteristic of the central regions of chromosomes II and III, and makes comparisons of data of far less value than for other regions.

As a check on the normality of the *cn en* stock, and as a control against the anticipated results with Notopleural, a rather extensive testcross of *cn en*/Oregon-Roseburg (Ore-R) ♀ ♀ was carried out (Exp. 610L; 26 cultures; LI; Feb. 13, 1936):

cn en	+	*cn*	*en*	n	*cn-en R*
4445	4646	322	324	9737	6.6%

A similar control of *blo en*/Ore-R ♀ × *blo en* ♂ ♂ (Exp. 610M; 30 cultures; LI; March 1, 1936) gave:

blo en	+	*blo*	*en*	n	*blo-en R*
2772	4793	142	199	7906	4.3%

Finally, a linkage experiment involving *cn en* and Notopleural was carried out (Exp. 610N; 34 fertile cultures; LI; Feb. 14, 1936):

cn en	*Np*	*cn Np*	*en*	*cn*	*Np en*	n	*R-1*	*R-2*
2931	2298	16	23	110	63	5441	0.7%	3.2%

The similar *blo*+*en*/+ *Np*+ testcrosses (Exp. 610P; 36 fertile cultures; LI; March 1, 1936) gave:

blo en	*Np*	*blo Np*	*en*	*blo*	*Np en*	n	*R-1*	*R-2*
450	6191	0	0	10	19	1098	0.0%	2.6%

These two experiments (*cn en/Np* and *blo en/Np*) and their controls (*cn en*/Ore-R and *blo en*/Ore-R) show that there is very probably a reduction of crossing over due to the deficiency. There are two measures of the amount of this reduction, namely: $6.6-3.9=2.7$ and $4.3-2.6=1.7$. Supplementary to these, we might give the calculations $4.3-2.9=1.4$ (where 2.9 is the mean value for the three experiments giving *Np-en* data) and $10.3-7.2=3.1$ (where 10.3 is the mean from the two *pr-en* controls and 7.2 is SKOOG's value for *pr-en* as modified by *Np*). The weighted mean from these four measures is about 2.0. But since all the experiments on which this value is based gave approximately 30 per cent more crossing over than expected from the standard map, a value of 1.5 would be more comparable with standard results as the amount of reduction due to Notopleural. Since the genetic length of the right limb of Chromosome II is about 52 units, and the salivary deficiency includes 4.4 percent of the right limb (see below), we might expect that the deficiency would make about 2.3 units reduction, if crossing over is equally distributed. But the general correlation of the linkage maps with the salivary maps shows that crossing over is only about half as free in this particular region as in the right limb as a whole. Hence, only about 1.2 would be the reduction expected, in agreement with the value 1.5 deduced from the experiments. While crossing over is probably entirely eliminated from within the deficient section, it may also be reduced somewhat in the immediate vicinity.

The locus of Notopleural cannot be definitely ascertained by linkage if there is crossing over reduction in the vicinity. If the 1.5 represents the length of the deficient section, the location should be described as extending between two points. These might be approximated at 1.8 to the left of engrailed, or at 60.2, for the right end, and 1.5 further to the left, or at 58.7, for the left end.

CHECK OF NOTOPLEURAL DEFICIENCY FOR POSSIBLE INCLUDED LOCI

In the general neighborhood of Notopleural (58.7–60.2) ±, the standard map (D.I.S. 3) showed the following mutants: staroid (58 ±), bloated (59 ±), engrailed (62.0), upward (62.5 ±) chaetelle (63 ±), Baroid (65 ±), Xasta (68 ±) and Abnormal-wing (65 ± 5). The mutants Baroid, Xasta and Abnormal-wing are associated with translocations. Tests showed that all of these loci are outside the deficiency. The closest neighbor is bloated to the left, and this agrees with the observation that *blo en/Np* gave no *blo-Np* recombinations. A more probable position of *blo* is thus at about 58.5.

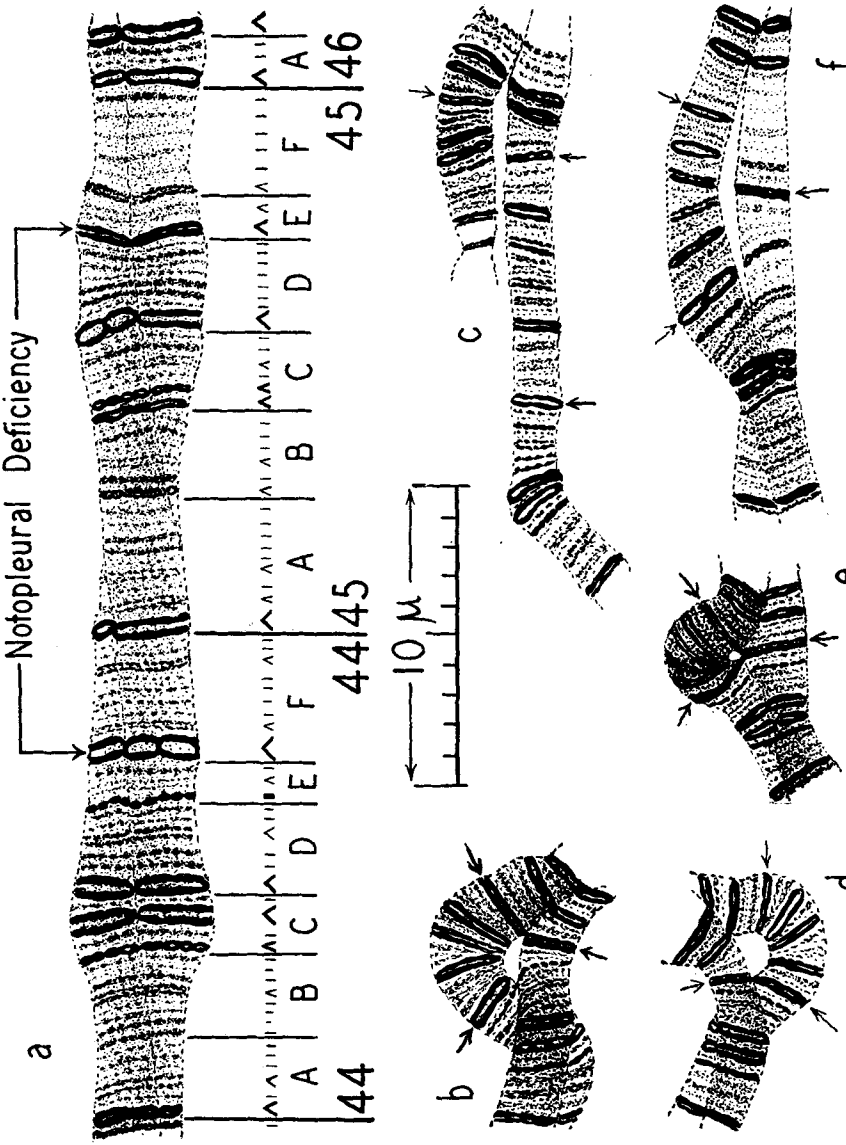

FIGURE 2. Salivary chromosomes of Notopleural heterozygotes. a. Revised normal map of sections 44 and 45 in 2R, with the Notopleural deficiency indicated. b. Synapsis at right limit. c. Non-synapsed strands, both lax. d. Synapsed at left limit. e. Synapsed at both limits. f. Well stretched, non-synapsed deficient strand. (C. B. Bridges, Del.)

SALIVARY ANALYSIS OF NOTOPLEURAL

The first confirmation of the hypothesis that Notopleural is a deficiency was made (Nov. 22, 1935) by LI, who observed a "buckle" and loop at about section 45 in the right limb of Chromosome II (BRIDGES 1935). For a more exact study, numerous permanent preparations of salivary chromosomes of Notopleural/Ore-R females were made. Studies of the normal morphology of sections 44 and 45 were carried out by BRIDGES on Oregon R and numerous other strains. By averaging the measurements and spacings of camera lucida drawings of the five most favorable stretched specimens, a revised map of this region was made (fig. 2a). Easy landmarks to the left of the deficiency are the three heavy doublets in 44 CD, while equally striking markers to the right are the two heavy doublets at the beginning of 46.

Study of the banding (fig. 2c, and especially 2f) showed that the Notopleural chromosome is normal through 44E and from 45E on. But at the rejunction is a doublet not exactly matching any band in the normal chromosome, being less heavy and less separated than the heavy doublet beginning 44F, and heavier than the close doublet beginning 45E. Study of the synapsis relations showed this anomalous band sometimes in synapsis with the 45E1 doublet (fig. 2b) but more frequently in synapsis with 44F1 doublet (fig. 2d) and occasionally with both at once (fig. 2e). Evidently both breaks of the deficiency split through the halves of doublets. The normal map shows 50 distinguishable transverse elements between the breaks of the Notopleural deficiency.

The interpretation to which we incline is that normal 44F1 doublet is a "repeat" (BRIDGES 1935) of two identical bands and that normal 45E1 doublet is similarly a case of identical twin bands. The new band ($\frac{1}{2}$ 44F1 doublet+$\frac{1}{2}$ 45E1 doublet) shows close union of the two parts, suggesting allelism. On this view the two normal doublets would be alleles, one derived from the other, or both modifications of a previous common ancestor.

It is suggested that "repeats" offer a generalized, and perhaps the most frequent, mechanism for further steps in rearrangement—either translocations, inversions or deficiencies—through a preliminary synapsis of homologous or allelic bands or sections, which are carried in separate chromosomes or localities, and subsequent crossing over to give the new configuration. Striking evidence for this general view will be presented in studies of certain other rearrangements.

SUMMARY

The dominant mutant "Notopleural" (symbol *Np*) was found as a spontaneous occurrence by ELEANOR NICHOLS SKOOG, February 20, 1933.

It is characterized by numerous slight departures from the wild type, especially by shortened notopleural, humeral and pretarsal bristles, by straggly microchaetae, by blunter wings with somewhat thickened and branched venation, by low production of eggs by *Np* females and by erratic mortality. The homozygote is completely lethal.

Linkage tests showed the locus to be in the right limb of Chromosome II, between the locus of bloated (58.5) and engrailed (62.0). Crossing over is locally reduced in the presence of *Np* by about 1.5 units. Since *Np* is a deficiency (see below) its limits on the normal map are about 58.7 to 60.2.

Salivary analysis shows a deficiency of 50 recognized bands. Both breaks are between the halves of doublets and the deficient chromosome shows a new doublet composed of the left half of 44E1 heavy doublet and the right half of 45E1 doublet which is slightly less heavy.

It is suggested that these two doublets are homologous, though one or both may have been altered by mutation from their common ancestor. The occurrence of such homologous "repeats" gives a reason for the exchange points or breaks of translocations, inversions and deficiencies coming at particular favored places.

LITERATURE CITED

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