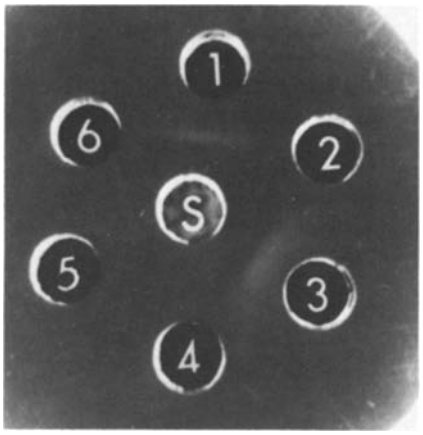

Fig. 3. Radial diffusion of lectins and trypsin-solubilized fibroin. See Table 1 for trypsin digestion procedure. Center well: trypsin digest supernatant. *1* Concanavalin A, *2 Pisum sativum, 3 Glycine max* (soybean), *4 Triticum vulgare* (wheat germ), *5 Arachis hypogaea* (peanut), *6 Tetragonolobulus purpureus*. All lectins were at a concentration of 1 mg lectin/ml phosphate-buffered saline. The gel was 1% agarose made in 0.85% saline

the core fibers may also have been digested. However, Andersen's data are from the spider, *Araneus diadematis;* corresponding data are not yet available for *Argiope*.

Samples of trypsin-solubilized fibroin were further analyzed by sodium dodecylsulfate (SDS)–polacrylamide electrophoresis according to [5]. Gels were stained with periodic acid-Schiff (PAS) or Coomassie brilliant blue according to [6]. A number of protein fragments were produced by trypsinization (Fig. 2) and these were separated by electrophoresis whereas the carbohydrate moiety remained in the stacking gel.

Samples of trypsin-solubilized fibroin were also studied by radial diffusion in agar gel (Fig. 3). By the method employed the carbohydrate moiety was seen to precipitate with Concanavalin A and the soybean lectin but not with other lectins studied. These data suggest the presence of galactosamine as well as glucopyranosyl or mannopyranosyl residues in the trypic digests of the web. The presence of the former was confirmed by automatic amino acid analysis where a ninhyrin-reactive peak appeared between leucine and phenylalanine. Both the form of the peak and its elution time were coincident with commercial galactosamine. Moreover, ninhyrin-degradation techniques [7] also revealed the presence of galactosamine [2].

We cannot account for the precipitation of web glycoproteins with Concanavalin A nor is it clear why the pea lectin, with similar carbohydrate affinities did not form a precipitate. Numerous assays using the Anthrone reagent [7] to detect neutral sugars were uniformly low in optical density, suggesting that neutral sugars are in very low quantities. This is probably the reason why carbohydrates were not reported earlier in analysis of webs [8].

All enzymes and lectins were products of Sigma Chem. Co. (St. Louis, MO). We have observed possible differences in lots of trypsin as regards digestion of the sticky spiral core fibers over the several years of our studies. The trypsin used in these experiments was product No. T-0134 lots 128C-0352, 59C-0012 and 92C-1550.

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Karyotypic Orthoselection in *Drosophila*

H.A. Ranganath and K. Hägele

Lehrstuhl für Genetik der Ruhr-Universität, D-4630 Bochum

In groups of related organisms frequently changes in the chromosome configuration have occurred during their phylogenetic history. Most of these changes can be attributed to translocations, inversions, centric fusions, fissions and alterations in the size of heterochromatic segments. In a number of evolutionary lineages there is a tendency for similar structural changes to establish themselves in one chromosome member of the karyotype after another. Frequently, this leads to a situation in which all the chromosome configurations are alike. Such a phenomenon is referred to as 'karyotypic orthoselection' [1].

In the present communication the different stages in the karyotypic evolution of the members of the *nasuta* subgroup of *Drosophila* are presented. Further, it is demonstrated that centric fusion has occurred repeatedly in the evolutionary history of one of its members, namely *D. nasuta albomicana*. Therefore it is concluded that the karyotype of *D. n. albomicana* is a product of 'karyotypic orthoselection'. To our knowledge, this is the first report of its type in *Drosophila*.

The *nasuta* subgroup of the *immigrans* species group of *Drosophila* is a cluster of morphologically almost identical forms. The members are: *D. nasuta nasuta, D. n. albomicana, D. n. kepulauana, D. kohkoa, D. pallidifrons, D. nixifrons, D. sulfurigaster sulfurigaster, D. s. bilimbata, D. s. albostrigata, D. s. neonasuta* and *D. pulaua* [2, 3].

In this subgroup three numerically different karyotypes are seen. *D. nixifrons* has $2n=10$ with one pair of metacentrics (chromosome 2), two pairs of acrocentrics (chromosomes 3 and 4), one pair of sex chromosomes and one pair of dots. The other forms with the exception of *D. n. albomicana* have $2n=8$ with one pair of metacentrics (chromosome 2), one pair of acrocentrics (chromosome 3), one pair of sex chromosomes and one pair of dots. *D. n. albomicana* has $2n=6$ with two pairs of metacentrics–one of them is chromosome 2 while in the other both the chromosomes 3 and the X or Y chromosomes are fused together–and one pair of long dots [2, 3].

The schematic representation of our considerations as to the stages in the karyotypic evolution within the *nasuta* subgroup is presented in Fig. 1. The primitive karyotypic constitution of *Drosophila* has five pairs of acrocentrics and one pair of dots, $2n=12$ (A to F). In the available members

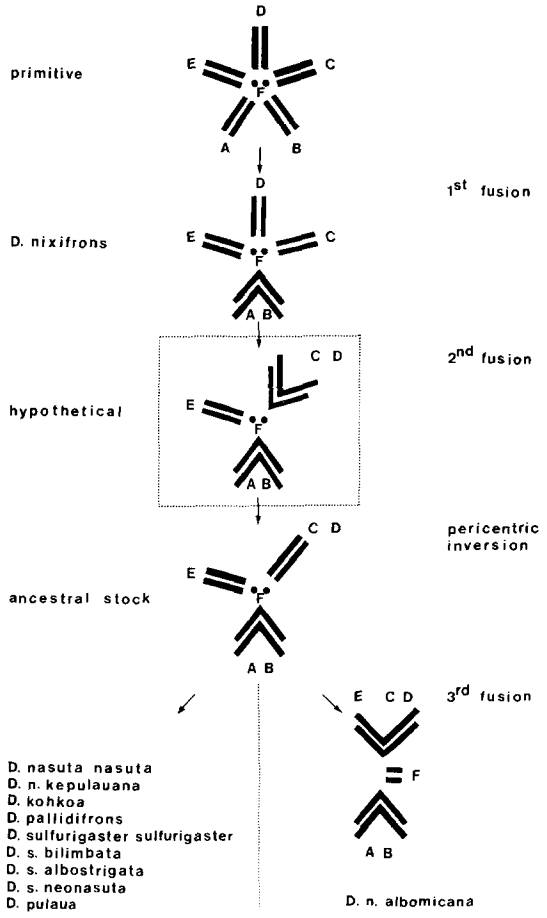

Fig. 1. In the ancestral stock: AB=chromosome 2; CD=chromosome 3; E=sex chromosomes; F=dot chromosomes. Chromosome 3 as double length rod is almost double the length of other acrocentrics in salivary gland chromosomes; in the highly condensed metaphase state it appears to be comparatively shorter

of the *nasuta* subgroup the homology of the karyotypic elements A to F has been confirmed by way of inter-racial and inter-specific hybridization experiments wherever crosses were successful as well as by comparison of the banding pattern in the polytene chromosomes [2, 6].

One centric fusion between A and B produced a metacentric which is represented as chromosome 2 in all the present-day forms of the *nasuta* subgroup. By this first change, the diploid number was reduced to $2n=10$. *D. nixifrons* can be taken as a representative of this stage in the karyotypic evolution of the group. Then, a further centric fusion between two other primitive elements (C and D) should have yielded a second pair of metacentrics to make $2n=8$. So far, this transitional stage with 'CD' metacentric has not been recorded in the members of the *nasuta* subgroup. Even though this karyotypic configuration forms a 'hypothetical stage' in the evolution of the *nasuta* subgroup, this is not uncommon in other groups of the genus *Drosophila* [4]. The 'CD' metacentric is supposed to have subsequently undergone a pericentric inversion to produce an acrocentric and this is seen as chromosome 3 in the present-day forms of the *nasuta* subgroup with the exception of *D. n. albomicana*. This acrocentric chromosome 3 is referred to as a 'double length rod' [2, 5] because it is derived from two of the six primitive elements. With these changes the diploid number remains $2n=8$ but the karyotypic composition is one pair of metacentrics (chromosome 2), one pair of acrocentrics (chromosome 3), one pair of sex chromosomes and one pair of dots. From this ancestral stock other members of the *nasuta* subgroup can be derived:

(a) on the one hand, changes in the size of the dot chromosomes, changes in the configuration of the Y chromosome and some inversion differences have yielded *D. n. nasuta, D. n. kepulauana, D. kohkoa, D. pallidifrons, D. s. sulfurigaster, D. s. bilimbata, D. s. albostrigata, D. s. neonasuta* and *D. pulaua* [2, 6];

(b) on the other hand, *D. n. albomicana* has emerged from this ancestral stock due to another centric fusion between the double length rods (chromosome 3) and sex chromosomes to form a new metacentric (CDE). In addition to this, we have found that there is a massive addition of heterochromatin to the dot chromosomes of *D. n. albomicana* [7]. Thus, as mentioned above, the karyotype of *D. n. albomicana* has $2n=6$ with two pairs of metacentrics (chromosome 2 and chromosome 3+sex) and one pair of long dots. This new complex, 3+sex comprises three of the six primitive elements of the *Drosophila* karyotype. When *D. n. albomicana* arrived on the evolutionary scene there were three centric fusions and one pericentric inversion.

As shown above, the karyotypic organization of *D. n. albomicana* involving three repeated centric fusions during its evolutionary history is well within the observations of White [1]. In the light of these, we propose that the karyotype of *D. n. albomicana* is an evolutionary product of 'karyotypic orthoselection'.

D. n. nasuta ($2n=8$) and *D. n. albomicana* ($2n=6$) represent two different products of karyotypic evolution. Inspite of this they are totally cross-fertile. Therefore, they are treated as chromosomal races [3, 8].

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