

distinguished from their paternal but not their maternal species by their sex-comb teeth number. Hybrids between *pseudoananassae* and *bipectinata*, and *pseudoananassae* and *parabipectinata* are distinguishable from their paternal species in sex-comb teeth number and from their maternal species except when the female parent is *parabipectinata* (i.e. *par* female \times *psn* male). Reciprocal hybrids for these two crosses are also distinguishable and this is unlike any other reciprocal hybrids formed within the complex.

In the remaining cross, *pseudoananassae* by *malerkotliana*, the only significant difference was between the sex-comb teeth number of the hybrid and that for *pseudoananassae* irrespective of whether this was the paternal or the maternal species. Hybrids in this cross are therefore distinguishable from *pseudoananassae* but not from *malerkotliana*.

Male abdominal tergite coloration is reported for dead mature flies. Variability of living forms obscures group differences. The general body color is tan. Tergites 5 and 6 are shiny black in *parabipectinata* and dull black in *malerkotliana*. Tergites 3 and 4 are also dark, more extensively so in *malerkotliana* than in *parabipectinata*. Markings on the abdominal tergites of *bipectinata* and *pseudoananassae* consist of a brown band along the posterior border of each tergite. Bands of color are wider in *pseudoananassae* giving it a darker appearance than *bipectinata*.

Hybrids were darker than their lightest parent and lighter than their darkest parent. In most crosses reciprocal hybrids were different being closest in color to their maternal species. Reciprocal hybrids which were least different were those between *bipectinata* and *parabipectinata*, dull brown, and *malerkotliana* and *parabipectinata*, shiny black to dull black. Hybrids between *pseudoananassae* females and *bipectinata* males were *pseudoananassae* like. Color in the reciprocal hybrid, which occurs infrequently, was not recorded.

The presence of teeth on the 3rd tarsal segment is a reliable character for distinguishing *malerkotliana* from the other species in the complex. Of the different kinds of *malerkotliana* hybrids, only two (*mal* female \times *bip* male and *par* female \times *mal*

male) lacked this character. Some of each of the other kinds of hybrid possessed it.

In conclusion, sex-comb teeth number distinguishes the species under study. It also distinguishes eight of the twelve kinds of hybrids from their maternal species and eleven from their paternal species. The twelfth hybrid (*psn* female \times *mal* male) can be identified because its color is like the female parent but it has 3rd tarsal segment teeth like the male parent. The latter character and color are not useful in distinguishing the four remaining hybrids from their maternal species.

The pattern of inheritance of sex-comb teeth suggests a polygenic model with genes located on the autosomes. The evidence is that no kind of hybrid differed from the mid parental value. In two kinds of crosses (*psn* \times *bip* and *psn* \times *par*) reciprocal hybrids were different. Therefore these three species (*psn*, *bip* and *par*) may also carry genes for sex-comb teeth number on the X-chromosome. A study of backcrosses between female hybrids and their paternal species is in progress to test genetic hypotheses.

The functional significance of teeth number in sex-combs is unknown. Elimination of tapping by removing fore tarsi decreases the sexual isolation between *D. malerkotliana* and *D. bipectinata*³. Perhaps the sex combs are scent receptors and teeth number reflects evolutionary divergence in responsiveness to species specific scents.

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Spontaneous aneuploidy of chromosome 4 in *Drosophila kikkawai* in Thailand¹

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Summary. In *D. kikkawai* ($2n = 8$), chromosome 4 aneuploidies up to heptasomy, possibly connected with spontaneous nondisjunction, translocation and centric dissociation, have been observed in one isofemale line collected from Mae Hong Son, north-west Thailand. This wide range of numerical and structural variation persisted in the laboratory for many generations.

Key words. *Drosophila kikkawai*; chromosome 4; aneuploidy.

The eukaryotic genome is usually invariable in terms of chromosome number. The gain or loss of heterochromatic material in chromosome complements may occur in nature without causing severe phenotypic or genetic effects^{2,3}. Conversely, the increase of euchromatin in the genome, even a small portion, might be expected to produce marked genetic effects. Yet chromosomal nondisjunction producing aneuploidy is not an uncommon phenomenon in higher organisms^{4,5}.

We report here some interesting cases of spontaneous aneuploidy of chromosome 4 in *D. kikkawai* from Thailand. In earlier studies of this species no such chromosomal variation was noted⁶.

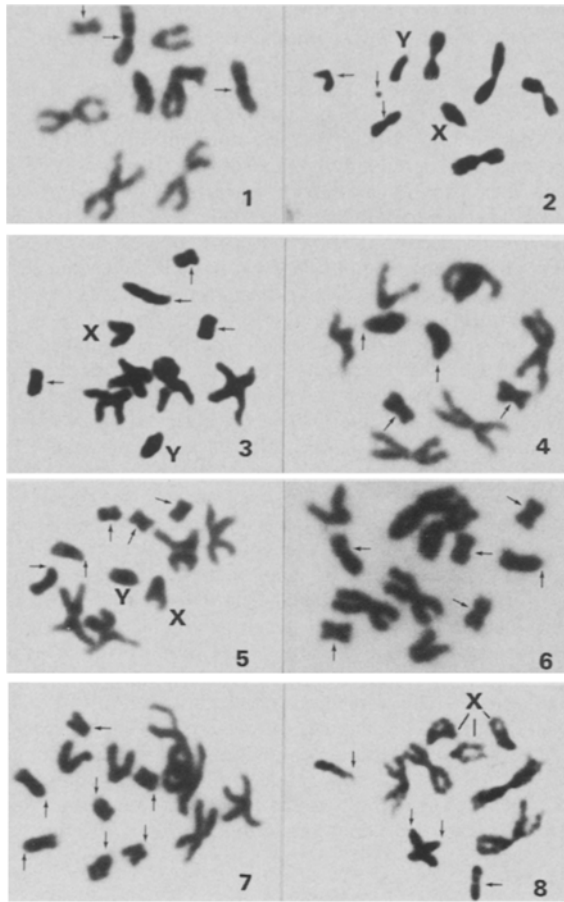
Materials and methods. F₁ larvae were cytologically examined from individual ovipositions of 42 females of *D. kikkawai* caught in Mae Hong Son, north-west Thailand. The larval mitotic chromosomes were prepared from brain ganglia using an air-dried Giemsa staining technique⁷. Three isofemale lines

showed variation in chromosome number with respect to microchromosome 4. One of the three families (A76-7) has been maintained in the laboratory as mass cultures as well as pair matings to follow the aneuploid condition in the culture stock until the 21st generation. Photomicrographs of metaphase chromosomes were made with Kodak High Technical film under oil immersion ($\times 670$) with a green filter.

Results and discussion. The normal metaphase karyotype of *D. kikkawai* is $2n = 8$. The 4th chromosome (microchromosome) varies in size and shape depending on the amount and distribution of major heterochromatic blocks, ranging from a small telocentric to a large metacentric configuration⁶. Natural populations of *D. kikkawai* in the Oriental region, including Thailand, generally exhibit three types of 4th chromosome; a large telocentric (Lt), a medium metacentric (Mm) and a large submetacentric chromosome (Lsm) (fig. 9). Of the 42 isofemale lines observed in this study three, including No. A76-7, showed

various conditions of aneuploidy, triplo-4 and tetra-4. Presumably such chromosomal aberrations occur spontaneously in natural populations. Only family No. A76-7 has been maintained in our laboratory for further observation on the transmission of the extra 4th chromosomes.

Interestingly enough, the progeny of the 3rd generation from one of the pair matings exhibited remarkable chromosome variation in the form of combinations of different types of chromosome 4 as trisomies up to a heptasomy (figs 1-8). The



Figures 1-8. Larval neuroblast metaphase chromosome of *D. kikkawai* showing the aneuploid condition: 1, trisomy (1Mm, 2Lsm); 2, trisomy (1D, 2Mm); 3, tetrasomy (1LLt, 3Mm); 4, tetrasomy (2Mm, 2Lt); 5, pentasomy (3Mm, 2Lt); 6, hexasomy (4Mm, 1Lt, 1Lsm); 7, heptasomy (4Mt, 3Lt); 8, tetrasomy for chromosome 4 (3Lt, 1Mm) and trisomy for chromosome X. The 4th chromosomes are indicated by arrows at centromeric positions. The male karyotype is shown by the presence of the X and Y chromosomes.



Figure 9. Diagrammatic representation of different 4th chromosome configurations involved in the aneuploid condition in *D. kikkawai*.

unique heptasomic larva manifested 3 medium and 4 small telocentric 4th chromosomes (fig. 7). The latter configuration was the rarest type of 4th chromosome so far encountered in this study. It is assumed that these 4th chromosomes arose spontaneously in this individual. However, the precise mechanism involved is still obscure. One possibility is that centric dissociations of two medium metacentric (Mm) 4th chromosomes could have occurred producing 4 medium telocentric chromosomes (Mt). Even though chromosomal change through centric dissociation is a rare phenomenon requiring special conditions⁵, it seems more probable than loss of heterochromatic blocks of the large telocentric chromosomes.

In addition, there was an interesting case of aneuploidy consisting of a tetrasomy for chromosome 4 and a trisomy for chromosome X (fig. 8). Further, two new figures of chromosome 4 were discovered among the progeny of the mass mating culture in the 17th generation. These were a small dot-like chromosome (D) and a very large telocentric (LLt) chromosome (figs 2 and 3, respectively). These two types of chromosome 4 were very rare. It is likely that they occurred in the laboratory population as a result of spontaneous translocation involving two telocentric 4th chromosomes. Thus, a long heterochromatic block of a telocentric chromosome could have been translocated to the distal end of another telocentric 4th chromosome transforming it into a very long telocentric form of approximately double the size of the original ones. The remaining centromere could then appear as a dot-like metaphase chromosome. As a general rule, translocated chromosomes produce reduced viability due to pairing problems in meiosis. Hence, they should be eliminated quickly from the population. This was indeed the case in the present study. These two types of chromosome 4 have not been recovered in the laboratory population since they were first detected in the 17th generation. Aneuploidy appears to have disappeared from this family by the 21st generation.

There were no major phenotypic effects observed in aneuploid individuals apart from the apparent reduction of body size. It has been demonstrated that triplo-4 and tetra-4 in *D. melanogaster* show reduction in viability^{8,9}. It may be noted that in *D. kikkawai*, at least to the 4th stage larvae, individuals could tolerate increased gene dosages in microchromosome 4 up to hexa- or heptasomy under laboratory condition. The tolerance of such a high degree of increase in gene dosage in chromosome 4 may be partially balanced by the correspondingly large amount of heterochromatin in the chromosome. This particularly striking variation in chromosome number is extremely rare in general. The results of this study may well reflect the fact that the observations were on laboratory populations, which are under selection pressures different from those in the wild. Whether such an aneuploid is restricted to just a few cases in the Mae Hong Son population or is widespread awaits further investigations.

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