Table II. Aniline hydroxylase in the nuclei, the nuclear membranes and the microsomes after induction with phenobarbital (PB), methylcholanthrene (MC) and pregenolone 16α-carbonitrile (PCN)

Fraction	Treatment C	PB	MC	PCN
	(nMoles *p*-aminophenol/min/mg protein)			
Nuclei	0	0	0	0
Nuclear membranes	0	0	0	0
Microsomes	0.52 ± 0.02	0.75 ± 0.06	0.76 ± 0.07	0.76 ± 0.06

Aniline hydroxylase was determined according to the method of SHENKMAN et al.[9]

zyme in the nuclear membranes a s compared to controls. Although the 3 substances are strong inducers of microsomal aniline hydroxylase, they did not induce this enzyme in either the nuclei or nuclear membranes. These results support the suggestion that the intracellular controls regulating the nuclear membrane enzymes upon action of methylcholanthrene differ from those which control and regulate the microsomal hydroxylase[8,9].

All the known inducers of aryl hydrocarbon hydroxylase have been divided into 2 distinct categories represented by phenobarbital and methylcholanthrene[3,6]. Although microsomes from pregnenolone 16α-carbonitrile- and phenobarbital-pretreated rats have properties in common[10], differences have been noted[3,4], thus raising the possibility of a new category of inducers. In this comparative study of the 3 inducers, we were interested to know whether the nuclear hydroxylases show different specificities with substrates such as benzo(a)pyrene and aniline. Our results concerning the induction of nuclear aryl hydrocarbon hydroxylase show that pregnenolone 16α-carbonitrile is similar to phenobarbital and different from methylcholanthrene. All 3 inducers enhanced aniline hydroxylase activity to the same extent in rat liver microsomes, but the enzyme was not induced in the nuclei or nuclear membranes, as shown in Table II[11].

Although the nuclear membranes from phenobarbital and pregnenolone 16α-carbonitrile-pretreated rats show common aryl hydrocarbon hydroxylase activity, this does not preclude the possible differences in the metabolite profile of a benzo(a)pyrene substrate when incubated with these nuclei. RASMUSSEN and WANG[12] recently demonstrated the dependence of the specific metabolism of benzo(a)pyrene on the inducer of hydroxylase activity. Further study of the induction of these enzymes in the nuclear membrane is thus of great importance in benzo(a)-pyrene carcinogenesis.

Résumé. L'aryl hydrocarbure hydroxylase nucléaire répond seulement au méthylcholanthrène tandis que le phénobarbital et la prégnénolone 16α-carbonitril ne l'affectent pas. Les trois substances n'induisent pas l'aniline hydroxylase nucléaire. Cependant elles sont des puissants inducteurs de l'aryl et l'aniline hydroxylase microsomiale.

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[8] D. W. NEBERT and H. V. GELBOIN, J. biol. Chem. *243*, 6242 (1968).
[9] J. B. SHENKMAN, H. REMMER and R. W. ESTABROOK, Molec. Pharmac. *3*, 113 (1967).
[10] R. E. TALCOTT and S. J. STOHS, Biochem. Pharmac. *23*, 1221 (1974).
[11] M. JACOBSON and R. KUNTZMAN, Steroids *13*, 327 (1969).
[12] R. E. RASMUSSEN and I. Y. WANG, Cancer Res. *34*, 2290 (1974).
[13] We thank Dr. H. GELBOIN for the 3-hydroxybenzo(a)pyrene, Dr. J. BABCOCK for pregnenolone 16α-carbonitrile, and Dr. M. BORNENS for his helpful discussions.

The Relationship Between Metaphase Heterochromatin and Polytene Inversions in *Drosophila*

It has long been a cytogenetic problem as to the circumstances under which an extra heterochromatin arises[1–3]. Recently, BAIMAI[4] has reported a one-to-one correlation between an extra heterochromatic segment and chromosome inversions in chromosome 4 of *D. disjuncta*. The present paper reports a further study of this important problem. *Drosophila formella* Hardy and Kaneshiro, a picture-winged species from the island of Hawaii, is a member of the *D. hawaiiensis* group. This species exhibits chromosomal polymorphism for inversion $4j^3/k^3$. In particular, the proximal break-point of inversion $4k^3$ has apparently occurred within the area of centromeric heterochromatin[5]. The present work provides additional evidence which supports the previous finding of a relationship between heterochromatin as determined by mitotic metaphase and a given chromosome inversion as seen in the polytene chromosome. Such parallel chromosomal changes can only be detected in material where both types of tissue may be simultaneously examined. It is hoped that the advanced hypothesis will, if confirmed, be of some genetic and evolutionary interest.

Materials and methods. In this study, use was made of the laboratory stock No. M87G1 which was derived from an individual wild-caught female collected from Puuwaawaa, Northwest slope of Hualalai, Hawaii (about 1290 meters altitude) by Prof. H. L. CARSON in December, 1969. The stock has been maintained in the laboratory at

[1] S. W. BROWN, Science *151*, 417 (1966).
[2] P. M. B. WALKER, Nature, Lond. *229*, 306 (1971).
[3] J. J. YUNIS and W. G. YASMINEH, Science *174*, 1200 (1971).
[4] V. BAIMAI, Can. J. Genet. Cytol. *17*, 15 (1975).
[5] F. E. CLAYTON, H. L. CARSON and J. E. SATO, Univ. Texas Publ. *7213*, 163 (1972).

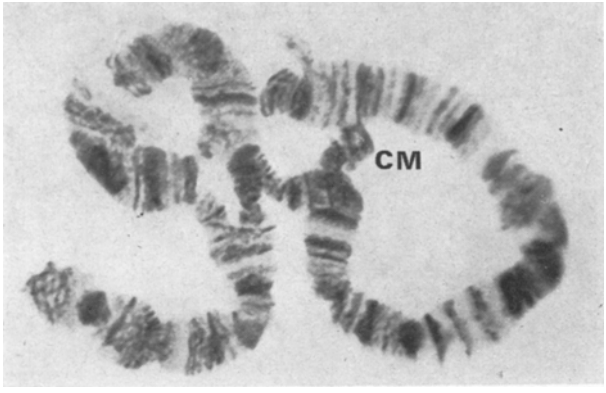

Fig. 1. Polytene elements of chromosome 4 of *D. formella* showing heterozygous condition for inversion $4j^3/k^3$ (×600). The proximal end of the polytene chromosome is indicated by CM.

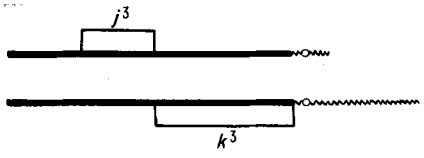

Fig. 2. A diagram of chromosome 4 showing the inversion j^3 (upper) and the inversion k^3 (lower) break-points, and centromeric heterochromatin (wavy lines).

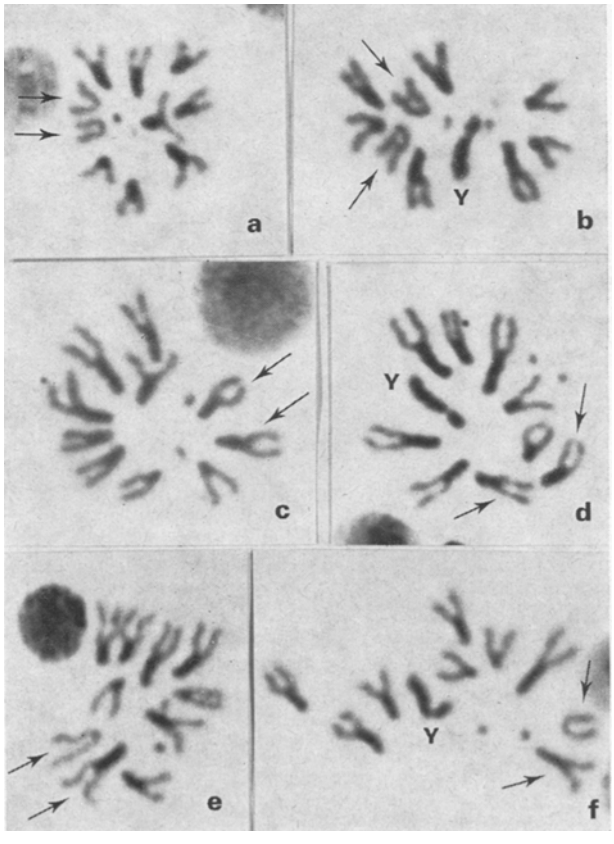

Fig. 3. Photomicrographs of larval ganglion metaphase chromosome complements of *D. formella*, ×600. The 4th chromosome pair is indicated by arrows in each figure. The metaphase chromosomes of larvae with $4j^3/j^3$ gene arrangements (a, b), with $4k^3/k^3$ (c, d), and with $4j^3/k^3$ (e, f). Females are shown on the left and males on the right.

approximately 18 °C. Each of a number of third instar larvae from this laboratory culture was examined for salivary gland chromosomes along with ganglion metaphase plate in the same manner as that previously described by BAIMAI[4].

Results and discussion. A total of 62 larvae from the same stock of *D. formella* were examined cytologically. The original isofemale had been inseminated in nature prior to capture and was found, by inference from F_1 larvae, to be heterozygous for inversions $4j^3/k^3$ (Figures 1 and 2). Homozygous ($4j^3/j^3$ and $4k^3/k^3$) and heterozygous ($4j^3/k^3$) conditions for these chromosome 4 gene arrangements have been observed in this study (Table). Furthermore, the homozygous state for the standard chromosome 4 gene order (+/+) has been encountered neither in natural populations[5] nor in the laboratory in this study. This is presumably due to the fact that the two inversions concerned are essentially in tandem. Recombination between these two separate sets of gene arrangements, therefore, seems unlikely or extremely rare.

Metaphase chromosome figures of *D. formella* contain unusually large amount of heterochromatin in autosomes as well as in the *X* chromosome (Figure 3). The *Y* chromosome is obviously a heterochromatic J-shape. The dot chromosomes are quite large. A detailed analysis of metaphase plates together with salivary chromosomes reveals a relationship between polytene chromosome 4 gene arrangements and 1 distinguishable pair of autosomes as determined by the ganglion metaphase figure. Thus a larva showing salivary chromosome $4j^3/j^3$ gene sequence possesses one unique pair of autosomes. These are clearly characterized by the absence of extra heterochromatic segments in comparison with other pairs of autosomes (Figure 3, a and b, arrows). On the other hand, a larva carrying the polytene homozygous state for inversion $4k^3/k^3$ displays the given autosome pair with distinct extra heterochromatin portions (Figure 3, c and d). However, a larva showing the heterozygous condition for inversion $4j^3/k^3$ always exhibits in metaphase plate only 1 autosome with very little centromeric as seen in the $4j^3/j^3$ gene arrangement; this exceptional chromosome is presumably a homologue of one of the autosomes containing marked centromeric heterochromatin (Figure 3, e and f). Such a clear-cut relationship is uniform in all cases examined (Table). These facts have thus led to the conclusion that the autosome with a relatively small amount of centromeric heterochromatin is the 4th chromosome which contains the j^3 gene order whereas the 4th chromosome exhibiting the k^3 gene sequence possesses an extra heterochromatic segment. It may be noted that the chromosome 4 showing the k^3 gene arrangement has the proximal break-point in the vicinity of centromeric heterochromatin (Figure 2). This observation is thus in agreement with the previous results in another Hawaiian *Drosophila* species, *D. disjuncta*[4]. The evidence from the present study, therefore, strongly supports the hypothesis that a chromosomal break occurring in the neighbourhood of centromeric heterochromatin seems, for some unknown reason, to have a profound effect in evoking the production of extra heterochromatin in this region. In view of the strong evidence that heterochromatic DNA as determined by satellite DNA in most organisms is species specific[6–8], centromeric heterochromatin may play a

[6] K. W. JONES, Nature, Lond. *225*, 912 (1970).
[7] M. L. PARDUE and J. G. GALL, Science *168*, 1356 (1970).
[8] J. A. MAZRIMAS and F. T. HATCH, Nature New Biol. *240*, 102 (1972).

Condition of polytene elements and metaphase plate figures of chromosome 4 of the same larvae of *D. formella*

Polytene gene sequence condition	Metaphase plate condition	No. of larvae	
		Female	Male
j^3/j^3	1 pair of autosomes with very little centromeric heterochromatin	6	5
k^3/k^3	The same autosome pair demonstrates large blocks of heterochromatin	4	5
j^3/k^3	A heteromorphic pair is present and only 1 chromosome shows little centrometic heterochromatin	22	20
Total		32	30

significant role in evolutionary diversity and species differentiation through chromosomal rearrangements by chromosomal breaks and refusions such as inversions and translocations. Thus the idea of the chromosome-break effect as evident in this and in the previous observations in Hawaiian *Drosophila* may shed some light on the problem of constitutive heterochromatin, and the phenomenon may be universal.

Zusammenfassung. Polytane Chromosomen und Metaphasen der Ganglionen von *D. formella*-Larven aus Hawaii wurden untersucht. Die Ergebnisse unterstützen das Konzept über die möglicherweise wichtigen zytologischen Wirkungen des Chromosomenbruches im Bereich des zentromeren Heterochromatins.

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Response of *Drosophila pavani*, *Drosophila gaucha* and their Hybrids to Larval Biotic Residues

Drosophila pavani is a neotropical species that occurs mainly in Central Chile, and its sibling species *D. gaucha* has been found in Southern Brazil, Uruguay, Argentina and Bolivia. The two species overlap their distributional area in a small zone near San Luis (Argentina). Crosses between the two species give an abundant hybrid offspring that is 100% sterile. In experiments on competitive ability of pre-adults of both species and their hybrids under crowding conditions in terms of food and space, it was found that the egg-to-adult survival of the hybrids is higher than that of the corresponding parental species when the pre-adults are reared alone. When these hybrids are bred together with the parental species, there is a significative decrease in their viability. On the other hand, *D. pavani* and *D. gaucha* increase their viability in the presence of the hybrids[1].

During the pre-adult stages, species or genotypes can be submitted to two aspects of competition: 1. Exploitation, the use by competing individuals of resources in short supply, mainly food and space, and 2. Interference, the harming of one or both species or genotypes living together, or the less efficient use of the resources as a result of coexistence[2,3]. A kind of interference observed in the *Drosophila* genus is the effect of the metabolic waste products over the larval viability[4,5]. For instance, recent experiments in which *D. pavani* were reared in culture media that had previously been used for the growth of larvae of the same species ('conditioned' medium[4-6]), revealed that *D. pavani* larval biotic residues inhibited the viability of its own as well as other species[6]. For the above reasons, the results observed when *D. pavani*, *D. gaucha*, and their hybrids are put together under competitive conditions, cannot be attributed entirely to food or space competition. During the course of investigations in which the 2 species and their hybrids were bred in an excess of food, it was observed that interference of the type described above also occurs.

In the experiments, groups of vials containing 10 cm^3 of basic *Drosophila* food media were sown each with 100 fertilized eggs of either *D. pavani*, *D. gaucha*, or from females of each one of the species inseminated by males of the other (hybrid eggs). The larvae were allowed to develop for 5 days at 25 °C, and then were killed by placing the vials in a temperature of −30 °C. After allowing the vials to thaw at room temperature ('conditioned' vials), they were sown each with 100 eggs of the same species previously used for conditioning the food media, or the other species or with hybrid eggs. The total number of eggs transferred in each group was of 1000. Series of vials were set up concurrently, containing fresh 'non-conditioned' culture media, and to which were sown independently an equal number of eggs of the parental species or hybrid eggs. All vials were placed in a constant temperature chamber at 25 °C and fresh yeast was added every 2 days in order to avoid food competition.

The Table indicated the total number of eggs sown, the number of emerging adults, and the mean values of viability per vial, with the corresponding standard errors. It is interesting to note that the metabolic waste products of *D. pavani*, *D. gaucha*, and their hybrids inhibit the

[1] D. Brncic and M. Budnik, Ecology *55*, 622 (1974).
[2] T. Park, Ecol. Monogr. *18*, 265 (1948).
[3] P. A. Parsons, *Behavioural and Ecological Genetics* (Clarendon Press, Oxford 1973).
[4] D. R. Weisbrot, Genetics *53*, 427 (1966).
[5] M. M. Dawood and M. W. Strickberger, Genetics *63*, 213 (1969).
[6] M. Budnik and D. Brncic, Ecology *56*, in press (1974).