Zoological Studies **37**(2): 75-88 (1998)

Zoological Studies

Review Article

Heterochromatin Accumulation and Karyotypic Evolution in Some Dipteran Insects

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CONTENTS

ABSTRACT

Visut Baimai (1998) Heterochromatin accumulation and karyotypic evolution in some dipteran insects. *Zoological Studies* **37**(2):75-88. Evolutionary divergence among eukaryotes always involves genetic changes at different levels of the genome. At the chromosomal level, heterochromatin differentiation resulting in karyotypic evolution provides a useful tool for cytotaxonomy of many groups of animals including the dipteran insects. In our studies, detectable differences in the amount and distribution of heterochromatin have been observed in several groups of closely related species and some sibling species complexes of *Drosophila, Anopheles*, and *Bactrocera*, for example, the *D. kikkawai* complex and the *montium* subgroup, the *An. dirus* complex and the *maculatus* group, and the *B. dorsalis* complex and the *Zeugodacus* group, respectively. Most cases, if not all, of our studies point to the fact that inter- and intraspecific differences in mitotic chromosomes are due to the acquisition of major block(s) of constitutive heterochromatin in the sex chromosome(s) and/or autosome(s), particularly at the pericentric region. Further, quantitative differences in heterochromatin of mitotic chromosomes can be successfully employed as genetic markers for separation of cryptic or isomorphic species in these groups of insects. Although the functional role and implications of heterochromatin in species differentiation is an unsolved problem, heterochromatin accumulation in the genome is clearly involved in genetic differentiation and karyotypic evolution of dipteran insects as demonstrated in the present study.

Key words: Constitutive heterochromatin, Mitotic chromosomes, Karyotypic evolution, Dipteran insects, Cytotaxonomy.

INTRODUCTION

The genetic constitution of a population may change through time and space, consequently leading to evolutionary divergence. It is also evident that the genetic constitution of a species varies to a greater or lesser degree from one population to another and from generation to generation. Therefore, different populations of a single species would be expected to be at different evolutionary stages of divergence mainly due to intrinsic genetic adaptation to their slightly different microenvironments and degrees of extrinsic isolation. Such genetic differentiation in different populations of a species may lead to speciation. Thus the process of speciation essentially involves a divergence of genetic constitution which must be sufficient to increase genetic incompatibility to various degrees between the newly evolved sibling species. These kinds of sibling species complexes are known in several groups of bisexually reproductive animals (Mayr 1963, White 1973), notably in insects (e.g., Carson and Kaneshiro 1976, Kitzmiller 1976).

The processes of genetic differentiation may occur at different levels of the genome during the course of evolutionary change. At the molecular level, it includes base substitution leading to changes in the unique DNA content. However, genetic changes that can be detected at the microscopic level include chromosomal rearrangements of gene sequences and heterochromatin differentiation resulting in quantitative changes in major blocks of repetitive DNA sequences. There have been numerous reports on inter- and intraspecific heterochromatin variation in relation to karyotypic evolution in both plants and animals (Pathak et al. 1973, Weimarck 1975, Imai et al. 1977, John 1981, Baverstock et al. 1982, Patton and Sherwood 1982). Furthermore, detectable differences in constitutive heterochromatin in mitotic chromosomes provide a useful criterion for separation of cryptic (isomorphic) species and of closely related species of some dipteran insects, as exemplified by the results of population cytogenetic studies of the Asian *Anopheles* (for a review, see Subbarao et al. 1983, Baimai 1988b). Thus, analysis of mitotic karyotype has been a useful tool for cytotaxonomic studies in some groups of eukaryotes (for a review, see White 1978).

The present review is concerned with quantitative changes in constitutive heterochromatin that lead to karyotypic evolution of certain groups of the dipteran insects belonging to the genera *Drosophila, Anopheles*, and *Bactrocera*. The possible role of heterochromatin differentiation occurring in natural populations in relation to evolutionary divergence at the chromosomal level is discussed.

THE GENUS *DROSOPHILA*

In the genus *Drosophila* (Diptera: Drosophilidae), variation in mitotic karyotype has been found not only among closely related species groups but also within the same taxon (Wilson et al. 1969, Clayton and Wheeler 1975, Baimai et al. 1983). This report presents a summary of our findings on inter- and intraspecific variation of heterochromatin in mitotic karyotype in some groups of *Drosophila*. Most cases, if not all, of these cytogenetic studies were based on isofemale lines derived from wild populations.

The *Drosophila montium* subgroup

The *Drosophila melanogaster* species group is the largest group of the genus *Drosophila* comprising about 173 species, of which 83 species belong to the *D. montium* subgroup (Bock and Wheeler 1972, Lemeunier et al. 1986, Toda 1991 pers. comm.). Most of the members of this subgroup are similar in external morphological characters, particularly those of the *D. kikkawai* complex (Tsacas and David 1977, Baimai 1979). Results of cytological studies of metaphase chromosomes of 22 species of the *montium* subgroup have been compiled by Clayton and Wheeler (1975). Although metaphase chromosomes of certain members of the *montium* subgroup had been included in the formal description of some species, detailed accounts on photomicrographs of their metaphase karyotypes suitable for cytological comparison were not available. Therefore, Baimai (1980) produced photomicrographs of metaphase karyotypes of 20 species of the *montium* subgroup which were obtained mainly from the laboratory stocks provided by the Department of Zoology, University of Texas at Austin.

All 20 species cytologically examined show a similar basic pattern of metaphase karyotype ($2n$ = 8). The most extensive variation in metaphase chromosomes with respect to the amount and distribution of constitutive heterochromatin was observed in the 4th (microchromosome) and the Y chromosomes, while the X was only slightly different among the species studied (Baimai 1980). Among these species, *D. birchii* and *D. kikkawai* have been extensively studied cytologically from

wild samples. *Drosophila birchii* is confined to the Australian and the Papua New Guinean regions whereas *D. kikkawai*, a subcosmopolitan species, is widely distributed ranging from Madagascar, the Indian subcontinent, across the Asian and Pacific regions to South America (Fig. 1). These 2 species show striking intraspecific variation in mitotic chromosomes due to the different amount and distribution of constitutive heterochromatin in sex chromosomes, and in the 4th chromosome (Baimai 1969, Baimai and Chumchong 1980, Baimai et al. 1986). Thus, *D. birchii* exhibits 2, 3, and 4 types of the 4th, the Y, and the X chromosomes, respectively, forming 7 distinct metaphase chromosome configurations occurring in natural populations (Fig. 1B).

Likewise, studies on population cytogenetics of *D. kikkawai* in Southeast Asia have revealed that it is actually a cluster of 3 closely related species (Tsacas and David 1977, Baimai 1979). Two newly described species are *D. leontia* Tsacas and David from Peninsular Malaysia and neighboring Singapore and *D. bocki* Baimai from Thailand. Cytogenetic evidence suggested that *D. kikkawai* is more closely related to *D. leontia* than to *D. bocki* (Kitthawee and Baimai 1979, Baimai et al. 1980). The 3 members of the *D. kikkawai* complex have been found to be sympatric in some collections in Southeast Asia (Baimai and Chumchong 1980, Baimai et al. 1980). Our collection records so far indicate that *D. kikkawai* is the most widespread species of the complex, occurring from Madagascar across the Asian region and continuing through the Pacific Islands to South America, while *D. leontia* and *D. bocki* seem to be restricted to Southeast Asia (Fig. 1).

The results of our extensive studies on mitotic karyotypes of these sibling species of the *D. kikkawai* complex have shown that *D. leontia* and *D. bocki* appear to be monomorphic in salivary gland chromosomes as well as in the metaphase karyotype (Kitthawee and Baimai 1979). On the contrary, *D. kikkawai* is highly polymorphic with respect to chromosomal rearrangements and constitutive heterochromatin in the Y and the 4th chromosomes (Fig. 1A) (Baimai and Chumchong 1980, Baimai et al. 1986). Our studies of the mitotic karyotypes of the *D. kikkawai* samples from many localities throughout its distribution range have revealed a remarkable variation of constitutive heterochromatin in the 4th chromosome and, to a lesser extent, in the Y chromosome (Baimai et al. 1986). There are 4 and 9 types of the Y and the 4th chromosomes, respectively, occurring in wild populations. Interestingly, the telocentric X chromosome is apparently uniform throughout the species distribution. This makes *D. kikkawai* the most variable species recorded to date within the genus *Drosophila* with respect to quantitative variation of heterochromatin in metaphase karyotype.

Moreover, during chromosomal analyses of some samples of *D. kikkawai*, a spontaneous tandem duplication was detected in a culture stock (no. J9) derived from a wild-caught female collected from Tananarive, Madagascar (Baimai and Kitthawee 1981). This rare phenomenon involved a large segment of the chromosome arm 3L. Additionally, among the 42 samples of *D. kikkawai* from wild populations collected from Mae Hong Son, northwest Thailand, 3 isofemale lines exhibited the aneuploid condition of the 4th chromosome (Traipakvasin and Baimai 1985). Such an aneuploidy persisted in one of the culture stocks (A76-7) until the 21st generation.

Our findings seem to indicate that genetic differentiation, at the chromosome level, is involved in the process of evolutionary divergence in this species complex. In this regard, *D. kikkawai* is certainly an excellent example for the study of a possible functional role of heterochromatin in the process of karyotypic evolution, particularly in the tropical environment of Southeast Asia.

The *Drosophila bostrycha-disjuncta* complex

Evolutionary biology of the picture-winged species of Hawaiian *Drosophila* has been extensively studied by Carson and his colleagues (see reviews by Carson and Kaneshiro 1976, Carson and Yoon 1982). Members of this unique group of *Drosophila* have large body sizes. They tend to form local colonies between which migration appears to occur only rarely under normal circumstances. The *D. grimshawi* subgroup of the picture-winged *Drosophila* is a cluster of some 60 chromosomally very similar species occurring on all the major islands of the Hawaiian Archipelago (Carson and Stalker 1968), including *D. bostrycha* on Molokai and *D. disjuncta s.l.* on Maui. These 2 species are homosequential with *D. grimshawi*, i.e., they have similar polytene chromosome banding sequences in all chromosome arms (Carson and Kaneshiro 1976). The *D. bostrycha-disjuncta* complex is particularly interesting because its members are very similar in external morphology (Hardy 1965). Even more interesting, they alone share a unique gene arrangement in chromosome 4, i.e., inversion 4v, which is an unusual event and a clue to their close relationship (Carson and Sato 1969). Mitotic

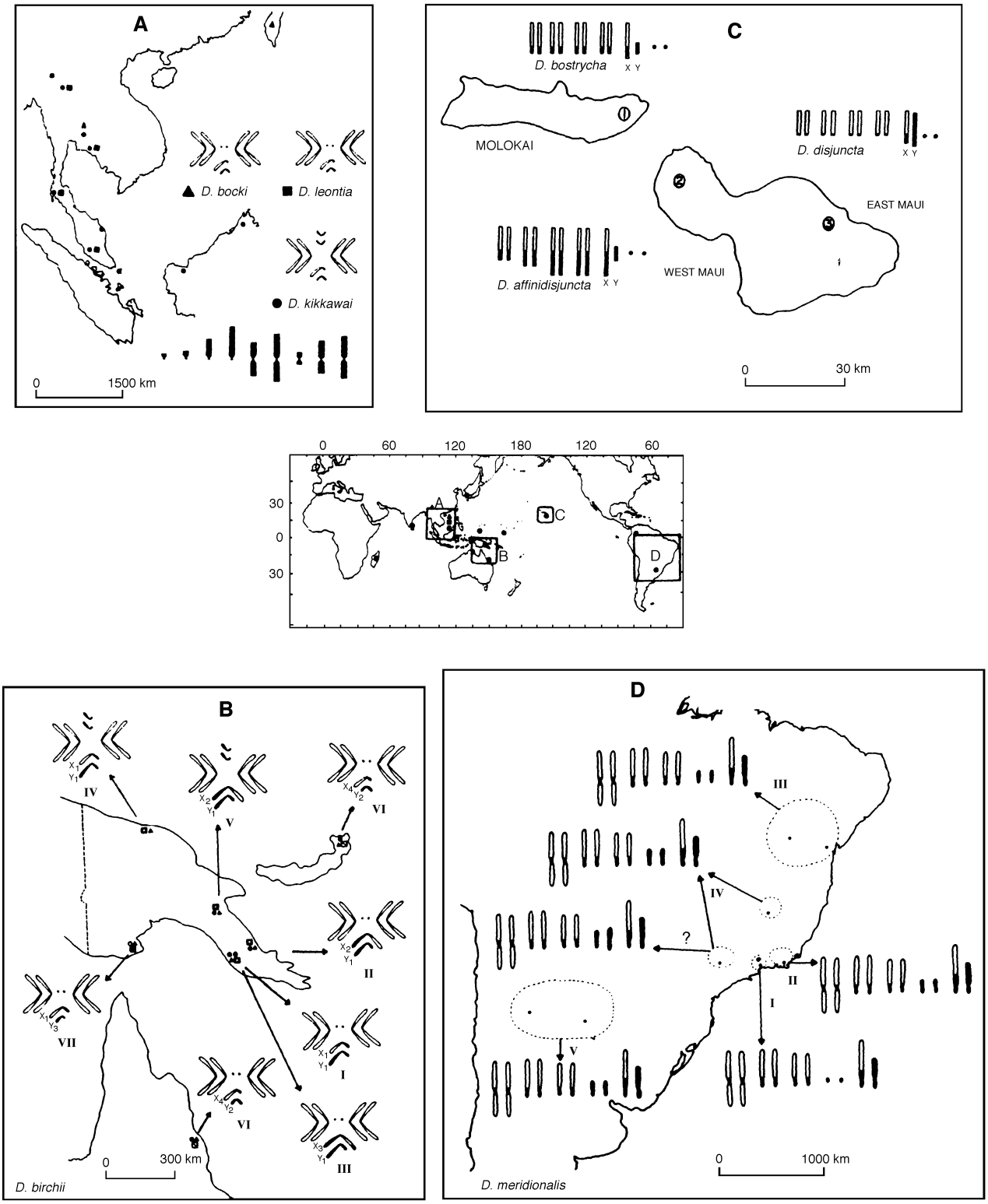

Fig. 1. World map showing geographical distribution and karyotypic variation of 4 groups of *Drosophila*. **A.** The *Drosophila kikkawai* complex includes *D. bocki* and *D. leontia* which are endemic to Southeast Asia; and *D. kikkawai*, the subcosmopolitan species. *Drosophila kikkawai* exhibits 9 types of 4th chromosome due to the different amount and distribution of heterochromatin. **B.** The *D. birchii* group, endemic to Australia and Papua New Guinea. **C.** The *D. bostrycha-disjuncta* group, the Hawaiian picture-winged species. **D.** The *D. meridionalis* group, the cactus-breeding species endemic to South America. Heterochromatin is shown in black.

chromosome analysis of the *D. bostrycha-disjuncta* complex from the wild samples from Molokai, and West and East Maui have led to the discovery of a new endemic species of Hawaiian *Drosophila* from the West Maui population, viz., *Drosophila affinidisjuncta* named by D.E. Hardy (1978). These 3 homosequential species clearly differ in the amount and distribution of constitutive heterochromatin in autosomes as well as in sex chromosomes (Fig. 1C) (Baimai and Ahearn 1978). *Drosophila disjuncta* has a standard metaphase karyotype showing a small amount of centromeric heterochromatin in all autosomes and a very large Y chromosome compared with those of *D. bo-strycha*. Conversely, *D. affinidisjuncta* exhibits prominent blocks of heterochomatin in the centromeric regions of all autosomes and the X chromosome compared with those of the other 2 sibling species. The Y chromosome of *D. affinidisjuncta* is similar to that of *D. bostrycha*. Detailed cytogenetic investigations involving exhaustive hybridization studies confirmed the existence of the 3 sibling species occurring allopatrically in the Molokai-Maui islands (Ahearn and Baimai 1987). Thus, the *D. bostrycha-affinidisjuncta-disjuncta* complex is an outstanding example of allopatric speciation involving karyotypic differentiation via gain of heterochromatin.

The *Drosophila meridionalis-serido* complex

A number of closely related neotropical species belonging to the *Drosophila repleta* group have been discovered and their phylogenetic relationships analyzed in terms of salivary gland chromosome banding patterns (for a review, see Wasserman 1982). Several members of this group are cactus-breeding species including *D. meridionalis* and *D. serido*. These 2 species are in close association with certain cactus species (*Opuntia*). Consequently, the distribution of these species closely reflects the sparse and discontinuous distribution of their host plants throughout South America. Each species thus consists of several geographically isolated populations and offers potential insights into the processes of genetic differentiation and speciation.

Analysis of metaphase chromosomes from both laboratory stocks and natural populations of *D. serido* and *D. meridionalis* shows a uniform "basic" metaphase karyotype. The 2 species include a number of different, geographically distinct, metaphase karyotypes involving differences in the major blocks of constitutive heterochromatin present in sex chromosomes and/or microchromosomes. These chromosomal differences are largely due to gain of extra heterochromatin. Moreover, the cytological evidence demonstrates that populations of both species are far from continuous in distribution. For example, *D. meridionalis* shows at least 5 patterns of heterochromatic difference in mitotic karyotype, in parallel with geographic isolation (Fig. 1D). Similarly, *D. serido* exhibits 6 different types of mitotic karyotype in terms of quantitative variation in heterochromatin in sex chromosomes and microchromosomes (Baimai et al. 1983). Thus, *D. meridionalis* and *D. serido* represent another interesting group of *Drosophila* which are undergoing species differentiation and karyotypic evolution through the acquisition of heterochromatin in the genome. Such cytological differences reflect the existence of subspecific or specific complexes within the taxa *D. meridionalis* and *D. serido* with minimal morphological differentiation.

THE GENUS *ANOPHELES*

The genus *Anopheles* (Diptera: Culicidae), comprising more than 400 described species, represents one of the most important groups of anopheline mosquitoes because of its vectorial capacity for disease transmission, particularly in tropical and subtropical regions. In Thailand, anopheline mosquitoes include some 72 known species (Harrison et al. 1991). Although considerable knowledge of systematics and geographical distribution of the known species of *Anopheles* in Thailand and Southeast Asia has been documented (Reid 1968, Harrison and Scanlon 1975), relatively few species are known cytologically, particularly mitotic karyotypes (Kitzmiller 1967, Kanda et al. 1983). During the past 15 years, we have carried out population cytogenetic studies of the *Anopheles* malaria vectors and some 40 nonvector species in Thailand and Southeast Asia. These studies provide exhaustive information on metaphase chromosomes of *Anopheles* and a better understanding of karyotypic evolution through the accumulation of heterochromatin in the genome.

The *Anopheles leucosphyrus* group

Our attention has been focused on the *An. leucosphyrus* group belonging to the Neomyzomyia series of the subgenus *Cellia*, since most of its

members are primary vectors of human malarial parasites. This species group exclusively inhabits forest and foothill areas and is widely distributed throughout the Oriental region. Early systematic studies (Peyton and Harrison 1979 1980) coupled with cytogenetic investigations of *An. balabacensis s.l.* (Baimai et al. 1981, Hii 1985, Baimai 1988b), *An. dirus* (=Thailand form of *An. balabacensis*) and *An. takasagoensis* (=Taiwan form of *An. balabacensis*) were recognized as distinct species from *An. balabacensis s.s.* from Balabac Island, the Philippines. However, further detailed studies on systematics and cytogenetics of natural populations of *An. dirus* in Thailand and southwest India have revealed that it is actually a species complex in itself consisting of at least 6 sibling species provisionally designated as *An. dirus* A, B, C, D, E, and F. Cytologically, these sibling species can be separated on the basis of distinct patterns of major blocks of constitutive heterochromatin in sex chromosomes, as appearing in the mitotic karyotype, and banding sequences, as well as the density of bands in salivary gland polytene chromosomes (Baimai et al. 1981 1984b 1987 1988a, Wibowo et al. 1984, Hii 1985, Sawadipanich et al. 1990). The 6 genetic species are almost indistinguishable on the basis of external morphological characters. Recently, species F has been formally named *An. nemophilous* by Peyton and Ramalingam (1988), but species F is used herein for a short designation of *An. nemophilous*. The results thus far also indicate that these sibling species have different geographical distributions in Southeast Asia (Fig. 2). Similarly, population cytogenetic studies of *An. leucosphyrus s.l.* have led to the recognition of 2 sibling species temporarily designated *An. leucosphyrus* species A and B (Baimai et al. 1988b).

Analyses of larval mitotic chromosomes of

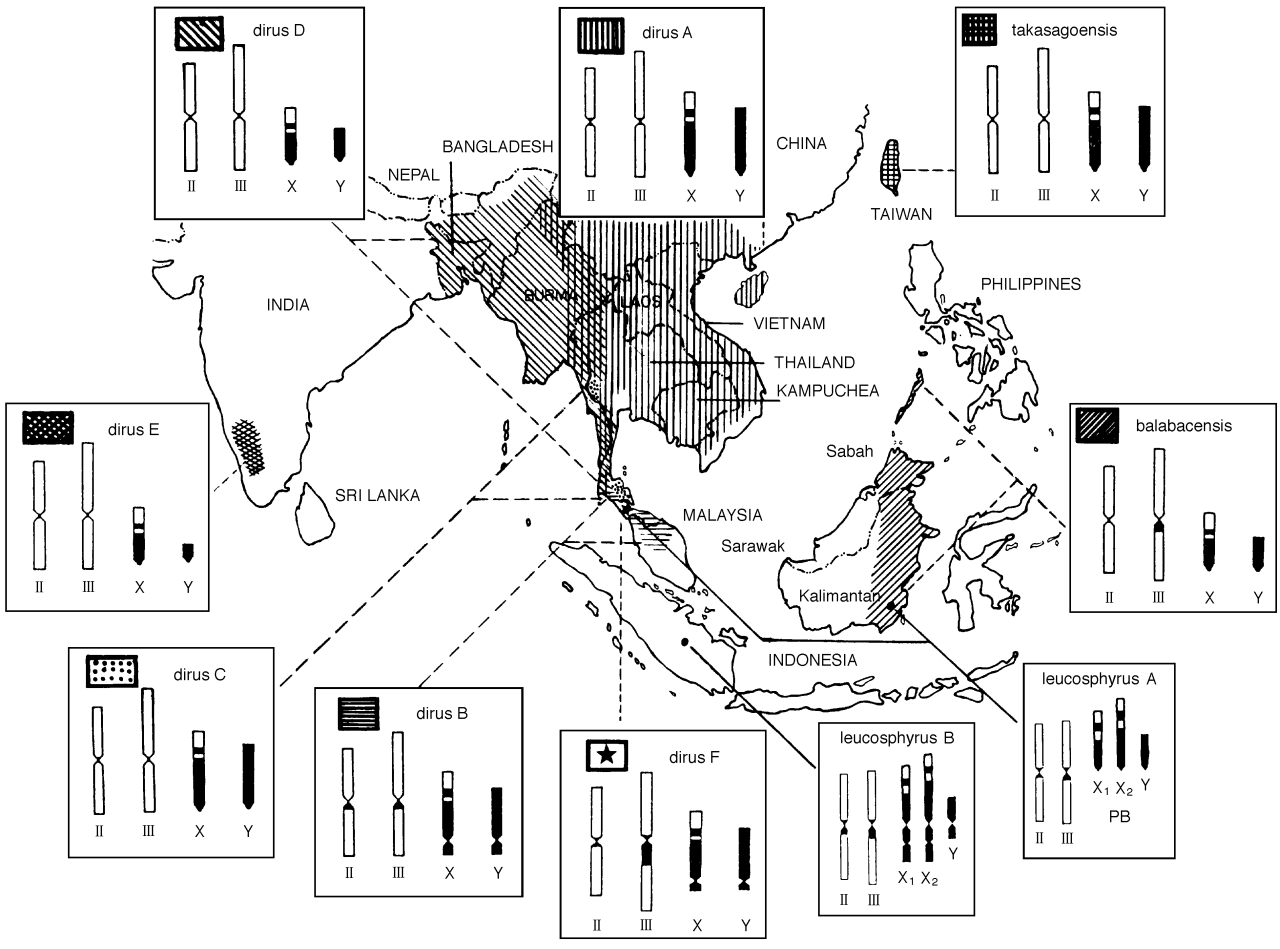

Fig. 2. Geographical distribution of 10 species of the *Anopheles leucosphyrus* group in Southeast Asia including *An. dirus* E in southwest India. Mitotic karyotypes are presented in the form of haploid idiograms (black represents the heterochromatin portion).

members of the *An. leucosphyrus* group using conventional Giemsa staining methods (Baimai et al. 1981) and/or Hoechst 33258 fluorescent banding techniques (Wibowo et al. 1984) have revealed marked differences in the amount and distribution of constitutive heterochromatin both in sex chromosomes and at centromeric positions of autosomes (see Fig. 2). The metaphase karyotype (*2n* = 6) of all members of this species group, except for *An. dirus* B, F, and *An. leucosphyrus* B, exhibits typical telocentric sex chromosomes of various sizes, which are clearly attributable to different amounts of heterochromatin in the vicinity of the centromere as well as qualitative differences of intercalary heterochromatin. Thus, the sex chromosomes of *An. dirus* D are relatively smaller than those of *An. dirus* A, C, and *An. takasagoensis*, each of which shows X and Y chromosomes of roughly similar size. Nevertheless, *An. dirus* A, C, and *An. takasagoensis* display significantly different fluorescent banding patterns of major blocks of intercalary heterochromatin in the X and Y chromosomes, although they cannot be readily distinguished by use of the Giemsa staining technique. The sex chromosomes of *An. dirus* B and F, on the other hand, are unique in having distinct subtelocentric (acrocentric) configurations. The short arm of the sex chromosomes is entirely heterochromatic, which is likely due to the acquisition of extra blocks of heterochromatin, although the possibility of pericentric inversion of the centromeric heterochromatin cannot be ruled out. *Anopheles dirus* B shows a considerable amount of centromeric heterochromatin in all autosomes, while *An. dirus* F exhibits a conspicuous block of heterochromatin in the centromeric region of autosome 3. This is a clear cytological landmark that can be used to separate species F from species B. Thus, the apparent heterochromatic differentiation is most extensive in *An. dirus* B and F compared with the other members of this species complex (Fig. 2). Furthermore, natural population samples of *An. dirus* A and B show even more extensive heterochromatin variation in the vicinity of the centromere of both X and Y chromosomes (Baimai et al. 1984a, Baimai and Traipakvasin 1987). The evolutionary role and epidemiological significance of such an extensive heterochromatic variation remain unknown (Baimai 1988a). *Anopheles dirus* D and E are allopatric species and are morphologically very similar. Cytologically, the Y chromosome of species E is clearly smaller than that of species D (Fig. 2). Although sex chromosomes of *An. balabacensis* appear to be similar to those of *An. dirus* D and E, they show a conspicuous block of centromeric heterochromatin in autosome 3 similar to that of *An. dirus* B and F (Fig. 2). These species are, of course, genetically quite remote and are geographically isolated. The remaining members of this species complex do not exhibit any detectable differences of centromeric heterochromatin of the autosomes.

Anopheles leucosphyrus A and B are allopatric species. Species A occurs in southern Thailand, Malaysia, and Borneo while species B is endemic to Sumatra, Indonesia. Although they are morphologically similar, species B shows a remarkable feature of submetacentric X and Y chromosomes which are entirely different from those of species A. This is obviously due to the acquisition of large blocks of heterochromatin in the presumed ancestral telocentric sex chromosomes. Such a gross chromosome difference with respect to constitutive heterochromatin is quite useful in separating these 2 sibling species of the *An. leucosphyrus* complex.

The *Anopheles maculatus* group

The situation of this species group is somewhat less complicated than that of the *An. leucosphyrus* group, and it has been recently resolved through chromosomal analyses and systematic studies (Green et al. 1985, Rattanarithikul and Green 1986, Rattanarithikul and Harbach 1990, Baimai et al. 1993b). *Anopheles maculatus s.l.* belongs to the Neocellia series of the subgenus *Cellia*. It is widespread thoughout the Oriental region and is regarded as an important vector of human malarial parasites in some parts of Indonesia and Peninsular Malaysia. In Thailand, it is recognized as a vector species only in the southern areas toward the Malaysia border, although it occurs throughout the country.

Cytologically, unlike the *An. leucosphyrus* group, the *An. maculatus* group has excellent polytene chromosomes in the ovarian nurse cells of half-gravid females which are more suitable for chromosomal analysis of the wild samples. Detailed analyses of polytene chromosomes and heterochromatin differences in sex chromosomes (Green et al. 1985) coupled with taxonomic studies (Rattanarithikul and Green 1986, Rattanarithikul and Harbach 1990) of Thai populations of members of the *An. maculatus* group have demonstrated that it is truly a species complex consisting of at least 8 closely related species and 2 forms: *An. sawadwongporni* (= species A), *An. maculatus s.s.* (= species B including forms E and F), *An.*

dravidicus (= species C), *An. notanandai* (= species G), *An. willmori* (= species H), *An. pseudowillmori* (= species I), *An. dispar*, and *An. greeni*. The first 6 species occur in central, north, and northeast Thailand while the last 2 species are known only from the Philippines.

Metaphase karyotypes of 8 species of the *An. maculatus* group exhibit inter- and intraspecific variation based on differences in the amount and distribution of constitutive heterochromatin in the sex chromosomes and/or in the centromeric regions of the autosome(s). Analysis of heterochromatin variation has revealed 3 distinct groups of mitotic sex chromosomes (Baimai et al. 1993b). Group 1, which includes *An. sawadwongporni*, *An. dravidicus*, and *An. pseudowillmori*, is characterized by telocentric or subtelocentric sex chromosomes. The only representative of Group 2 is *An. notanandai*, which has a metaphase karyotype showing a unique chromosome X with a very small amount of centromeric heterochromatin. Group 3 includes *An. dispar*, *An. maculatus*, *An. willmori*, and *An. greeni* showing subtelocentric, submetacentric, or metacentric sex chromosomes.

Furthermore, analysis of mitotic chromosomes of *An. willmori* derived from individual wild-caught females collected from Chiangmai Province, northern Thailand, has revealed 4 isofemale lines showing variation in the X chromosome, including the normal X_1 and 3 aberrant types (X_3, X_4, and X_L). It is postulated that these different types of X chromosomes could have arisen as a result of spontaneous chromosomal rearrangements involving tandem translocation and paracentric inversions followed by acquisition of constitutive heterochromatin (Baimai et al. 1996c). Such rare events of chromosomal changes have become established in the natural population of *An. willmori* in northern Thailand.

Other species groups of the Oriental *Anopheles*

During our population cytogenetic studies of the Oriental *Anopheles* which have spanned a period of 15 yr, we have had the opportunity to analyze metaphase karyotypes of 40 species and 20 cytological forms of the subgenera *Cellia* and *Anopheles* (excluding the *An. leucosphyrus* and the *An. maculatus* groups). Most cases, if not all, show mitotic karyotype variation due to the different amount and distribution of constitutive heterochromatin in the sex chromosomes and, to a lesser extent, in the autosomes (Baimai et al. 1993a 1994 1995a 1996a,b). Such a cytological approach can be a useful cytotaxonomic tool in separating closely related species or isomorphic species of *Anopheles*, at least in the Southeast Asian region.

THE GENUS *BACTROCERA*

Bactrocera (Diptera: Tephritidae), one of the most important genera of fruit flies causing damage to vegetables and fruits, is widely distributed throughout Southeast Asia, Australia, and the Pacific region (Drew 1989). These fruit flies are mainly endemic to tropical and subtropical rain forests of the regions. The genus *Bactrocera* includes 4 groups of subgenera, i.e., *Bactrocera, Melanodacus, Queenslandacus*, and *Zeugodacus*. Among these groups, the *Zeugodacus* and the *Bactrocera* groups are most diversified comprising 11 and 8 subgenera, respectively. In Thailand, the subgenus *Bactrocera* is the most prominent group consisting of some 36 known species followed by the subgenus *Zeugodacus* containing 18 described species. Extensive surveys and systematic studies have been made recently by Drew (1989) and Drew and Hancock (1994). Among these fruit flies there are some cryptic or isomorphic species which cause great taxonomic problems because of their similarity in external morphology. Nevertheless, such sibling (cryptic) species may exhibit different behaviors in mating, feeding, and ovipositing on specific host plants that can be readily observed. Moreover, some sibling species can be easily separated, based on mitotic karyotype analysis. Our ongoing project has focused on population cytogenetic investigations particularly on karyotypic differentiation due to the different amount and distribution of constitutive heterochromatin in the centromeric regions of mitotic chromosomes.

Analysis of mitotic chromosomes on the basis of G- and H-bandings has revealed striking differences in metaphase karyotypes with respect to the quantitative variation of pericentric heterochromatin of autosomes and sex chromosomes among closely related species of the *B.* (*B.*) *dorsalis* complex and some members of the different subgenera of *Bactrocera* (Baimai et al. 1995b 1996d). For example, wild samples of 5 species of the *B. dorsalis* complex, including those described by Drew and Hancock (1994) and a new species (E) discovered in our studies, clearly show distinctive patterns of mitotic karyotype based on quantitative differences in constitutive heterochromatin in the centromeric regions of the autosomes and the sex chromosomes (Fig. 3) (Baimai et al. 1995b). Like-

wise, comparison of mitotic chromosomes of 5 distinct species belonging to 4 different subgenera also shows karyotypic evolution involving heterochromatin differentiation, notably in the centromeric regions of autosomes and sex chromosomes (Fig. 3) (Baimai et al. 1996b). Such quantitative differences in constitutive heterochromatin represent cytological characteristics of closely related species or even isomorphic species. In fact, the discovery of some new genetic species in this study is primarily the result of mitotic karyotype analysis coupled with careful observations of morphological characters as well as specific host plants (Baimai et al., unpubl. data). Generally, the evolutionary divergence in mitotic karyotypes of the fruit flies in Thailand tends toward the gain of constitutive heterochromatin in the genome. Our ongoing project on genetics of fruit flies in Thailand will certainly provide more cytological data pertaining to the role of heterochromatin in karyotypic evolution of this important group of dipteran insects in this region.

CONCLUDING REMARKS

In the view of modern evolutionary biology, through studies on the genetics of natural populations, e.g., Dobzhansky (1970), Carson (1982), and White (1978), it is believed that genetic differentiation via reorganization at molecular and/or karyotypic levels over time and space constitutes a fundamental mechanism in the evolutionary process. Further, allopatric speciation is the most common mode of species divergence among eukaryotes (Mayr 1963). In this regard, different geographical populations are subjected to divergent selective pressures in separate microhabitats. Thus, allopatric speciation results in biological species which usually differ in morphology, genetics, and ecology. Yet genetic differentiation, which probably directly influences the mating behavior of allopatric populations, may result in sibling species that are morphologically very similar. If this happens, uncertainty may exist within the species or taxon. Hence, bi-

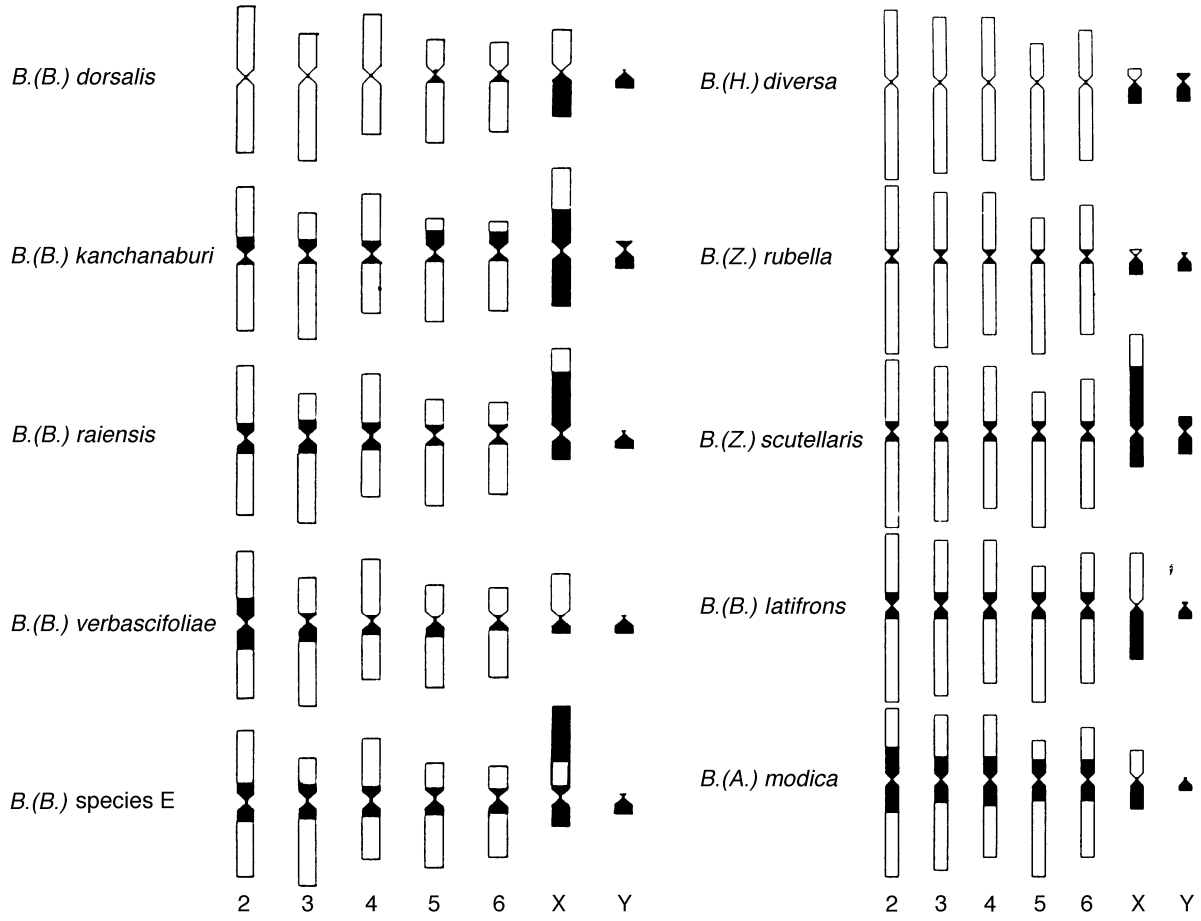

Fig. 3. Diagrammatic representation of haploid idiograms of mitotic karyotypes of 5 species of the *Bactrocera dorsalis* complex (left) and 5 distinct species representing 4 subgenera of the genus *Bactrocera* (right). Heterochromatin portions are depicted in black.

ologists are quite often not too sure whether individuals of similar gross morphology from different populations or even in the same supposed population belong to the same gene pool. Thus, in such complexes, study of genetic variation in natural populations becomes an indispensable method for determining degrees of evolutionary divergence. These research approaches to species problems have largely concentrated on *Drosophila* (Dobzhansky 1970), on *Anopheles* (Kitzmiller 1976), and quite recently on *Bactrocera* (Baimai et al. 1995b 1996d, unpubl. data). Our studies on these groups of dipteran insects in the Oriental region described here lend support to this paradox of allopatric speciation and to possible involvement of heterochromatin differentiation in the genome.

In an earlier study on the *D. melanogaster* group, we examined mitotic karyotypes of *D. birchii*, an endemic species of northern Australia and Papua New Guinea, and *D. kikkawai*, a subcosmopolitan species. These 2 species exhibit extensive variation in mitotic karyotypes which are expanded by heterochromatin additions into the 4th chromosome (microchromosome) and sex chromosomes. A similar phenomenon of heterochromatin variation has been observed in *D. meridionalis* and *D. serido* of the *D. repleta* group which are endemic to South America. None of these studies thus far has led to new species designations. However, our detailed cytogenetic studies of the Hawaiian *Drosophila*, *D. bostrycha* and *D. disjuncta*, have led to a discovery of a new sibling species, *D. affinidisjuncta*, allopatrically occurring in West Maui. Such presumed allopatric speciation of the Hawaiian *Drosophila* correlates with heterochromatin accumulation in autosomes as well as in sex chromosomes. A similar phenomenon of heterochromatin accumulation in the genome has been reported in a number of species groups of *Drosophila*, for example, the *D. immigrans* group (Wilson et al. 1969, Ranganath and Hagele 1982, Wakahama et al. 1983), the *D. pachea* group (Ward and Heed 1970), and the Hawaiian picture-winged *Drosophila* (Clayton 1969 1988, Yoon and Richardson 1978). Likewise, we have observed heterochromatin additions to autosomes and sex chromosomes in the Oriental *Anopheles* and *Bactrocera* as briefly described above. Such cytological evidence seems to suggest that heterochromatin accumulation is somehow involved in species differentiation of these dipteran insects. Heterochromatin variation in natural populations seems to be a general phenomenon in higher organisms (John 1981).

Ever since the discovery of heterochromatin by Heitz (1928), constitutive heterochromatin has been generally observed in higher organisms, both plants and animals, particularly in the insects of the order Diptera (White 1978). Constitutive heterochromatin always appears in mitotic chromosomes, especially in the pericentric regions, as blocks of dark staining flanking the centromeres. In the dipteran insects, constitutive heterochromatin might extend more than 60% of the metaphase length of the X chromosome and as much as 50% of certain autosome pairs, as demonstrated in our studies. Structurally, it was later discovered to consist of highly repetitive DNA or satellite DNA arranged in tandem in the eukaryotic genome (Britten and Kohne 1968, Peacock et al. 1977 1981, Appels and Peacock 1978, Bonaccorsi and Lohe 1991, Lohe et al. 1993). Further, modern cytogenetic techniques and molecular analysis of constitutive heterochromatin in *D. melanogaster* chromosomes have revealed structural genetic content and its functional role. Recently, some 30 active genes have been found in the heterochromatin region of *D. melanogaster* chromosomes (for a review, see John and Miklos 1979, Gatti and Pimpinelli 1992). For example, there are the *suppressor of forked* gene at the proximal region of the X chromosome, the *light* gene at the distal region of chromosome arm 2L, and the *rolled* gene within the proximal region of chromosome arm 2R (Le et al. 1995, Berghella and Dimitri 1996). However, the Y chromosome contains certain active genes, particularly the fertility factors. Thus the presence of heterochromatin in eukaryotic chromosomes suggests its significant role in the regulatory function and concerted evolution of the genome (Dover 1982, Dover and Flavell 1984, Pardue and Hennig 1990, Irick 1994, Zuckerkandl and Hennig 1995).

One of the proposed functional roles of heterochromatin is the proper recognition and segregation of homologous chromosomes during meiosis (John and Miklos 1979, John 1988). Additional copies of repetitive elements of DNA sequences in homologous heterochromatin may be an evolutionary response to additive affects. It is my perception that heterochromatin differentiation is probably established simultaneously with or even before any other genetic, ecological, or behavioral differences that might also contribute to partial reproductive isolation. Selection against the structural heterozygotes in cytological hybrids may be strong enough to initiate a reinforcement process that could lead to species differentiation and speciation.

The dramatic evidence available so far seems

to suggest that heterochromatin differentiation often plays an important role in karyotypic evolution in dipteran insects, at least in the Oriental region, as described here. Although the formulation of models for speciation in relation to heterochromatin differentiation is inevitably in the realm of speculation, the foregoing cytogenetic data indicate some implications of heterochromatin in the phylogenetic affinity and consequently the evolutionary divergence of sibling species or closely related species of these dipteran insects. Therefore, detailed investigation into the dynamics of heterochromatin accumulation, particularly at the molecular level, and its evolutionary significance, remains intriguing and challenging.

Acknowledgments: I wish to thank P. Grote and M. Paetkau for reading the manuscript. I also thank the Institute of Zoology, Academia Sinica, Taiwan, for providing a travel grant. The work was partially supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, US Army Component, AFRIMS Bangkok, the Thailand Research Fund, and the National Center for Genetic Engineering and Biotechnology.

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異染色質之累積與一些雙翅目昆蟲之細胞核型之進化

Visut Baimai[1]

真核細胞的生物之分歧進化包括染色體組之各個不同層次的遺傳改變。在染色體的層次上，異染色質的分化導致核型的改變提供了許多動物包括雙翅目的昆蟲一個非常有用的細胞分類學的工具。本文介紹果蠅屬（果蠅科）、瘧蚊屬（蚊科）及果實蠅屬（果實蠅科）昆蟲其親緣關係很近的種或同胞種之異染色質的量的改變與異染色質在染色體上的分布來探討其分歧進化，如雙翅目昆蟲果蠅屬之 *montium* 種亞群之吉川氏果蠅(*D. kikkawai*)種族、瘧蚊屬之 *maculatus*（斑腳瘧蚊）種群之惡兆瘧蚊(*Anopheles dirus*)種族及果實蠅屬之東方果實蠅(*Bactrocera dorsalis*)種族及雙果實蠅(*Zeugodacus*)種群。

本研究的結果顯示在有絲分裂中期的染色體不管種間或種內的差異在主要部分之性染色體或體染色體的結構異染色質之增得，尤其是在染色體的臂間部分(pericentric region)。再者，有絲分裂中期異染色質之量的改變，可以很成功的做為遺傳標記來細分蔭蔽種(cryptic species)或型態相近的物種。雖然異染色質在物種的分化上所扮演的色角至今未明，但是異染色質在染色體組(genome)上的累積明顯的與雙翅目昆蟲其遺傳上的分化及染色體的進化有關。

關鍵詞：結構異染色質，有絲分裂染色體，核型的進化，雙翅目昆蟲，細胞分類學。

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