Cytogenetic Relationships of Drosophila Affinidisjuncta Hardy[1]

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ABSTRACT: Cytological investigations of the mitotic metaphase chromosomes in the *disjuncta - bostrycha* species complex have led to the discovery of a new species of the picture-winged group, *Drosophila affinidisjuncta* Hardy from West Maui. Interspecific variation of metaphase karyotype is due to the amount and distribution of heterochromatin in the chromosome complement. The results of hybridization tests among these three homosequential species verifies the full-fledged species status of the new species. The cytological method is useful as a diagnostic tool for the recognition of species differences in this cluster of morphologically very similar species. It is further suggested that this metaphase chromosome technique has potentially wide application in systematic and evolutionary studies of the endemic Hawaiian drosophilids.

INTRODUCTION

Evolutionary biology of the picture-winged species of Hawaiian *Drosophila* (Drosophilidae) has been extensively studied by Carson and his colleagues (*see* reviews by Carson *et al.*, 1970, and Carson and Kaneshiro, 1976). This unique group of *Drosophila* has members which have large body sizes. They tend to form local colonies between which migration appears to occur only rarely under normal circumstances. The *grimshawi* subgroup of the picture-winged *Drosophila* is a cluster of 60 chromosomally very similar species. They occur on all the major islands of the Hawaiian Archipelago (Carson and Stalker, 1968). Included in this subgroup are nine species that are homosequential with *D. grimshawi, i.e.*, they have identical polytene chromosome banding sequences in all chromosomes (Carson and Kaneshiro, 1976). Of these, *D. disjuncta* on Maui and *D. bostrycha* on Molokai are particularly interesting for they 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) (Carson and Sato, 1969), which is an unusual event and a clue to their close relationship.

In spite of the available data on systematic and cytological differentiation in this species complex, critical detailed cytogenetic studies

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have not been made until recently. Yang and Wheeler (1969) reported preliminary studies on interspecific hybridization of all possible combinations of certain picture-winged species. Recently, Craddock (1974a) reported hybridization experiments among the homosequential species of the *Drosophila planitibia* subgroup. Furthermore, Clayton (1968, 1969) and Clayton *et al.* (1972) have extensively investigated metaphase karyotypes of most of the endemic picture-winged species. Intra- and interspecific variations in metaphase karyotype have been recorded. In all cases, the variation is due to the presence or absence of extra heterochromatin. This kind of chromosomal variation cannot be observed in salivary gland chromosomes since heterochromatic material does not undergo polytenization (Rudkin, 1969). However, recent studies on mitotic chromosomes of certain species belonging to the *grimshawi* subgroup using a colcemid technique reveal more conclusive information about the apparent amount of heterochromatin present in the chromosome complement (Baimai, 1975a, b). Although heterochromatin polymorphisms may occur within a species, extensive fixed differences between two populations strongly suggest specific differentiation. Thus, in cases where morphological differences are small, the genetic divergence of the entities concerned can frequently be assessed by recourse to metaphase analysis. This approach has proved useful in *D. birchii* (Baimai, 1969) and in *D. athabasca* (Miller and Roy, 1964).

This report presents the application of the cytological method, together with hybridization experiments, to help in the recognition of species differences in several cases where morphological separation is very difficult, if not impossible. The results of these investigations have led to the discovery of a new endemic species of Hawaiian *Drosophila, Drosophila affinidisjuncta* named by D. E. Hardy (*see* the preceding article). This new species was formerly considered to represent a population of *D. disjuncta* in West Maui because specimens were indistinguishable either by external morphology or by polytene chromosomes (Carson and Sato, 1969). This article will give an account of the cytogenetic relationships between *D. affinidisjuncta, D. disjuncta* and *D. bostrycha.*

MATERIALS AND METHODS

Metaphase plate chromosomes were prepared from ganglion cells of third instar larvae deriving from individual wild-caught females using the simple technique of Baimai (1976) (Table 1). The method essen-

TABLE 1.—Number of isofemale lines from each locality examined for metaphase chromosomes

Species	Locality	Number of isofemale lines examined
D. bostrycha	Apee, East Molokai	11
D. affinidisjuncta	Kaulalewelewe, West Maui	12
D. disjuncta	Waikamoi, East Maui	12

tially involves pretreatment of larval ganglion cells with colcemid solution before swelling the cells in a hypotonic solution of sodium citrate and fixing in acetic alcohol. Cell dissociation and suspension were obtained in 60% acetic acid. The cells were spread onto microscope slides, heat-dried and stained with Giemsa's solution.

Laboratory stocks derived from isofemale lines were used in interspecific hybridization experiments: two lines of *Drosophila bostrycha* from Apee, one line of *D. affinidisjuncta* from Kaulalewelewe and two lines of *D. disjuncta* from Waikamoi. These three species were crossed in all possible combinations. At least four replicates were made for each cross with two to four pairs placed in each replicate vial. The F_1 hybrids were separated by sex and aged for 3-5 weeks. F_1 hybrid females were backcrossed to both parental species in order to determine fertility. F_1 male hybrids were dissected and examined for the status of spermiogenesis and presence of motile sperm, an indication of potential fertility. In each cross, from 15 to 25 F_1 males were dissected.

RESULTS

Detailed analysis of larval ganglion metaphase figures of these species reveals a striking difference in the chromosome complements (Fig. 1). The two extreme cases with respect to the amount of heterochromatin are found in the chromosome complements of *Drosophila disjuncta* and *D. affinidisjuncta*. Rather surprisingly, these two extreme cases are not geographically the most separate populations. Thus all lines of *D. disjuncta* examined show a metaphase karyotype consisting of five pairs of rods and one pair of dots. All the autosomes are apparently acrocentric and have a very small amount of centromeric heterochromatin. The sex chromosomes are readily distinguishable because of the presence of an extra heterochromatic arm on the X chromosome. The large Y chromosome is conspicuous and almost totally heterochromatic (Fig. 1, a and b). In contrast, *D. affinidisjuncta* from West Maui exhibits a marked difference in metaphase chromosome condition from that in *D. disjuncta*. The difference is obviously due to the presence of a large heterochromatic block on each of the autosomes as well as on the X chromosome. Thus this species shows three pairs of large V-shaped, one pair of small J-shaped, and a pair of dot-like chromosomes. Furthermore, the Y chromosome is comparatively shorter than that of *D. disjuncta* and is easy to recognize (Fig. 1, c and d).

Drosophila bostrycha from Molokai exhibits yet another form of metaphase karyotype, *i.e.*, with the presence of an intermediate amount of heterochromatin. All the four pairs of major autosomes contain a considerable amount of extra heterochromatin which makes them appear as small J-shaped configurations (Fig. 1, e and f). The X chromosome cannot be easily separated from the autosomes. The Y is a small, heterochromatic chromosome which is apparently similar to that of *D. affinidisjuncta*.

The evidence, therefore, is that these three closely related species

have different metaphase chromosome complements. Furthermore, samples of the three species from other locations within their respective ranges totally conform to the pattern presented here. (The detailed geographical information, including inversion frequencies in various populations, will be presented in a paper now in preparation.) Thus, the metaphase karyotype may be used in this case as a diagnostic character for distinguishing the species. Salivary gland chromosomes are indistinguishable and external morphology only differentiates *Drosophila bostrycha* from the other two.

Hybridization experiments among these homosequential species yield further evidence for the species status of the three members of this complex (Table 2). Without exception, all possible crosses gave F_1 hybrids of both sexes in approximately equal numbers. The fertility of the F_1 hybrids was tested. The results in all cases are consistent and show that the male hybrids are sterile while the female hybrids are

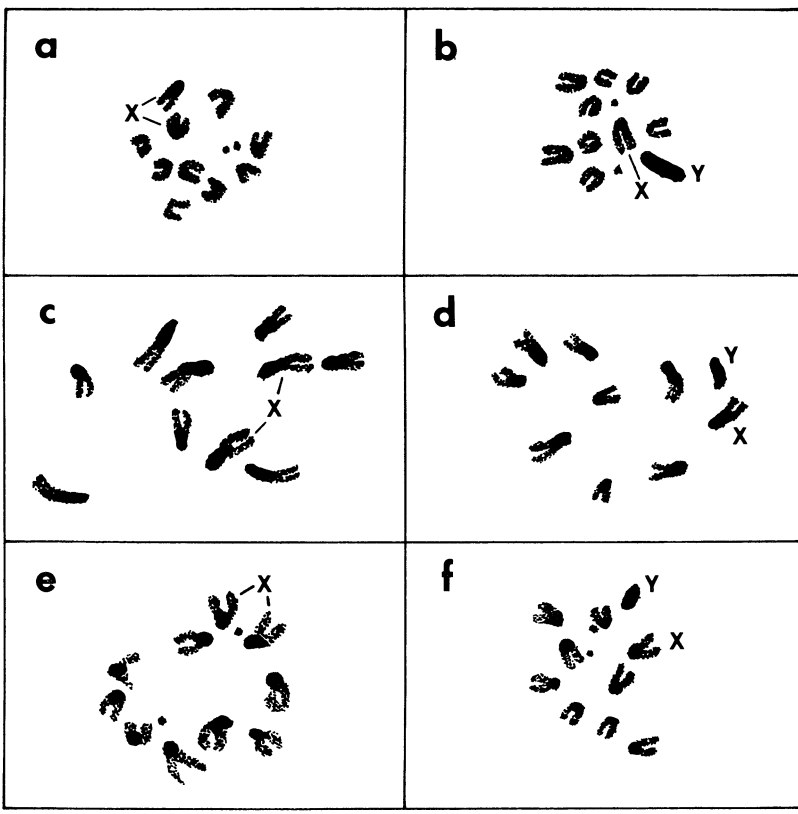

Fig. 1.—Drawings of mitotic metaphase chromosomes. a-b, *D. disjuncta;* c-d, *D. affinidisjuncta;* e-f, *D. bostrycha.* Females are shown on the left, males on the right (magnification ca. 600 X)

fertile. It is interesting that the degree of sterility in male hybrids, using Craddock's (1974a) criteria, varies depending on the parents involved in matings. For instance, the cross between *Drosophila affinidisjuncta* female and *D. disjuncta* male yielded sterile F_1 male hybrids of Class I. In this extreme type of sterility no sperm differentiate; only degenerating cellular debris is found within the testes. Furthermore, in the crosses reported here, Class I sterility is accompanied by abnormally formed testes—small, dark brown and with few coils. The reciprocal cross, on the other hand, yielded sterile male hybrids of Class II. The testes contain all stages of spermiogenesis through coiled sperm bundles (*see* Tokuyasu *et al.*, 1972). However, sperm are apparently not liberated from the cysts since the vasa deferentia are empty. Likewise, a cross between *D. affinidisjuncta* female and *D. bostrycha* male gave sterile male hybrids of Class I, whereas the reciprocal cross yielded sterile male hybrids of Class II. Finally the cross between female *D. disjuncta* and male *D. bostrycha* generated sterile male hybrids of Class I while male F_1 hybrids from the reciprocal cross were Class II sterile.

DISCUSSION

From the geographical distribution, *Drosophila bostrycha, D. disjuncta* and *D. affinidisjuncta* are allopatric species. Each occurs on one of the three isolated volcanoes of the Maui complex of islands (Fig. 2). *Drosophila disjuncta* is confined to East Maui, whereas *D. affinidisjuncta* is restricted to West Maui. These two parts of Maui island are in fact separated by an isthmus of agricultural lowland, primarily sugar cane plantations, ca. 30 km wide. *Drosophila bostrycha* occurs only on the eastern part of Molokai which is separated from Maui by the Pailolo Channel, approximately 14 km wide. Geological data indicate that these separate volcanoes have been alternately joined and then separated by changes in sea level at least twice during recent geological time (Stearns, 1966). Such circumstances may have provided the geographic isolating mechanism which aided the speciation process of these closely related species.

Structural changes of chromosomes such as fusions, whole-arm translocations and pericentric inversions provide drastic changes in organization of the genetic material. These changes may have a regu-

TABLE 2.—Interspecific hybridization experiments

Hybridization			Total	♂♂ / 100♀♀	F_1 Hybrids Fertility		
Female		Male			Female	Male	(Class)
disjuncta	x	*bostrycha*	89	85.4	fertile	sterile	(I)
bostrycha	x	*disjuncta*	141	104.4	fertile	sterile	(II)
affinidisjuncta	x	*bostrycha*	68	88.9	fertile	sterile	(I)
bostrycha	x	*affinidisjuncta*	128	106.4	fertile	sterile	(II)
affinidisjuncta	x	*disjuncta*	124	82.3	fertile	sterile	(I)
disjuncta	x	*affinidisjuncta*	285	88.7	fertile	sterile	(II)

latory function with regard to gene action. Thus, significant changes in developmental processes may occur without altering genic constitutions. These kinds of structural reorganization of the genome evidently play an important role in speciation in many groups of animals (White, 1969; Crozier, 1970; Jackson, 1971; Kerr and da Silveira, 1972; Pathak *et al.*, 1973; Craddock, 1974b; Goodpasture and Grissel, 1975).

In endemic Hawaiian *Drosophila,* with the exception of a few cases of centric fusions in the *D. crassifemur* complex (Yoon *et al.*, 1975), evidence from extensive cytological studies by Clayton (1968, 1969) and Clayton *et al.* (1972) show that no major structural changes of this type have occurred in the course of the speciation process. Nonetheless, intra- and interspecific variation in metaphase karyotype due to the addition of extra heterochromatin can be found among the picture-winged species group. Within the *grimshawi* subgroup both *D. formella* (Baimai, 1975a) and *D. recticilia* (Baimai, 1977) exhibit a polymorphism for additional heterochromatin on one metaphase chromosome which is correlated with an inversion polymorphism. One of the species discussed here, *D. disjuncta,* shows a similar situation with respect to heterochromatin but this is limited to one population in Kipahulu Valley (Baimai, 1975b).

The present study, of course, documents a case of interspecific variation in heterochromatin on all metaphase chromosomes. It may be

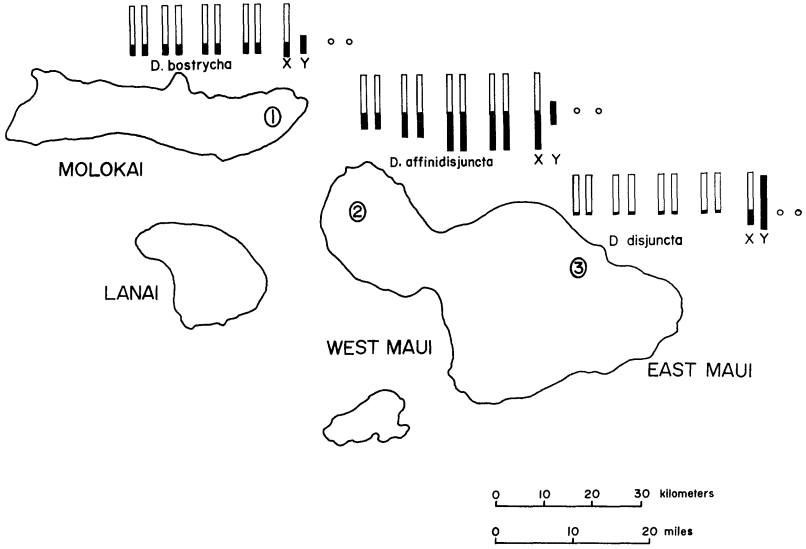

Fig. 2.—Map of the Maui island complex of the Hawaiian Archipelago. Localities mentioned in text include: (1) Apee, East Molokai (*D. bostrycha*); (2) Kaulalewelewe, West Maui (*D. affinidisjuncta*), and (3) Waikamoi, East Maui (*D. disjuncta*). The metaphase karyotype of each species is shown in the accompanying diagram. Heterochromatin is indicated in black

suggested that cytological differentiation involving heterochromatic material could play a significant role, at least at certain stages, during the actual process of species divergence itself. The presence of an extra heterochromatic portion might provide a flexibility for adaptation and possibly for a new genetic potential in various environmental niches. Although precise mechanisms for the origin of extra heterochromatin in one or all chromosomes of the complement are still obscure, this kind of cytological differentiation has been very useful in the recognition of the taxa studied here as separate species. This investigation suggests the potential value of cytological data in taxonomy, especially in the homosequential and morphologically similar species of the endemic Hawaiian *Drosophila*.

The presence of strong reproductive isolation is usually taken as conclusive evidence for genetic divergence between biological species. Hybridization tests among the three homosequential species discussed here show that there is reproductive isolation at the postmating level. The F_1 male hybrids were completely sterile in all cases. These results confirm the full-fledged species status for *Drosophila affinidisjuncta* from West Maui.

Evidence presented in this report shows that each of the three allopatric populations may be regarded as full species. Additional supporting evidence comes from a study of electrophoretic variability made by Rockwood *et al.* (1971). They reported a significant difference in esterase-β phenotypic frequencies as well as the heterozygosity value between the western and eastern Maui populations of what was then called *Drosophila disjuncta*. At this stage it is impossible to draw conclusions as to the actual pathways of chromosomal changes involving heterochromatin and the direction of species divergence. Further cytogenetic, electrophoretic and behavioral investigations of this unique species complex are required to elucidate the problem. Detailed accounts of the intra- and interspecific variability and microevolution of these remarkably close taxa will be presented in later communications.

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