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Metaphase Configurations in Drosophila: A Comparison of Endemic Hawaiian Species and Non-Endemic Species

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ABSTRACT

The metaphase configurations of 400 strains from 63 species of Hawaiian *Drosophila* were determined from squash preparations of larval brain tissue or spermatogenic cells from adult testes. These karyotypes include configurations from seven species not previously described. Metaphases of 148 Hawaiian species have been recorded, including species of the "picture-wing" group, the "modified mouthpart" group, and the "bristle-foot" group. A comparison between Hawaiian species and non-endemic species was made on the basis of chromosome numbers and configurations. Among the Hawaiian species, 85.8% have retained the primitive haploid configuration of five rods and one dot compared with only 34.8% of species from the rest of the world. In only 4.7% of Hawaiian species is the chromosome number reduced from the basic haploid number of six, whereas it is reduced in 47.6% of the species from other areas. Most of the changes in chromosome size and shape among the Hawaiian species seem to be the result of added heterochromatin or chromosome fusions; no evidence of pericentric inversions has been found in modified karyotypes.

INTRODUCTION

Wheeler and Hamilton (1972) tabulated the valid species in the genus *Drosophila* and reported that one-fourth of a total of 1,254 described species are from the islands of Hawaii. Hardy (1974) estimated that the total fauna in the family Drosophilidae in Hawaii may consist of 750 to 800 different species. Before 1963, almost no information was available on the genetics or cytology of the Hawaiian species of *Drosophila*. At that time, the University of Hawaii and the University of Texas began sponsoring a research project which involved several senior investigators studying various aspects of the evolution and genetics of the Drosophilidae of Hawaii. A summary of the major accomplishments through the first few years was given in a review article by Carson et al. (1970). A symposium on the "Evolution in the Hawaiian Drosophilidae," presented at the XIVth International Congress of Entomology in 1972 (see White 1974), provided background information and described achievements in such areas as cytology, mating behavior, morphology, reproductive isolation, habitat selection, and competition. The present study reports karyotype findings from 1972 to 1976, bringing the total number of metaphases described from 141 to 148 different Hawaiian species in the genus *Drosophila*. Prior to this report, metaphases were described by Clayton (1966, 1968, 1969, 1971), Carson et al. (1967), and Clayton et al. (1972).

MATERIALS AND METHODS

Metaphase configurations were determined from spermatogenic cells of adult males or from cells of larval brains. Tissues were stained in aceto-orcein and transferred to 50% acetic acid for squash preparation. Adults were collected from localities on Oahu, Kauai, Maui, Molokai, Lanai, and Hawaii and brought into the laboratory where females were placed singly into vials of a special high-protein medium (Wheeler and Clayton 1965) to establish "iso-female" lines. Third instar larvae from these iso-females were used for the cytological study. If larvae were not available, adult males of the species were dissected; the testes were removed and stained for examination of spermatogonia or primary spermatocytes. Species collected in the wild as larvae were maintained in the laboratory until mature enough for dissection and cytological study.

RESULTS AND DISCUSSION

Metaphase configurations were recorded from larvae of iso-female lines, from larvae collected in the wild, and from spermatogenic material of adult males. The results of the chromosome analyses are given in Table I. Included in the tabulation are configurations of 400 strains from 63 species of Hawaiian *Drosophila* which were analyzed during the period 1972-1976 and metaphases from seven species not previously described. Among the latter are five species undescribed at the time of the chromosome analyses, *D. digressa, D. gynmophallus, D. lasiopoda, D. psilotarsalis,* and *D. differens* (Hardy and Kaneshiro 1972a, b), and two species not previously analyzed cytologically, *D. anomalipes* (Hardy 1965) from Kauai and *D. cilifemorata* (Hardy 1965) from West Maui. Larval material of *D. anomalipes* was made available for study by Dr. H.T. Spieth, who developed a technique for raising this species in the laboratory. The metaphase configuration of *D. cilifemorata* was analyzed from primary spermatocyte cells of an adult male.

In Table II, a comparison is made between the metaphase configurations of species of Hawaiian *Drosophila* and those of species from other parts of the world. Hardy (1965) placed all Hawaiian *Drosophila* species into the subgenus *Drosophila* and the comparison therefore is based on Hawaiian species and non-endemic species belonging to this subgenus. The Hawaiian species which have been studied cytologically have been placed into groups based upon certain characteristics such as "picture-wing," "modified mouthpart," and "bristle-foot" groups. The numbers in Table II are derived from the listing of metaphase configurations by Clayton and Wheeler (1975) and from Table I.

The basic, or primitive, metaphase configuration in *Drosophila* consists of a haploid set of five rods and one dot. Speciation has been accompanied by modifications of this primitive karyotype, involving alteration of the number of chromosomes and/or change of chromosome size and shape. Patterson and Stone (1952) summarized the means by which such chromosome alterations could have occurred. A pericentric inversion results in a change in the shape of a metaphase chromosome if the position of the centromere is altered. Translocations result in detectable changes if there is a mutual exchange involving large segments of unequal length. A fusion results when there are two simultaneous breaks adjacent to centromeres on nonhomologous chromosomes and two long segments fuse. The centromere of this "translocated" chromosome is contributed by one of the long segments and the other centromeric fragment is either retained as a supernumerary chromosome or lost. In addition, the gain or loss of heterochromatic segments may account for changes in the appearance of somatic metaphase chromosomes.

The metaphase configurations listed in Table II are those which have been found among the Hawaiian species. For comparison the number of non-endemic species with similar configurations is given. Thirty-two percent of the non-endemic species have metaphase configurations not found among those Hawaiian species that have

been studied cytologically. The primitive configuration has been retained in 85.8% of the Hawaiian species but in only 34.8% of the species from other regions. In only 4.7% of the Hawaiian species is the chromosome number reduced from the basic haploid number of six, whereas it is reduced in 47.6% of the other species.

Among the Hawaiian species there has been no evidence of change in metaphase configurations resulting from pericentric inversions or translocations. The modifications may be explained by fusions, resulting in reduced numbers and V-shaped chromosomes, or by addition of heterochromatin. The latter type of change is the most common, found in both the "picture-wing" and "modified mouthpart" groups, but absent from the metaphase figures of the 17 other species examined. It can be seen from Table 2 that, within the "picture-wing" group, all species have retained the haploid number of six and modified karyotypes may be explained on the basis of heterochromatin added to dots or rods. One species, *D. cyrtoloma*, apparently has heterochromatin added to every chromosome in the set, which results in five V-shaped and one J-shaped chromosome. This configuration has not been described previously for any other *Drosophila* species. The karyotypes of six species within the "modified mouthpart" group have been altered from the primitive by fusions, the resultant configurations having one V-shaped chromosome (3R, 1V, 1D) or two V-shaped chromosomes (1R, 2V, 1D).

As is apparent in Table II, most of the species examined cytologically have been members of the "picture-wing" group. A chromosome phylogeny based on inversion differences was developed by Carson (Clayton et al. 1972) for 96 species of this species group. On the basis of this phylogeny, it appears that metaphase chromosome modifications of the species were distinct events rather than a type of speciation in which closely related species share chromosomal changes through a common ancestor. The situation seems to be different among the non-endemic *Drosophila* species. Stone (1962) discussed metaphase relationships among approximately 300 species that had been analyzed cytologically. Considering groups in which related species may share a common ancestral chromosome modification, he estimated that there had been 32 pericentric inversions, three translocations, 58 fusions, and 38 cases of added heterochromatin. Therefore, the percentages in Table II are probably too high for non-endemic species because no attempt was made to consider common ancestral configurations. A comparison of data on Hawaiian karyotypes with Stone's estimates reveals the conservative trend within the Hawaiian species. According to Stone, heterochromatin addition had occurred in approximately 12.5% of the species. Among the Hawaiian species this addition has been observed in 9.5%. Chromosome fusions have been found in 4.7% of the Hawaiian *Drosophila* compared with 19.3% of non-endemic species. These observations must be considered preliminary because the number of species available for cytological studies has been very limited except in the "picture-wing" group. Analysis of chromosome relationships among the different groups of the Hawaiian species can be expanded as additional species are cultured and studied in the laboratories.

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Table I. Karyotypes of Hawaiian Drosophilidae, 1972-1975

Species (Metaphase)	Locality and Collection No.
Drosophila	
adiastola (5R, 1D)	Puu Kukui, W. Maui (Q30G9) Waikamoi, Maui (Q33S3, S4) [1]Hanaula, W. Maui (R10M10; R82B5, B7, B8, B9) Waihoi Valley, Maui (R22G1)
aglaia (5R, 1D)	Puu Kaua, Oahu (P72G4)
**anomalipes* (5R, 1D)	Kokee, Kauai (T48B)
assita (5R, 1D)[2]	Near Moanuiahea, Hawaii (Q65F4) Moanuiahea, Hawaii (R4B11, B13, B14)
balioptera (5R, 1D)	Manawainui Gulch, W. Maui (Q34B1, G16) S. of Hanalilolilo, Molokai (R83B9)
bostrycha (5R, 1D)	S. of Hanalilolilo, Molokai (R83B14) Mapuleha Gulch, Molokai (Q86G2)
**cilifemorata* (5R, 1D)	Puu Kukui, W. Maui (Q30B**)
claytonae (5R, 1D)[2]	Olaa Forest Reserve, Hawaii (P105G8) Laupahoehoe Forest Reserve, Hawaii (Q57S10)
conspicua (5R, 1D)	Near Moanuiahea, Hawaii (Q65F10; Q66Q7) Keaa District, Hawaii (R1G1)
crucigera (5R, 1D)	Mokuleia Rd. to Kaena Pt., Oahu (P95G1, G2) Near Pali Lookout, Oahu (Q26R2)
cyrtoloma (5V, 1J)	Waikamoi, Maui (Q52B100; R9M22)
**differens* (5R, 1D)	S. of Hanalilolilo, Molokai (Q84G9)
**digressa* (5R, 1D)	[3]Olaa Forest Reserve, Hawaii (P105G**, Q49G24) [4]Olaa Forest Reserve, Hawaii (Q49G α; Q55G α1)
discreta (5R, 1D)	Waikamoi, Maui (R9M5, M12)
fasciculisetae (5R, 1D)	Waikamoi, Maui (R9M6)
flexipes (5R, 1D)	Wailupe Gulch, Oahu (Q24Q3, Q4)
formella (5R, 1D)[5]	Pauahi, Kona, Hawaii (Q17F4, F5, G6, J2) Near Moanuiahea, Hawaii (R5S23)
gradata (5R, 1D)	Mokuleia Jeep Rd., Oahu (P77-#1) Puu Kaua, Oahu (Q12G α1)
grimshawi (5R, 1D)	Kawela Gulch, Molokai (Q7Q14, Q15; Q81G28, G29, G30, G31) Near Kawela Gulch, Molokai (Q82Q5) S. of Hanalilolilo, Molokai (Q84G6; R83B10, B11, B12, B13) Kaiholena Gulch, Lanai (Q20Q1, Q2; Q20Q β(1), γ(1), Δ(1) Manawainui Gulch, W. Maui (Q32S2, G6; Q34G8, G17, G18, G20, G21; Q80B4) Pohakia Gulch, W. Maui (Q35G3, G4, G5, G7, G8) Kahuahua Gulch, Maui (Q37G5, G9, G10) Halemanu Valley, Kauai (Q76M3)
gymnobasis (5R, 1D)[1,2]	Auwahi, Maui (R8B16, B α(1)
**gynmophallus* (5R, 1D)	Puu Pane, Oahu (P24Q4) Makaleha Valley, Oahu (L92Q1)
hawaiiensis (5R, 1D)	Kilauea Forest Reserve, Hawaii (P104G1) Hanaula, W. Maui (R10M10; R96Q1) Puuwaawaa Summit, Hawaii (Q75Q1) Laupahoehoe Forest Reserve, Hawaii (Q57M5)
musaphilia (5R, 1D)	Halemanu Valley, Kauai (Q76M1)
neopicta (5R, 1D)	Paliku, Haleakala, Maui (Q51B) Hanaula, W. Maui (Q79M36, M37)
nigribasis (5R, 1D)	Mt. Kaala, Oahu (Q96B4) Konahuanui Peak, Oahu (Q11-1a; Q11Q α(1a)
oahuensis (5R, 1D)	Mt. Kaala, Oahu (Q96B9) Kahana Valley, Oahu (Q23Q α(1)
obscuripes (5R, 1D)	Paliku, Haleakala, Maui (Q51F2)
ochracea (5R, 1D)	Keaa Forest, Hawaii (Q53F1, G3) Puna Forest Reserve, Hawaii (R13Q1)
ochrobasis (5R, 1D)[2]	Kipuka 9, Saddle Rd., Hawaii (Q15G2; Q46G1, G2, G3, Q46F α(1-2); Q69F1) Kipuka 14, Saddle Rd., Hawaii (Q88F14, F15, F16, G15; R16M2, M3, M4)
odontophallus (5R, 1D)	Auwahi, Maui (R8M11, M12) Manawainui Gulch, W. Maui (Q32B1, B4, B7, B8, B9, G1, G2, G3, S3, G α(5-6), B α(5)
ornata (5R, 1D)	Mt. Kahili, Kauai (Q78B2, B3)
orphnopeza (5R, 1D)	Waihoi Valley, Maui (R22G α(1,2), γ(13), β(1-4)
orthofascia (5R, 1D)	Kawaipapa Gulch, Hana Forest Reserve, Maui (Q25Q1, Q2, Q4, Q5) Auwahi, Maui (R8B1, B4, B5, D2, D4) Kaoholena Gulch, Lanai (Q20Q ε (1-3)
paenehamifera (5R, 1D)	Trail to Puu Kukui, W. Maui (Q30B3)
paucipuncta (5R, 1D)	Olaa Forest Reserve, Hawaii (P105G4), G6, G7, MSC)
peniculipedis (5R, 1D)[2]	Hanaula, W. Maui (R10B13, B14, B15, B16, B17)
picticornis (5R, 1D)	Halemanu Valley, Kauai (Q76B α(1-2); Q76B β(1-3)
planitibia (5R, 1D)	Waikamoi, Maui (R63B2) Hanaula, W. Maui (R92B3)
primaeva (5R, 1D)	Mt. Kahili, Kauai (Q78B4, B5, B6)
**psilotarsalis* (5R, 1D)	Near Moanuiahea, Hawaii (R5B3)
recticilia (5R, 1D)	Kahuahua Gulch, Kaupo Gap, Maui (Q37B α(1-27)
setosifrons (5R, 1D)	Olaa Forest Reserve, Hawaii (P105G2, G5)
setosimentum (5R, 1D)	Kipuka 9, Saddle Rd., Hawaii (Q15G3) Laupahoehoe, Hawaii (Q57M1) Haleuanui, 2200', Hawaii (R96G1) [3]Pauahi, Hawaii (R2S2, S3, S4) [3]Pawaina, Hawaii (R3G1, G2, G4) Kipuka at 4140', Hawaii (Q58M1, M2, M3; Q70Q1, M1, M2, M3, M4, M6) [4]Moanuiahea, Hawaii (Q64B1, B2, B3, M1; R4B3; B4, B5, B6, B7, B8, B10; Q66Q2) Kipuka 14, Saddle Rd., Hawaii (Q68F2; Q88Q1) Olaa Forest Reserve, Hawaii (Q43G28, G29; Q49F3, F4, F7, G2, G3, G4, G5, G6, G7, G8, G10, M2, M3, M4, M5, M7, M8, M9, M10; Q60G3, G4, G5, G6, G7; Q55F1, F2, F3, F4, G4, M2, M3, M4, M5, M8, M12; Q56G2,3,4 G3, G4, G6, G8, G9; Q71G1, G2, G3, G7, G8, G9, G10, M1, M4, M5, M7, M12, M13, M14, S7; Q74S1, S3, S4, S5, S6, S9, S11, S13, S14, S16, S17)

	Near Moanuiahea, Hawaii (Q65B13; R5S4, S5, S7, S10, S12, S15) Honaunau Forest Reserve, Hawaii (R6B4, B5, S4, S5, S7, S10, S12, S15) Papaloa, Hawaii (R7B7) Kipuka Ki, Hawaii (Q91Q α(1-3) Poliokeawe Pali, Hawaii (Q95Q α) Kipuka Puaulu, Hawaii (Q72S1)
heedi (6R)[1]	Ahumoa, Hawaii (P97G1, G2, B λ[1], B λ[2], B λ[3], B μ[2] , B**; Q44BΥ) Kipuka near Puukole, Hawaii (P101Q α2) Pohakuloa St. Park, Hawaii (P103G α)
heteroneura (5R, 1D)*	Pauahi, Kona, Hawaii (Q17G2; R79G1, G4, G5) Keahou Ranch, Hawaii (R60G50, G75, G76; R71G1)
hirtipalpus (5R, 1D)[1]	Waikamoi, Maui (Q33S6; Q52M16, M17, J3; Q79M32)
**lasiopoda* (6R)*	Waikamoi, Maui (Q52B2, J3, J8, J9, J10, J11, J13, M15)
limitata (6R)	Waikamoi, Maui (Q52B12) Manawainui Gulch, W. Maui (Q80B2, B3)
lineosetae (5R, 1D)	Hanaula, W. Maui (Q79B1)
liophallus (5R, 1D)	Kawela Gulch, Molokai (Q7Q2) Manawainui Gulch, W. Maui (Q34B3, G9, G12, G13; Q32 α(1-5), Q32B α(1,3)
macrothrix (5R, 1D)	Olaa Forest Reserve, Hawaii (Q55G α(2)
melanocephala (5R, 1V)	Waihoi Valley, Maui (R22G)
montgomeryi (6R)	Puu Kaua, Oahu (P72Q1, Q4, Q5, G7, G8)
murphyi (5R, 1D)	Olaa Forest Reserve, Hawaii (P105G9) Pauahi, Kona, Hawaii (Q17F8, G9, G10, G11; R2B12, B13, B16, B17, G3) Moanuiahea, Hawaii (Q64B5) Near Moanuiahea, Hawaii (Q65F11; R5F5, B5) Honaunau Forest Reserve, Hawaii (R6B3) Keahou Ranch, Hawaii (R60G55)
silvarentis (5R, 1D)[1]	Kilauea Forest Reserve, Hawaii (Q48G1, G3, G5) Humuula Saddle Road, Hawaii (P102G1, G5, B3) Papa Kapa, Hawaii (Q10Q7, Q8) Near Moanuiahea, Hawaii (Q65B3, B7, B8, B9, B10, B11) Papaloa, Hawaii (R7B1, B2, B3, B5) Pauahi, Kona, Hawaii (Q17J1) Ahumoa, Hawaii (P97B β, P97B**, P97Gθ, P97GΔ, P97Gβ, P99Bβ, P97Bμ[1], P97Bα, P97BΥ; Q44BΔ1, Bα5, Bβ, Q44Gβ2, Q44ψ2, Q44Gψ1; Q45Bα1)
silvestris (5R, 1D)	Olaa Forest Reserve, Hawaii (P105G3)
sobrina (5R, 1D)	Kahana Valley, Oahu (Q23Q α3)
sodomae (5R, 1D)	Kawela Gulch, Molokai (Q7Q13; Q82Q2, Q3) Kahuahua Gulch, Maui (Q37QB)
spectabilis (5R, 1D)[1]	Hanaula, W. Maui (R10M1)
sproati (5R, 1D)	Honaunau Forest Reserve, Hawaii (R6B2) Kipuka at 4140', Hawaii (Q70Q(1)
substenoptera (5R, 1D)	Makaha, Waianae, Oahu (P74**)
turbata (5R, 1D)[1,3]	Wailupe Gulch, Oahu (Q24Q6) Kaunala Gulch, Oahu (Q22B α(1a)(2a)(3); Q22β(1-10)
villitibia (5R, 1D)[2]	Kawela Gulch, Molokai (Q81G7)

*Metaphase for this species reported here for first time.
**Metaphase determination from adult male.
[1] One rod double-length, or longer.
[2] One rod longer, not double-length.
[3] Two rods longer, not double-length.
[4] Two rods half-length.
[5] Large dots.
[6] Very small dots.

Table II. Comparison of Metaphase Configurations of Hawaiian *Drosophila* and Non-Endemic Species Belonging to the Subgenus *Drosophila*

Haploid Karyotypes	Species Endemic to Hawaii						Non-endemic Species*	
	"picture-wing"	"modified mouthpart"	"bristle foot"	Other	Total No.	Total %	No.	%
Primitive:								
5R, 1D	92	19	2	14	127	85.8	85	34.8
Fusion:								
3R, 1V, 1D	0	3	0	0	3	2.0	26	10.7
1R, 2V, 1D	0	3	0	1	4	2.7	27	11.1
Added heterochromatin:								
6R	6	4	0	0	10	6.7	10	4.1
5R, 1V	1	0	0	0	1	0.7	4	1.6
5V, 1J	1	0	0	0	1	0.7	0	0.0
4R, 1V, 1D	1	0	0	0	1	0.7	13	5.3
4R, 1J, 1D	0	1	0	0	1	0.7	1	0.4
4R, 1J, 1D	0	1	0	0	1	0.7	1	0.4
Other:	0	0	0	0	0	0.0	78	32.0
total	101	30	2	15	148		244	

*Numbers taken from tabulation of species in Clayton and Wheeler (1975) and Clayton (this publication).