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**Genetics of speciation in the *Aedes (Stegomyia) scutellaris* subgroup  
(Diptera:Culicidae). 4. Chromosomal relationships of *Aedes cooki* with  
four sibling species<sup>1</sup>**

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Morphology and behavior of chromosomes and development of testes and sperm were examined in hybrids from interspecific crosses involving *Aedes cooki* and four sibling species of the *Aedes (Stegomyia) scutellaris* subgroup of mosquitoes. The degree of abnormality in hybrid spermatogenesis in interspecific crosses involving *Aedes cooki* males and females of four sibling species paralleled the geographic distributions of these species and the genetic divergence indicated by other genetic studies. Hybrids from crosses involving *Aedes malayensis* females and *Aedes cooki* males were characterized by atrophied testes and extensive chromosome breakage. Hybrids from crosses involving *Aedes alcasidi* females and *Aedes cooki* males suggested a possible pericentric inversion distinguishing the largest autosome of *Aedes alcasidi* from that of *Aedes cooki*. Hybrids from interspecific crosses involving females of *Aedes polynesiensis* and *Aedes pseudoscutellaris* and males of *Aedes cooki* showed high percentages of univalents of the smallest chromosome pair. Hybrid spermatogenesis in two interspecific crosses involving *Aedes cooki* females differed from results of reciprocal crosses. Data were scant, however, and interpretation was difficult in view of negligible hatch in all interspecific crosses involving *Aedes cooki* females.

*Key words:* *Aedes*, inversion, phylogeny, hybrid dysgenesis.

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On a étudié la morphologie et le comportement des chromosomes et le développement des testicules et du sperme chez les hybrides de croisements interspécifiques entre *Aedes cooki* et quatre espèces issues des mêmes parents du sous-groupe de moustiques *Aedes (Stegomyia) scutellaris*. Il y avait corrélation entre le taux d'anomalie de la spermatogenèse hybride dans les croisements interspécifiques impliquant les mâles et femelles d'*Aedes cooki* de quatre espèces issues de mêmes parents et la distribution géographique de ces espèces ainsi que la divergence génétique signalée dans d'autres travaux génétiques. Les hybrides de croisements impliquant des spécimens femelles d'*Aedes malayensis* et des spécimens mâles d'*Aedes cooki* se caractérisaient par des testicules atrophiés et un taux élevé de bris de chromosomes. Les hybrides de croisements impliquant des spécimens femelles d'*Aedes alcasidi* et des spécimens mâles d'*Aedes cooki* ont suggéré la présence probable d'une inversion péricentrique permettant la distinction entre le plus gros autosome d'*Aedes alcasidi* et celui d'*Aedes cooki*. Les hybrides de croisements interspécifiques impliquant des femelles d'*Aedes polynesiensis* et d'*Aedes pseudoscutellaris* et des mâles d'*Aedes cooki* ont révélé des taux élevés d'univalents de la plus petite paire de chromosomes. La spermatogenèse hybride de deux croisements interspécifiques impliquant des femelles d'*Aedes cooki* a donné des résultats différents de ceux de croisements réciproques. Cependant les données étaient limitées et le travail d'interprétation difficile étant donné le faible taux d'éclosion de tous les croisements interspécifiques impliquant les femelles d'*Aedes cooki*.

*Mots clés:* *Aedes*, inversion, phylogénie, dysgénésie des hybrides.

[Traduit par le journal]

### Introduction

*Aedes cooki* Belkin belongs to the *Ae. (Stegomyia) scutellaris* subgroup of mosquitoes, a complex of approximately 30 sibling species distributed from Taiwan south and east to Tahiti (Huang 1972; Huang and Hitch-

cock 1980). All species in the group are allopatric in relation to *Ae. cooki*, including those used in the present study. *Aedes cooki* occurs only on a few small islands in the Tonga group of Polynesia, while the Tahitian strain of *Ae. polynesiensis* Marks represents the eastern range of this widespread Polynesian species. *Aedes pseudoscutellaris* Theobald is found on the Fiji Islands of Melanesia, *Ae. malayensis* Colless occurs in south-east Asia and Taiwan, and *Ae. alcasidi* Huang is found

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on Taiwan and the Philippines (Huang and Hitchcock 1980).

Electrophoretic data (Pashley and Rai 1983) and crossing relationships (Hoyer and Rozeboom 1977; Dev and Rai 1982) indicated a geographic gradient of genetic compatibility in the *Ae. scutellaris* subgroup. Sherron and Rai (1983) correlated high hatch rates with geographic proximity in interspecific crosses involving *Ae. cooki* and *Ae. malayensis*, *Ae. alcasidi*, *Ae. polynesiensis*, and *Ae. pseudoscutellaris*. Low hatch rates in some backcrosses were considered the result of strong genetic divergence between *Ae. cooki* and geographically remote species such as *Ae. malayensis* and *Ae. alcasidi*.

Chromosomal relationships in the genus *Aedes* have been described to some extent. Rai (1963) found that the basic complement of one small pair and two relatively larger pairs was conserved across several subgenera, though there were some differences in chromosomal lengths and centromeric positions between species. Motara and Rai (1978) found by Giemsa C-banding that certain members of the *Ae. scutellaris* subgroup, *Ae. (Stegomyia) aegypti* L., and *Ae. albopictus* Skuse had an intercalary band on the putative female-determining chromosome; as well, all chromosomes had centromeric bands.

Few studies have focused on meiotic homologies in interspecific hybrids of mosquitoes. Rooney (1980) attributed meiotic aberrations in interspecific hybrids in the *Ae. scutellaris* subgroup to chromosomal differences between species. Motara and Rai (1977) found that differences in heterochromatin activation between *Ae. aegypti* and *Ae. (Stegomyia) mascarensis* led to intersex formation in some interspecific hybrids. L. J. Szymczak (unpublished data) reported that *Ae. (Ochlerotatus) epactius* and the closely related *Ae. atropalpus* differed by three paracentric inversions and one pericentric inversion. Sherron and Rai (1983) demonstrated strong genetic compatibility and chromosomal homology in hybrids of *Ae. cooki* with *Ae. kesseli* Huang and Hitchcock, in spite of significant differences in chromosomal lengths between the species.

The purpose of the present study was to examine chromosomal differentiation between *Ae. cooki* and four sibling species: *Ae. malayensis*, *Ae. alcasidi*, *Ae. polynesiensis*, and *Ae. pseudoscutellaris*. This was accomplished by studying the following: (i) testicular development in interspecific hybrids and parental strains; (ii) relative chromosomal lengths in *Ae. cooki* and *Ae. alcasidi*, which represents the western extreme of the *Ae. scutellaris* distribution; (iii) mean chiasmata frequencies per primary spermatocyte in interspecific hybrids and parental strains; (iv) chromosomal evidence of hybrid dysgenesis or breakdown in re-

TABLE 1. Percentages of pupae with normal and atrophied testes in crosses involving *Ae. cooki* and four sibling species

Cross (♀ × ♂) <sup>a</sup>	No. of pupae	Testes development	
		Both normal	One or both atrophied
C × C	71	85.9	14.1
Po × Po	32	87.5	12.5
Ps × Ps	25	92.0	8.0
A × A	40	90.0	10.0
M × M	73	92.2	7.8
Po × C	97	98.2	1.8
C × Po	23	30.4	69.6
Ps × C	56	98.2	1.8
C × Ps	17	58.8	41.2
A × C	41	34.1	65.9
C × A	10	50.0	50.0
M × C	158	9.5	90.5
C × M	8	25.0	75.0
F <sub>1</sub> (M/C) × C	42	92.9	7.1
C × F <sub>1</sub> (M/C)	19	84.2	15.8
F <sub>1</sub> (M/C) × M	17	0.0	100.0
M × F <sub>1</sub> (M/C)	23	8.7	91.3

<sup>a</sup>Frequencies differed significantly between crosses, based on a G-test independence evaluated at  $p = 0.05$ .

productive development, in the form of univalents and chromosome breakage; (v) chromosomal homologies in interspecific hybrids as indications of structural heterozygosity (e.g., inversions) between species. Data were integrated with other genetic information to detect a possible chromosomal basis of reduced fertility in interspecific crosses involving *Ae. cooki* and these four sibling species.

### Material and methods

One strain each of four species of the *Ae. scutellaris* subgroup was crossed with *Ae. cooki*. Three stocks were obtained from the World Health Organization International Reference Center for *Aedes*, Vector Biology Laboratory, University of Notre Dame. They were as follows: (i) *Ae. malayensis* (M), collected in 1968 from Prachnap, Khiri Khan Province, Thailand; (ii) *Ae. polynesiensis* (Po), collected in 1969 in Tahiti; and (iii) *Ae. alcasidi* (A), collected in 1972 at Peiyun, Tung-ho, Taiwan. *Aedes pseudoscutellaris* (Ps) was obtained from a laboratory colony of the Vector Research Unit, Public Health Office, in Suva, Fiji, in 1980. The Johns Hopkins University provided an autogenous strain of *Ae. cooki* (C) collected on Niue Island near Tonga in 1973.

Colonies, reciprocal interspecific crosses, and backcrosses involving *Ae. cooki* consisted of mass matings of 48–100 virgin females and equivalent numbers of males in 3.8–L cages containing paper cups with wet paper towels for oviposition. Females were mated 2–3 days postemergence. The rearing of all species was done in an insectary maintained at  $25 \pm 2^\circ\text{C}$  and  $80 \pm 5\%$  relative humidity.

Two days after the establishment of the mating cages, fe-

TABLE 2. Mean chiasmata frequencies per primary spermatocyte in crosses involving *Ae. cooki* and four sibling species

Cross (♀ × ♂)	No. of pupae examined	No. of cells examined	Mean chiasmata frequency		
			I	II/III	$\bar{X}$ total ± SE
C × C	16	611	1.05	1.40	3.84 ± 0.449
Po × Po	5	145	1.23	1.65	4.53 ± 0.990 <sup>a</sup>
Ps × Ps	6	180	1.17	1.18	3.52 ± 0.316
A × A	5	209	1.20	1.46	4.13 ± 0.265
M × M	8	284	1.30	1.61	4.51 ± 0.378 <sup>a</sup>
Po × C	15	624	0.829	1.41	3.66 ± 0.866
Ps × C	6	278	0.891	1.25	3.39 ± 0.335 <sup>a</sup>
C × Ps <sup>b</sup>	3	48			2.10 ± 0.624 <sup>a</sup>
A × C	10	94			2.07 ± 0.686 <sup>a</sup>
M × C	5	22			3.06 ± 1.50 <sup>a</sup>
C × M	3	24			4.10 ± 0.721
F <sub>1</sub> (F/M) × C	4	122			4.15 ± 0.288
C × F <sub>1</sub> (M/C)	8	102			4.41 ± 0.844 <sup>a</sup>

<sup>a</sup>Significantly different from C × C, based on *t*-tests for equal means evaluated at *p* = 0.05.

<sup>b</sup>Identities of individual chromosomes not distinguishable in this and subsequent crosses.

TABLE 3. Percentages of cells with chromosome breakage and univalents in crosses involving *Ae. cooki* and four sibling species

Cross (♀ × ♂)	No. of cells examined	% chromosome breaks	% univalents		Total % abnormal cells
			I	II/III	
C × C	611	3.6	11.0	1.9	16.4
Po × Po	145	1.4	11.9	1.5	14.8
Ps × Ps	180	0.6	2.2	0.9	3.7 <sup>a</sup>
A × A	209	1.9	1.9	0.7	4.5 <sup>a</sup>
M × M	284	3.2	5.6	1.3	10.1 <sup>a</sup>
Po × C	624	1.8	36.2	6.0	40.8 <sup>a</sup>
Ps × C	278	4.3	26.3	5.1	35.6 <sup>a</sup>
			I/II/III <sup>b</sup>		
C × Ps	57	91.2	7.0		98.2 <sup>a</sup>
A × C	161	29.8	35.4		60.0 <sup>a</sup>
M × C	71	70.4	42.3		81.7 <sup>a</sup>
C × M	85	8.1	8.1		16.2
F <sub>1</sub> (M/C) × C	218	2.8	3.2		6.0 <sup>a</sup>
C × F <sub>1</sub> (M/C)	166	4.2	6.6		10.8 <sup>a</sup>

<sup>a</sup>Significantly different from C × C at *p* = 0.05, based on *t*-tests of total percentages of abnormal cells.

<sup>b</sup>Individual chromosomes not identifiable in this and subsequent crosses.

males were bloodfed. Crosses were maintained until oviposition nearly ceased. The egg papers obtained were embryonated in the insectary for 2 days and stored in an egg chamber maintained at 13°C. After 5–7 days, eggs were hatched in deoxygenated water containing small amounts of liver powder.

Male pupae (12–18 h old) from colonies of *Ae. cooki* and from interspecific crosses involving *Ae. cooki* were dissected in distilled water and their testes squashed in 1% acetolactic orcein stain. Cells in prophase I from each pupa were scored for univalents, chromosomal breaks, and chiasmata frequencies. The presence of univalents was considered abnor-

mal because homologous chromosomes in *Aedes* are paired even in somatic cells and prepachytene spermatocytes (Rai 1963). Where possible, percent univalents and chiasmata frequencies were scored separately in the smallest chromosome pair and the larger pairs. Available mitoses were scored for chromosomal breaks as well. Testes were scored according to whether they appeared atrophied or developed. Photomicrographs were taken at 100× under oil immersion using a Zeiss microscope and camera with Panatomic-X black and white film. Prints were made at a total magnification of 2000×.

For chromosome measurements, a photomicrograph of the

TABLE 4. Comparison of chromosome lengths and arm ratios between *Ae. cooki* and *Ae. alcasidi*

Species	No. of cells examined	Mean chromosome lengths ( $\mu\text{m}$ ) and arm ratios											
		I				II				III			
		Arm a	Arm b	Total	Arm a/ arm b	Arm a	Arm b	Total	Arm a/ arm b	Arm a	Arm b	Total	Arm a/ arm b
<i>Ae. cooki</i>	27	3.44	3.48	6.92	0.99	4.97	4.96	9.93	1.00	4.63	4.17	8.80	1.11
<i>Ae. alcasidi</i> *	10	4.98	4.95	9.93†	1.01	7.92	7.91	15.83†	1.00	6.81	6.80	13.61†	1.00

\*Based on data from V. Dev and K. S. Rai (unpublished data).

†Indicates significant differences between species based on *t*-tests for equal means evaluated at  $p = 0.05$ .

ocular micrometer was taken at the same magnification as that of the chromosome preparations. A print was made and used as a scale to measure the lengths of individual chromosomes from the photomicrographs. Measurements were made from 27 somatic metaphases in *Ae. cooki* and were compared with similar measurements in *Ae. alcasidi* recorded by V. Dev and K. S. Rai (unpublished data).

Data were analyzed by the methods of Sokal and Rohlf (1969). Frequencies of atrophied and developed testes were compared for independence between crosses using the *G*-test. Percentages of abnormal cells, mean cell chiasmata frequencies, and chromosomal lengths were compared with equivalent data for parental *Ae. cooki*, using *t*-tests for equality of means. All statistics were evaluated at the 0.05 level of significance.

### Results

Table 1 shows percentages of pupae with normal and atrophied testes in parental species and interspecific crosses involving *Ae. cooki*. Statistical analysis revealed significant differences between crosses in frequencies of pupae with normal versus atrophied testes ( $p < 0.05$ ). *Aedes cooki* had 85.9% pupae with both testes normal in size. Percentages of pupae with normal testicular development in interspecific crosses ranged from 9.5 (M ♀ × C ♂) to 98.2% (Po ♀ × C ♂, Ps ♀ × C ♂). Pupae from backcrosses involving F<sub>1</sub> (M/C) and *Ae. cooki* had high percentages of normal testes (84.2 and 92.9%). Pupae from backcrosses involving F<sub>1</sub> (M/C) and *Ae. malayensis* had low percentages of normal testes (0.0 and 8.7%).

Table 2 lists mean chiasmata frequencies per primary spermatocyte in parental species and interspecific crosses involving *Ae. cooki*. Means for individual chromosomes are listed for parental strains and crosses involving females of *Ae. polynesiensis* and *Ae. pseudoscutellaris* and males of *Ae. cooki*. There was considerable variation among pupae in several parental colonies and interspecific hybrids (e.g., C ♀ × C ♂, Po ♀ × C ♂) and was incorporated in tests for statistical significance. The mean chiasmata frequency per cell in *Ae. cooki* was 3.84; *Ae. polynesiensis* and *Ae. malayensis* were significantly different from *Ae. cooki* among parental strains with mean chiasmata frequencies of 4.53 and 4.51, respectively. Five interspecific crosses showed significantly different mean chiasmata frequencies from *Ae. cooki*. They were C ♀ × Ps ♂ (2.10), Ps ♀ × C ♂ (3.39), A ♀ × C ♂ (2.07), M ♀ × C ♂ (3.06), and C ♀ × F<sub>1</sub> (M/C) ♂ (4.41).

Table 3 lists percentages of abnormal cells in pupal testes from parental colonies and interspecific crosses involving *Ae. cooki*. Cells in stages other than prophase I or metaphase I are included in crosses in which dividing cells were rare (e.g., M ♀ × C ♂). Two classes of chromosomal abnormalities are emphasized, univalents and chromosomal breaks. Univalents are also

listed for individual chromosomes in parental strains and crosses involving females of *Ae. polynesiensis* and *Ae. pseudoscutellaris* and males of *Ae. cooki*. Four crosses had too few dividing cells to yield meaningful data. They were C ♀ × Po ♂, C ♀ × A ♂, F<sub>1</sub> (M/C) ♀ × M ♂, and M ♀ × F<sub>1</sub> (M/C) ♂. Data are pooled in each cross because cytological abnormalities observed in a given cross were observed in all pupae in that cross and some hybrids (e.g., M ♀ × C ♂) showed too few primary spermatocytes per individual to permit meaningful within-group comparisons.

All parental strains had significantly fewer abnormal cells than *Ae. cooki* with the exception of *Ae. polynesiensis* (14.8%). The presence of univalents of the smallest chromosome was the most common abnormality in parental strains, reaching 11.9% of all spermatocytes in *Ae. polynesiensis*. Percent abnormal cells in interspecific crosses ranged from 6.0% in the cross F<sub>1</sub> (M/C) ♀ × C ♂ to 98.2% in the cross C ♀ × Ps ♂. Percentages of cells showing univalents ranged from 3.2% in the cross F<sub>1</sub> (M/C) ♀ × C ♂ to 42.3% in the cross M ♀ × C ♂. Breakage in chromosomes in hybrids ranged from 1.8 (Po ♀ × C ♂) to 91.2% (C ♀ × Ps ♂).

Table 4 lists mean chromosomal lengths and arm ratios for *Ae. cooki* and *Ae. alcasidi*. In both species,  $2n = 6$  with a small metacentric pair and two large metacentric pairs. These are denoted by I, II, and III, respectively, in keeping with the nomenclature for *Ae. aegypti*. Arm ratios were approximately 1.0 for all chromosomes of both species. All somatic chromosomes of *Ae. alcasidi* were significantly longer than corresponding chromosomes of *Ae. cooki*, with total chromosome lengths of 25.65 μm in *Ae. cooki* and 39.37 μm in *Ae. alcasidi*.

Figures 1a and 1b show diakinesis cells of *Ae. cooki* and *Ae. malayensis*, respectively. Figures 1c–1f show chromosomes from pupal testes from the cross, M ♀ × C ♂. Figures 1c and 1e show chromosome breakage in diplotene cells, while Fig. 1f shows breakage of somatic chromosomes in the same cross. Figure 1d shows heteromorphic bivalents in a diplotene cell. Figure 1g shows an anaphase I bridge from the cross F<sub>1</sub> (M/C) ♀ × C ♂.

Figures 1h–1j show meiotic cells and spermatids from the cross C ♀ × M ♂. Figures 1h and 1j show diakinesis and diplotene cells, respectively, with obvious length differences between paired chromosomes. Figure 1i shows normal spermatids from the cross C ♀ × M ♂. This cross had relatively more pupae with normal testes and normal chromosomes than did the reciprocal cross. The few spermatids observed were not stunted and misshapen as in the reciprocal cross.

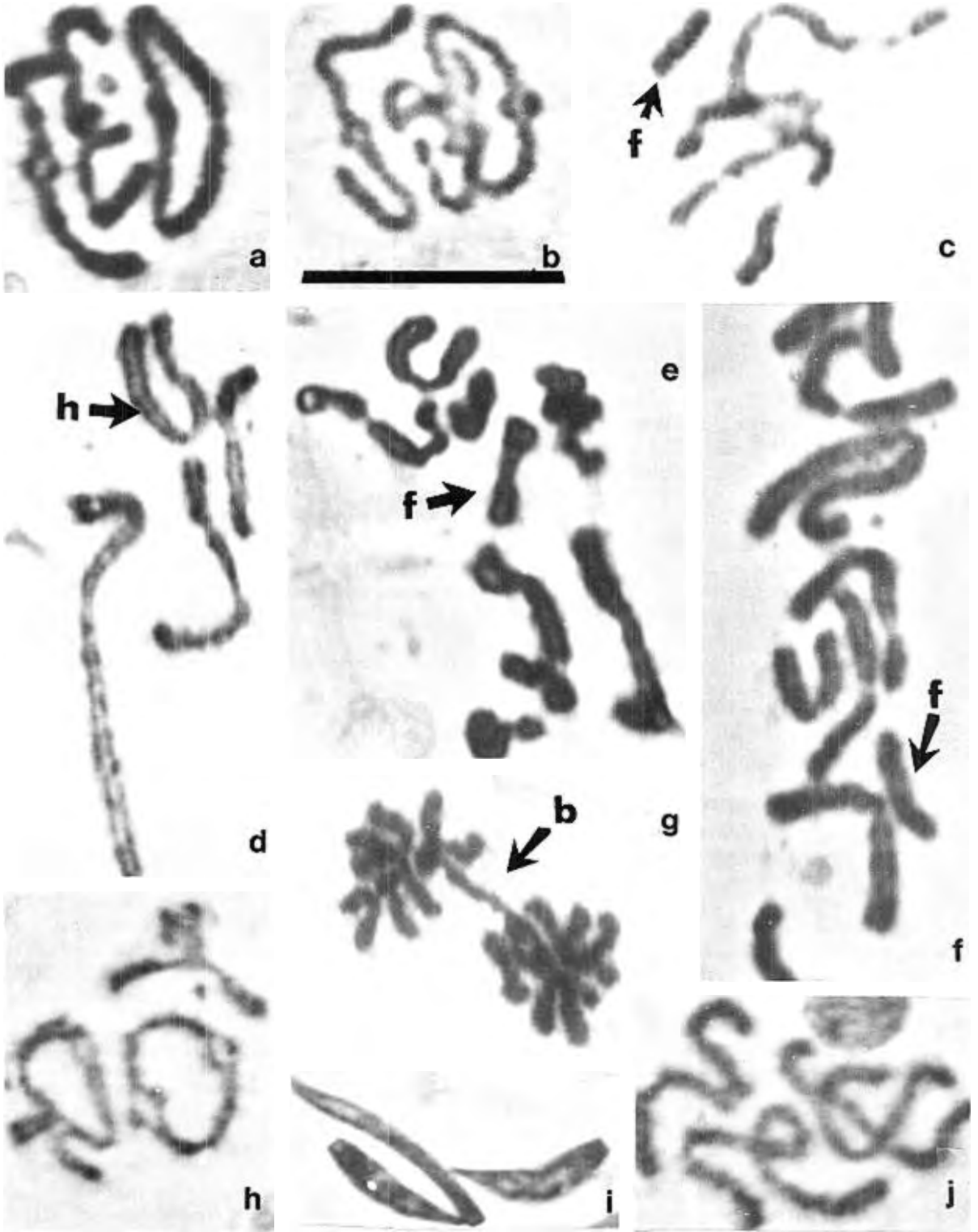
Figures 2a and 2b show diakinesis cells of *Ae. cooki* and *Ae. alcasidi*, respectively. Figures 2c–2i include six diplotene cells and one pachytene cell from the cross A ♀ × C ♂. All cells contained three heteromorphic pairs of chromosomes and there were univalents and chromosome fragments in many cells. Comparisons of somatic chromosome lengths in *Ae. cooki* and *Ae. alcasidi* with members of heteromorphic bivalents in hybrids indicated synapsis of chromosomes of the same relative size as shown in Figs. 2d and 2i. Figures 2h and 2i show a possible pericentric inversion loop in the largest autosomal bivalent.

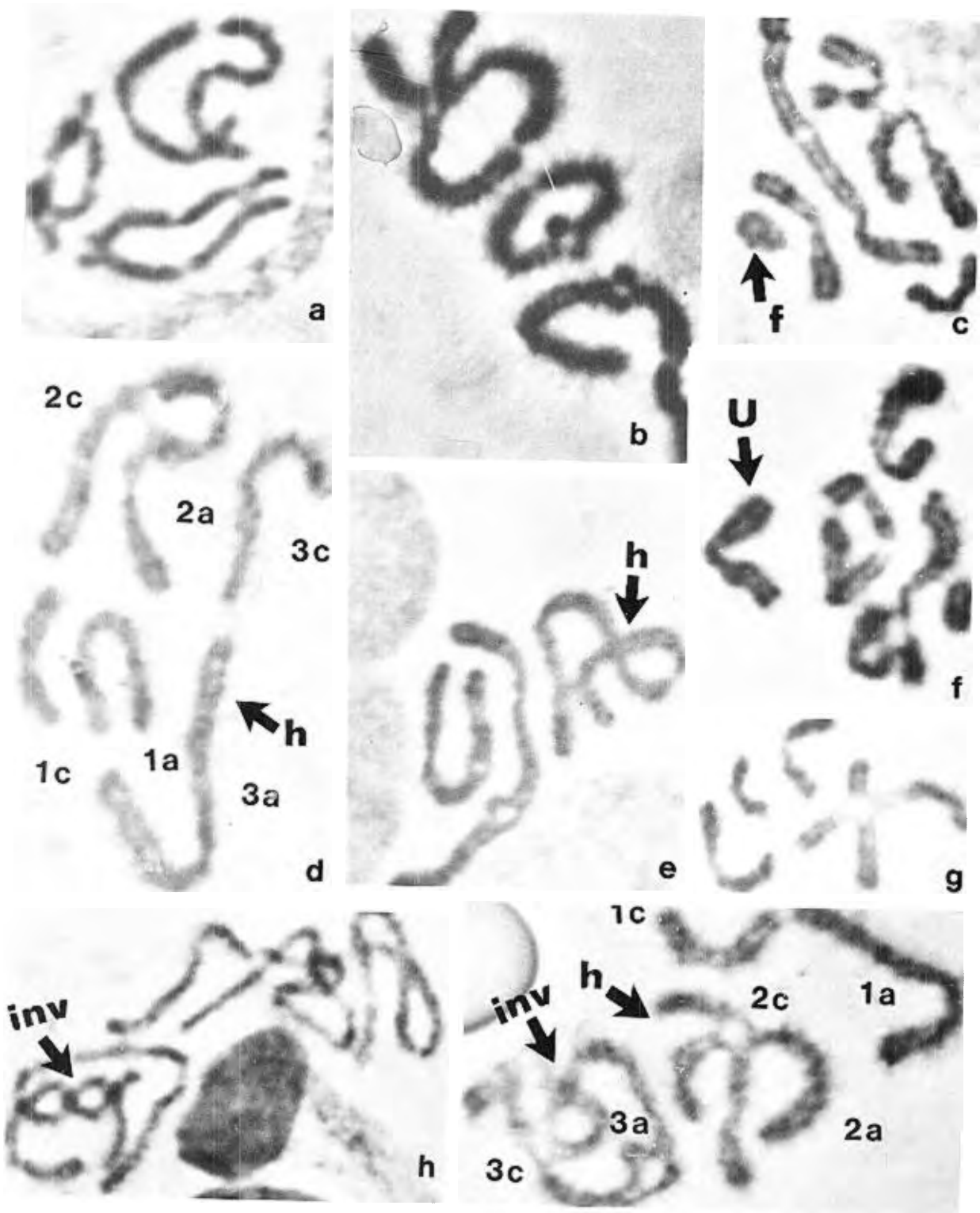
Hybrid male cytology and spermatids from crosses involving *Ae. cooki* and *Ae. polynesiensis* are shown in Figs. 3l–3f. Figures 3a and 3b show diakinesis cells of *Ae. polynesiensis* and *Ae. cooki*, respectively. Figure 3c represents somatic pairing in two of three chromosome pairs in the cross Po ♀ × C ♂. Figures 3d and 3e show univalents of the smallest chromosome pair in the same cross. The presence of univalents was the most common abnormality in the cross Po ♀ × C ♂ and was observed most often in the smallest pair. Figure 3f includes two deformed spermatids observed in this cross.

Figures 4a–4i represent male cytology in crosses involving *Ae. cooki* and *Ae. pseudoscutellaris*. Figures 4a and 4b show diakinesis cells of *Ae. pseudoscutellaris* and *Ae. cooki*, respectively. Figures 4c and 4d show extensive chromosome breakage typical of the cross C ♀ × Ps ♂. Figures 4e–4i represent cytology in the cross Ps ♀ × C ♂. Differences between lengths of paired chromosomes were seen in this cross as in other interspecific crosses involving *Ae. cooki*. The most common abnormality in the cross Ps ♀ × C ♂ was the presence of univalents of the smallest chromo-

FIG. 1. Spermatogenesis in crosses involving *Ae. cooki* and *Ae. malayensis*. (a) Diakinesis cell of *Ae. cooki*. (b) Diakinesis cell of *Ae. malayensis*. (c–e) Diplotene configurations from the cross M ♀ × C ♂. (f) Somatic chromosomes from the cross M ♀ × C ♂. (g) Anaphase I from the cross F<sub>1</sub> (M/C) ♀ × C ♂. (h) Diakinesis configuration from the cross C ♀ × M ♂. (i) Spermatids from the cross C ♀ × M ♂. (j) Early diplotene configuration from the cross C ♀ × M ♂. f, acentric fragment; h, cell showing three heteromorphic pairs; b, anaphase I bridge. Bar = 10 μm.

FIG. 2. Male cytology in crosses involving *Ae. cooki* and *Ae. alcasidi*. (a) Diakinesis cell of *Ae. cooki*. (b) Diakinesis cell of *Ae. alcasidi*. (c–g) Diplotene configurations from the cross A ♀ × C ♂. (h) Pachytene cell from the cross A ♀ × C ♂. (i) Diplotene cell from the cross A ♀ × C ♂. u, univalent; inv, pericentric inversion; a, *Ae. alcasidi* chromosomes, c, *Ae. cooki* chromosomes.





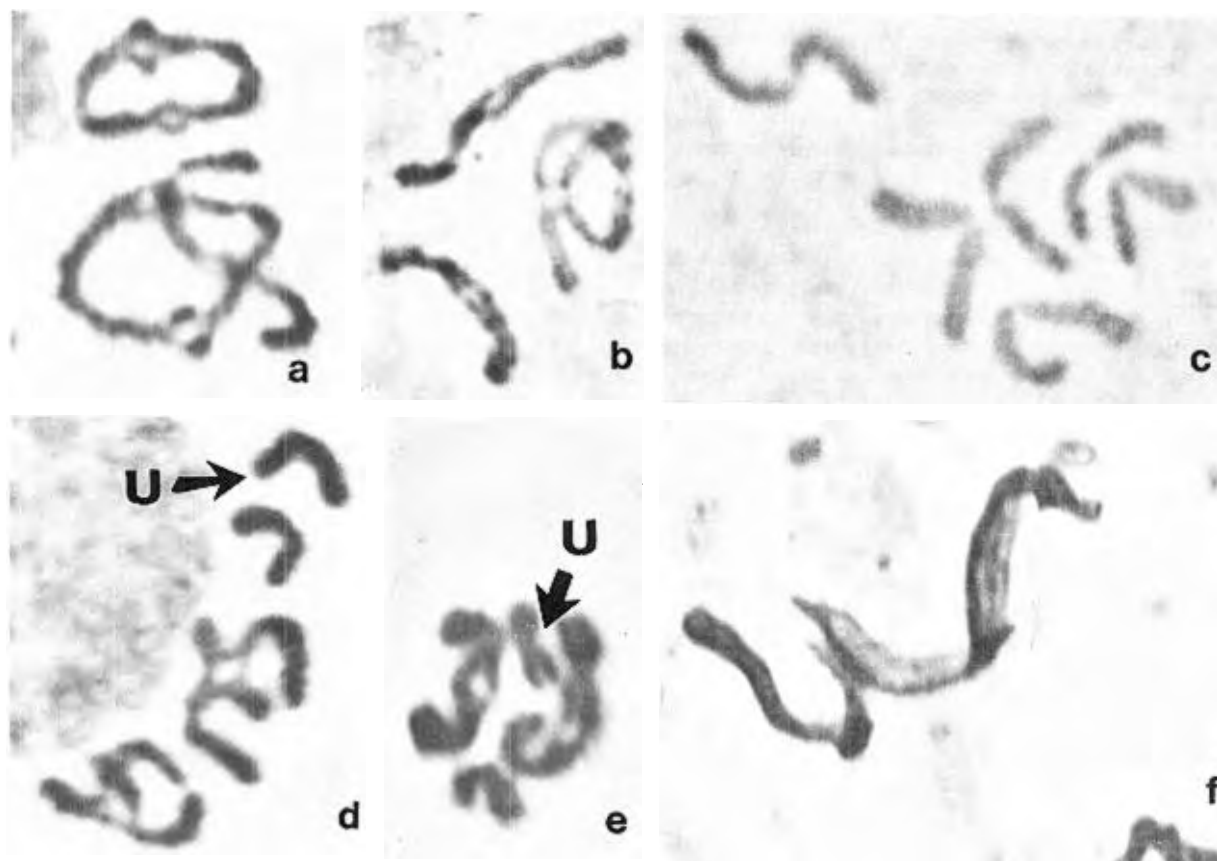


FIG. 3. Spermatogenesis in crosses involving *Ae. cooki* and *Ae. polynesiensis*. (a) Diakinesis cell of *Ae. polynesiensis*. (b) Diakinesis cell of *Ae. cooki*. (c) Somatic chromosomes from the cross Po ♀ × C ♂. (d) Spermatids from the cross Po ♀ × C ♂.

some pair (Figs. 4a and 4i). Somatic pairing was also observed in this cross (Fig. 4h).

### Discussion

*Aedes alcasidi*, *Ae. malayensis*, *Ae. pseudo-scutellaris*, and *Ae. polynesiensis* form a west-east gradient of increasing geographic proximity with *Ae. cooki* (Huang 1972; Huang and Hitchcock 1980). Sherron and Rai (1983) found a corresponding gradient of genetic compatibility in hybridization experiments involving *Ae. cooki* and these four species. Electrophoretic data (Pashley and Rai 1983) supported this gradient of genetic similarity. The present study indicated that hybrid spermatogenesis and species chromosomal relationships reflect this pattern of genetic compatibility in crosses involving *Ae. cooki* males. Hybrid reproductive development was increasingly disturbed with increasing species geographic distance from the natural range of *Ae. cooki* in the Tonga Islands.

In crosses involving *Ae. cooki* and *Ae. malayensis* or *Ae. alcasidi* only 9.5% of F<sub>1</sub> (M/C) pupae and 34.1% of F<sub>1</sub> (A/C) pupae had normal testes (Table 1). The few

spermatids observed were often irregular and distorted in shape. Testis development in backcrosses using F<sub>1</sub> (M/C) suggested a pattern of hybrid dysgenesis. High percentages of pupae from backcrosses involving F<sub>1</sub> (M/C) and *Ae. cooki* showed normal testes (92.9 and 82.4%, respectively). On the other hand, low percentages of pupae from backcrosses involving F<sub>1</sub> (M/C) and *Ae. malayensis* showed normal testes (0.0 and 8.7%, respectively).

In addition, preliminary observations of 30 F<sub>1</sub> females from each of the crosses A ♀ × C ♂ and M ♀ × C ♂ revealed that only seven had normal-sized ovaries.

Cytological data suggested a chromosomal basis for hybrid male sterility in crosses involving *Ae. cooki* and *Ae. malayensis* (Tables 2 and 3). Hybrid testes from the cross M ♀ × C ♂ showed chromosomal breakage and (or) univalents in 81.7% of cells (Table 3; Figs. 1c-1f), whereas the reciprocal cross showed either breakage or univalents in only 16.2% of cells. Backcrosses involving F<sub>1</sub> (M/C) and *Ae. cooki* showed high chiasmata frequencies (Table 2) and significantly fewer

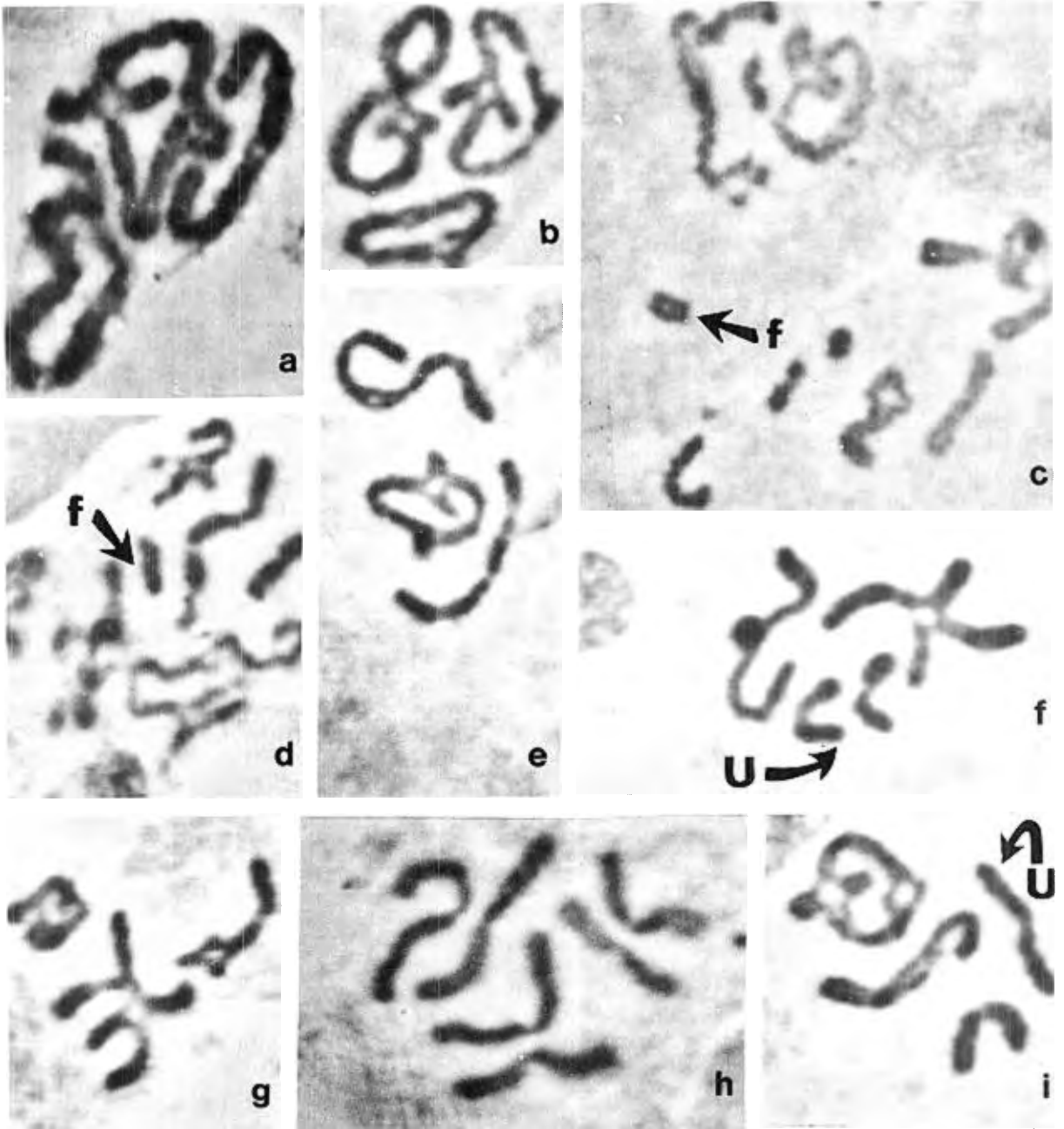


FIG. 4. Male cytology in crosses involving *Ae. cooki* and *Ae. pseudoscutellaris*. (a) Diakinesis cell of *Ae. pseudoscutellaris*. (b) Diakinesis cell of *Ae. cooki*. (c-d) Diakinesis configurations from the cross C ♀ × Ps ♂. (e-g) Diakinesis configurations from the cross Ps ♀ × C ♂. (h) Somatic configurations from the cross Ps ♀ × C ♂. (i) Diakinesis configuration from the cross Ps ♀ × C ♂.

abnormal cells than parental *Ae. cooki*, but backcrosses involving F<sub>1</sub> (M/C) and *Ae. malayensis* produced too few meiotic cells to yield meaningful data.

There appeared to be two major sources of genetic breakdown in hybrids from interspecific crosses involving *Ae. cooki* and *Ae. malayensis*. First, there

seemed to be a general incompatibility between the genomes of the two species that led to low hatch rates in the F<sub>1</sub> and most backcrosses (Sherron and Rai 1983). Second, spermatogenesis was especially aberrant when sons had an *Ae. cooki* male chromosome and at least one-half of the genome was derived from *Ae. malay-*

*ensis* (M ♀ × C ♂, M ♀ × F<sub>1</sub> (M/C ♂). The more normal crosses (C ♀ × M ♂, C ♀ × F<sub>1</sub> (M/C) ♂, F<sub>1</sub> (M/C) ♀ × C ♂) could not produce such males or would produce them only rarely.

Similar examples of hybrid dysgenesis, or breakdown in reproductive development, are well known in Diptera. Motara and Rai (1977) found that genomic interaction in certain backcrosses of *Ae. aegypti* and *Ae. mascarensis* led to aberrant male development. Curtis (1982) demonstrated that sex chromosome – autosome incompatibility was responsible for male hybrid sterility in interspecific crosses in the *Anopheles gambiae* complex. Henderson et al. (1978) reported extensive chromosomal breakage in hybrids of certain strains of *Drosophila melanogaster*. Zouros (1981) found that a single heterozygous chromosome was sufficient to suppress viability in backcrosses involving hybrids of *Drosophila mojavensis* and *D. arizonensis*. Chromosomal incompatibility in hybrids of *Ae. cooki* and *Ae. malayensis* may involve specific genes or many small structural differences because there was no cytological evidence of inversions or other rearrangements in crosses between them.

It should be noted that viable hybrids from crosses involving *Ae. cooki* and four sibling species showed no developmental abnormalities, such as intersexuality or noticeable larval–pupal mortality. This indicates that chromosomal incompatibility in F<sub>1</sub> hybrids was expressed only in germ cells (atrophied testes and ovaries) and during meiosis, and not in somatic tissues. In contrast, hybrid incompatibility at the gene level was also expressed as embryonic death, though this was intensified in backcrosses owing to abnormal gamete production in F<sub>1</sub> meiosis. Engels and Preston (1979) also found that hybrid dysgenesis between strains of *D. melanogaster* was restricted to the germ line in the form of atrophied testes and ovaries.

Cytology in hybrids from the cross A ♀ × C ♂ was not as disturbed as that from the cross M ♀ × C ♂. Percentages of cells with univalents were 35.4% in F<sub>1</sub> (A/C) males versus 42.3% in F<sub>1</sub> (M/C) males (Table 3). However, F<sub>1</sub> (A/C) males showed much fewer cells with chromosomal breakage (29.8 vs. 70.4%).

The most striking cytological feature of the cross A ♀ × C ♂ was the abundance of heteromorphic bivalents (Figs. 2c–2i) and this reflects species differences in chromosome lengths. Somatic chromosomes were about 35% shorter in total length in *Ae. cooki* than in *Ae. alcasidi* (Table 4) and comparisons demonstrated that the smallest, intermediate, and largest chromosome of *Ae. cooki* were paired with homologous but larger chromosomes of *Ae. alcasidi*. Ueshima (1963) found numerous heteromorphic bivalents in hybrids of geographic races of *Cimex pilosellus* (Hemiptera: Cimicidae) but concluded that these associations were

nonhomologous, in spite of the formation of single chiasma. The heteromorphic bivalents encountered in the cross A ♀ × C ♂ undoubtedly represent true homology because multiple chiasmata were formed (Figs. 2e and 2i); chiasmata were often interstitial (Figs. 2c and 2i), and large portions of chromosomes were involved in pairing (Fig. 2h).

In spite of some degree of chromosomal stability in F<sub>1</sub> hybrids, backcrosses using F<sub>1</sub> (A/C) gave very poor hatches (Sherron and Rai 1983). Perhaps recombination between unequal chromosomes in the F<sub>1</sub> upset blocks of coadapted or regulatory genes. Shaw et al. (1982) showed this to be the case in infertile backcrosses using interspecific hybrids of grasshoppers of the genus *Caledia*. Considering the marked length differences between chromosomes of *Ae. cooki* and *Ae. alcasidi* (Table 4), it is likely that recombination in the F<sub>1</sub> produced unbalanced gametes. Genetic imbalance may also explain poor embryonation in backcrosses using F<sub>1</sub> (A/C).

The excess chromatin present in *Ae. alcasidi* relative to *Ae. cooki* may represent an ancestral condition. Hinegardner (1975) noted that some primitive or ancient animals tend to have more DNA than other members of their taxa.

There is some evidence of a structural rearrangement in addition to length differences between chromosomes of *Ae. cooki* and *Ae. alcasidi*. Figures 2h and 2i show a distinct loop in one arm of the largest bivalent in a hybrid from the cross A ♀ × C ♂. Crossing-over in this possible autosomal pericentric inversion would contribute to the production of unbalanced gametes.

*Aedes alcasidi* and *Ae. malayensis* may represent ancestral species within the *Ae. scutellaris* subgroup. Both species are distributed in the western extreme of the *Ae. scutellaris* subgroup (Huang 1972) and both species have the largest chromosomes of any species examined in this subgroup (Table 4; V. Dev and K. S. Rai, unpublished data). *Aedes alcasidi* may be a direct ancestor of *Ae. cooki* while *Ae. malayensis* may be derived independently from *Ae. alcasidi*. There appears to be greater chromosomal affinity between *Ae. cooki* and *Ae. alcasidi* than between the former and *Ae. malayensis* (Table 3; Figs. 1 and 2).

Observations of spermatogenesis in hybrids from crosses involving *Ae. cooki* males and females of the relatively proximal species, *Ae. pseudoscutellaris* and *Ae. polynesiensis*, suggest a higher degree of genetic compatibility (Sherron and Rai 1983). Crosses involving these species and *Ae. cooki* males showed high percentages of pupae with normal-sized testes (Table 1). Mean chiasmata frequencies were not significantly different between the cross Po ♀ × C ♂ and parental *Ae. cooki* (Table 2). Crosses involving females of *Ae. polynesiensis* or *Ae. pseudoscutellaris* and males of *Ae.*

TABLE 5. Summary of observations of spermatogenesis in parental colonies and interspecific crosses involving *Ae. cooki*

Cross (♀ × ♂)	Mean cell chiasmata	% univalents	% breaks	% normal testes
C × C	3.84	12.9	3.6	85.9
Po × Po	4.53	13.4	1.4	87.5
Ps × Ps	3.52	3.1	0.6	92.0
A × A	4.13	2.6	1.9	90.0
M × M	4.51	6.9	3.2	92.2
Po × C	3.66	42.2	1.8	98.2
C × Po	—	—	—	30.4
Ps × C	3.39	31.4	4.3	98.2
C × Ps	2.10	7.0	91.2	58.8
A × C	2.07	35.4	29.8	34.1
C × A	—	—	—	50.0
M × C	3.06	42.3	70.4	9.5
C × M	4.10	8.1	8.1	25.0

*cooki* showed significantly more abnormal cells than parental *Ae. cooki* (Table 3); however, this was mostly in the form of univalents of the smallest chromosome pair rather than chromosome breakage (Figs. 3*d*, 3*e*, 4*f*, and 4*i*).

Significant percentages of cells with univalents in interspecific hybrids of *Ae. cooki* may represent some degree of structural divergence between chromosomes of *Ae. cooki*, *Ae. polynesiensis*, and *Ae. pseudoscutellaris*. Colonies of *Ae. cooki* and *Ae. polynesiensis* showed 11.0 and 11.9% univalents, respectively, in the smallest chromosome pair and low levels of univalents appear to be characteristic of these inbred colonies. However, much higher levels of univalents in hybrids may have lowered backcross hatch rates by producing aneuploid gametes (Burnham 1979). Kitzmiller et al. (1967) attributed sex chromosome univalents in hybrids in the *Anopheles maculipennis* group to extensive minor rearrangements. Univalent sex chromosomes in hybrids of *Anopheles maculatus* with *Anopheles stephensi* showed strong differences in banding patterns (Narang 1972, 1973). One or more small paracentric inversions could also inhibit pairing in the smallest pair of hybrid chromosomes.

Spermatogenesis in interspecific hybrids was different between reciprocal crosses involving *Ae. cooki*. Most notably, univalent sex chromosomes were common in the cross Ps ♀ × C ♂ while chromosomes were sometimes pulverized in the cross C ♀ × Ps ♂ (Table 3; Fig. 4*c*). It is unclear whether these differences have a genetic basis because data were relatively scant from interspecific crosses involving *Ae. cooki* females. Fertility in such crosses does not exceed 2.0% hatch (Sherron and Rai 1983) and is due to the effects of ovarian rickettsiae in *Ae. cooki* (Wright and Wang 1980; Trpis et al. 1981). The possibility that ovarian rickettsiae also

influence hybrid meiosis deserves further investigation.

Table 5 summarizes data from parental colonies and interspecific crosses involving *Ae. cooki* and four sibling species. *Aedes malayensis* and *Ae. alcasidi* are geographically remote from *Ae. cooki* in the western extreme of the *Ae. scutellaris* subgroup distribution. The present study indicates considerable genetic divergence of these two species from *Ae. cooki*, in agreement with hybridization experiments (Sherron and Rai 1983). Hybrids showed extensive chromosome breakage and atrophied testes and in the case of *Ae. malayensis* there is evidence of sex chromosome – autosome incompatibility.

*Aedes polynesiensis* and *Ae. pseudoscutellaris* are much closer geographically to *Ae. cooki*; hybrid spermatogenesis in crosses with *Ae. cooki* males suggests a greater genetic affinity between these species. The occurrence of univalents appears to be the only major abnormality in hybrid spermatogenesis. This suggests some degree of chromosomal divergence from *Ae. cooki* leading to inhibited pairing of the smallest chromosome in hybrids.

Data from the crosses C ♀ × Po ♂ and C ♀ × Ps ♂ do not conform to the geographic gradient of genetic compatibility with *Ae. cooki* suggested by other crosses. It is unclear whether hybrid breakdown in these crosses is due to genetic factors alone.

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