A SECOND SPECIES OF *HOLOTHYRUS* (ACARINA:HOLOTHYROIDEA) FROM AUSTRALIA.

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(One Text-figure.)

[Read 29th September, 1954.]

Synopsis.

Holothyrus constrictus, n. sp., from South Queensland, is described and discussed. The key of Womersley (1935) to the known world species is amended to include it.

The rare suborder Holothyroidea includes only the genus *Holothyrus* Gervais, 1842, with seven described species, of which one is from Mauritius, three from Seychelles, two from New Guinea, and one (*H. australasiae* Wom., 1935) from South Australia and the North Island of New Zealand. An eighth species from South Queensland is described in the present paper.

The history of the genus is fully discussed by Thon (1906), and summarized by Womersley (1935). All the species are forest dwellers, living in humus and the like. The Australian forms are the smallest known, not exceeding 3 mm. It is also of note that the female genital shields of the Australian forms differ from those figured by Thon, and copied by Trägårdh (1938), the anterior and lateral shields being much more definite and well developed, as shown by Womersley (Plate VIII), and in Figure 1A below. The two Australian forms are also the only ones described as having distinct processes on the femur, genu and tibia of leg II in the male. However, in all other respects they are typical of the genus, and there seems no good reason to separate them. Full generic details are given by Thon and Womersley, and minor details by Vitzthum (1941) and Baker and Wharton (1952).

I wish to thank Mr. H. Womersley, of the South Australian Museum, Adelaide, where this work was done, and Dr. I. M. Mackerras, of this Institute, for their kind advice during the preparation of this paper.

Family HOLOTHYRIDAE Thorell, 1882.

Genus Holothyrus Gervais, 1842.

Holothyrus constrictus, n. sp.

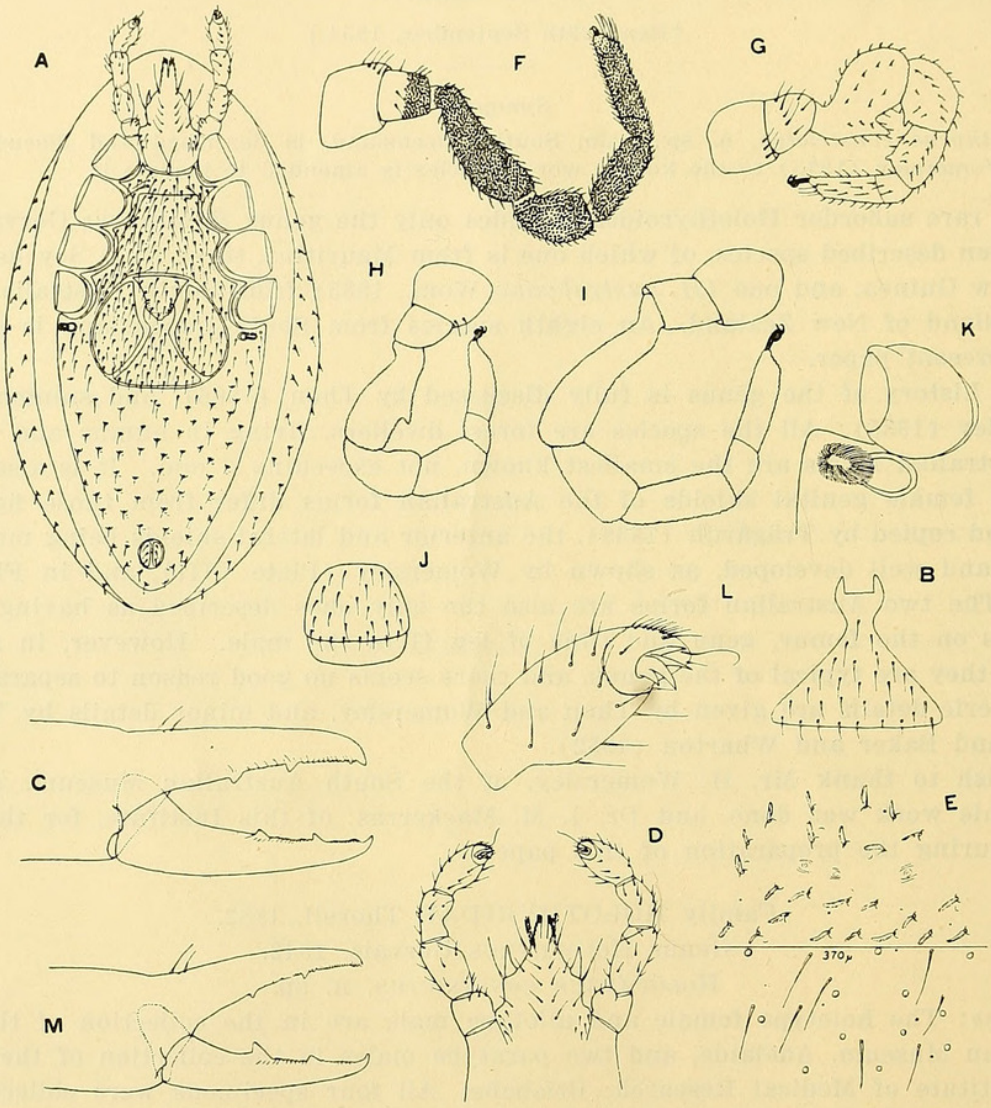
Types: The holotype female and allotype male are in the collection of the South Australian Museum, Adelaide, and two paratype males in the collection of the Queensland Institute of Medical Research, Brisbane. All four specimens were collected in a composite lot of soil and litter from Brookfield, Brisbane, Queensland, 21st May, 1949. by Dr. E. H. Derrick.

Female.

A small, dark brown, heavily chitinized species; *idiosoma* length 2080 μ, breadth 1150 μ. *Dorsal shield* completely covering dorsum, and underlapping venter as shown; raised into small, irregularly-spaced tubercles, each carrying a short seta 18 to 25 μ long, which has one edge with a double row of short ciliations, while the other edge is smooth; shield very finely granulated, with punctate, linear semi-circular, and peculiar eye-like pores.

Venter (Fig. 1A), sternal, metasternal, endopodal, ventral and anal shields fused to form holoventral shield, 1488 μ long, 720 μ wide at greatest width (behind coxae IV), markedly thickened around acetabula (representing the endopodal shields), and especially so at the anterior sternal part of the shield; sternal section of shield concave anteriorly, broadening to behind coxae IV, whence ventri-anal portion tapers slowly

to posterior of venter; at least two pairs of longer sternal setae and pores distinguishable from the accessory setae. *Genital shield* (Fig. 1B) wider basally than high, 352 μ × 304 μ, with two anterior horns, behind which the shield constricts markedly, and then expands broadly to its base, as figured. *Lateral shield* (Fig. 1A) longer than broad, 352 μ × 192 μ, narrower anteriorly, and with rounded ends, as figured. *Median shield* (Fig. 1A) broader than long, 208 μ × 160 μ, tapering twice to posterior. Two *anal valves* (Fig. 1A) roughly semi-circular, 96 μ × 46 μ, each with three or four simple, short setae. Sternal and genital shields covered by thickly set, simple setae to 54 μ long, posterior part of venter with simple setae on tubercles, except at extreme posterior where a few setae



Text-figure 1.—*Holothyrus constrictus*, n. sp.

A-E, female, F-M, male.—A, ventral view; B, genital shield; C, chelicera; D, ventral view of gnathosoma; E, dorsal shield of *H. constrictus*, n. sp., above, and *H. australasiae* Wom., below; F, leg I; G, leg II; H, leg III; I, leg IV; J, genital shield; K, pit behind coxa IV; L, palpal tibia + tarsus; M, chelicera.

are ciliated like those on dorsum. Behind each coxa IV a sub-spherical pit (Fig. 1K) with aperture guarded by heavy brush of fine setae. Cuticle between dorsal and holoventral shield without setae. *Tritosternum* present, but small, with two weakly ciliated laciniae.

Gnathosoma (Fig. 1D) enclosed in camerostome formed by anterior part of dorsal shield. *Palpi* with five free segments, palpal tibia expanded medially, tibia + tarsus 176 μ × 78 μ, with 4-tined sensory seta at tip of tarsus, palpal claws indistinct. *Chelicerae* (Fig. 1C) large, fixed finger with two main teeth, and finely serrate edge between teeth, and with two setae dorsally at base, movable finger 264 μ, also with two teeth and

serrations. *Legs* fairly long and slender, closely tuberculate like dorsum (except on coxae), all with two claws, caruncle and pad (caruncle reduced on leg I); leg I 1490 μ, leg II 1170 μ, leg III 1180 μ, leg IV 1740 μ.

Male.

Somewhat smaller than female, heavily chitinized; *idiosoma* length 1760 μ, breadth 960 μ. *Dorsal shield* of same structure as female, entirely covering dorsum, and underlapping venter by approximately 80 μ. *Holoventral shield* of similar composition to female, 1360 μ × 688 μ (behind coxae IV). *Genital shield* (Fig. 1J) quadrate with rounded corners, broader than long, 168 μ × 116 μ; genital aperture sub-circular, 168 μ in diameter. *Anal valves* longer than broad, 80 μ × 42 μ, with three or four pairs simple setae. Glands present behind coxae IV. Ventral cuticle ornamented posteriorly with tubercles as in female. *Tritosternum* present, small (only very narrow space between sternal margin and gnathosoma).

Gnathosoma and *palpi* as in female; palpal tibia + tarsus (Fig. 1L) 176 μ × 88 μ. *Chelicerae* (Fig. 1M) very similar to female, but second tooth on movable finger slightly stronger; length of movable finger 256 μ. *Legs* (Fig. 1F–I) fairly long, slender, except leg II; all with caruncle, two claws and pad (caruncle of leg I reduced). Femur II with large process ornamented as general leg surface, femur III with similar, smaller process, genu II and tibia II with simple, small process, as figured, coxae of all legs smooth; leg I 1490 μ, leg II 1200 μ, leg III 1280 μ, leg IV 1810 μ.

Distribution.—Known only from the type locality in South Queensland.

Taxonomic notes.

This species may be readily separated from the other known Australian species, *H. australasiae* Wom., by the great difference in cuticular ornamentation (Fig. 1E), and from the other species by its very small size and armature of leg II in the male. In *H. constrictus*, n. sp., the cuticle of the dorsal shield in both sexes has numerous irregularly-spaced tubercles, each bearing a single, short seta, 18 μ to 25 μ long. These setae are stout, and have a double row of ciliations on one side only. Between the tubercles are punctate, semi-circular and eye-like pores. The tubercles are present ventrally on the posterior half, but only the setae on the extreme posterior tubercles are ciliated. The setae on the anterior half of the venter are simple and not set on tubercles, to 35 μ in the male and to 50 μ in the female. In *H. australasiae* Wom. the tubercles are smaller and not set so closely together, and the setae are not set on them, but directly in the cuticle. The setae are much longer, to 90 μ, and simple. The ventral surface is rather similar to the dorsal, with somewhat shorter setae. The general cuticular surface in both species is very minutely granulated.

The key of Womersley may be amended to include the new species by inserting the following caption:

4*a*. Leg II of male with brushes of setae on femur, genu and tibia in addition to processes; female genital shield not constricted behind anterior horns; male 2·9 mm., female 2·8 mm. (South Australia and New Zealand).....*H. australasiae* Womersley, 1935.
Leg II of male without such brushes; female genital shield markedly constricted behind anterior horns; male 1·76 mm., female 2 mm. (Queensland).....*H. constrictus,* n. sp.

There have been two errors in recent literature concerning this genus which should be noted. Radford (1950) in his checklist gives the reference to the genotype as "*Ann. Soc. ent. France*, 2", etc., but this should read "11". Secondly, in Baker and Wharton's book (1952), Fig. 33 (page 39) is labelled as "*Holothyrus longipes* Thorell, 1882. (After Hirst, 1922.)". Hirst's figure 77, page 92, is simply labelled "*Holothyrus* sp. (After Thorell.)". Vitzthum (1941, Abb. 478, S. 752) also reproduces the same figure by Thorell, and labels it (correctly) "*Holothyrus nitidissimus* Thorell, 1882, nach Thorell". Thorell described four forms of the genus in 1882 (Descrizione di alcuni Aracnidi inferiori dell' archipelago malese, in: *Ann Mus. civ. Stor. nat Genova*, 18), viz., *H. longipes*, *H. l.* var. *ferrugineus*, *H. nitidissimus* and *H. scutifer*. Of these, Thorell himself said *H. l.* var. *ferrugineus* was a nymphal form, and that "*nitidissimo* haec forma

[*scutifer*] valde similis est, et forsitan alter sexus ejus". Thon regards this to be true, and only two of Thorell's four forms are valid, *H. longipes* and *H. nitidissimus*. Thon re-examined *H. longipes* and stated: "Die Füsze sind sehr lang und auffallend schlank wie bei keiner andern Art. Thorell hat den Artnamen sehr treffend gewählt. Der 1. Fusz ist der längste, unbedeutend länger als der letzte." He gives the ratio of body length: length leg I: length leg IV as 17:41:40. The figure given by Baker and Wharton, then, cannot be *Holothyrus longipes* Thorell, 1882, but is really *Holothyrus nitidissimus* Thorell, 1882, as given by Vitzthum.

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THE NATURE AND SIGNIFICANCE OF NON-RECIPROCAL FERTILITY IN *AËDES SCUTELLARIS* AND OTHER MOSQUITOES.

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(Three Text-figures.)

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Synopsis.

When crosses are made between two subspecies of *Aëdes scutellaris*, the matings prove fertile or infertile according to the direction in which the matings are made. In the mating of subsp. *scutellaris* (S) females with subsp. *katherinensis* (K) males, fully viable eggs are produced. In the reciprocal mating, copulation, insemination and egg laying are normal, but the eggs are totally inviable.

This non-reciprocal fertility shows strictly maternal inheritance. Backcrosses of F_1 females to subsp. *katherinensis* males are viable and the B_1 progeny are of *scutellaris* mating type. In successive backcrosses to *katherinensis* males, to the B_6 generation, the *scutellaris* mating type is retained. B_{6K} males, derived from repeated backcrosses to subspecies *katherinensis*, are still incompatible with *katherinensis* females.

The genetic system determining the inheritance of mating type must depend either on anomalous meiosis in oogenesis or on nucleus-independent cytoplasmic factors. There is no critical evidence enabling a choice between these two hypotheses.

A survey of the available data on non-reciprocal fertility between species, subspecies, and races of *Aëdes* and *Culex* suggests that it has had significance as a source of incipient speciation in mosquitoes.

Introduction.

The natural history of mosquitoes has long held a place of importance in entomological research, and the systematics of the Culicidae has advanced to a stage where the limits between species are often finely drawn on the bases of morphological, physiological and ecological criteria. The application of the genetic concept of species, which emphasizes intrinsic isolation, is likely to cause some reorientation of the taxonomy of the group in the near future.

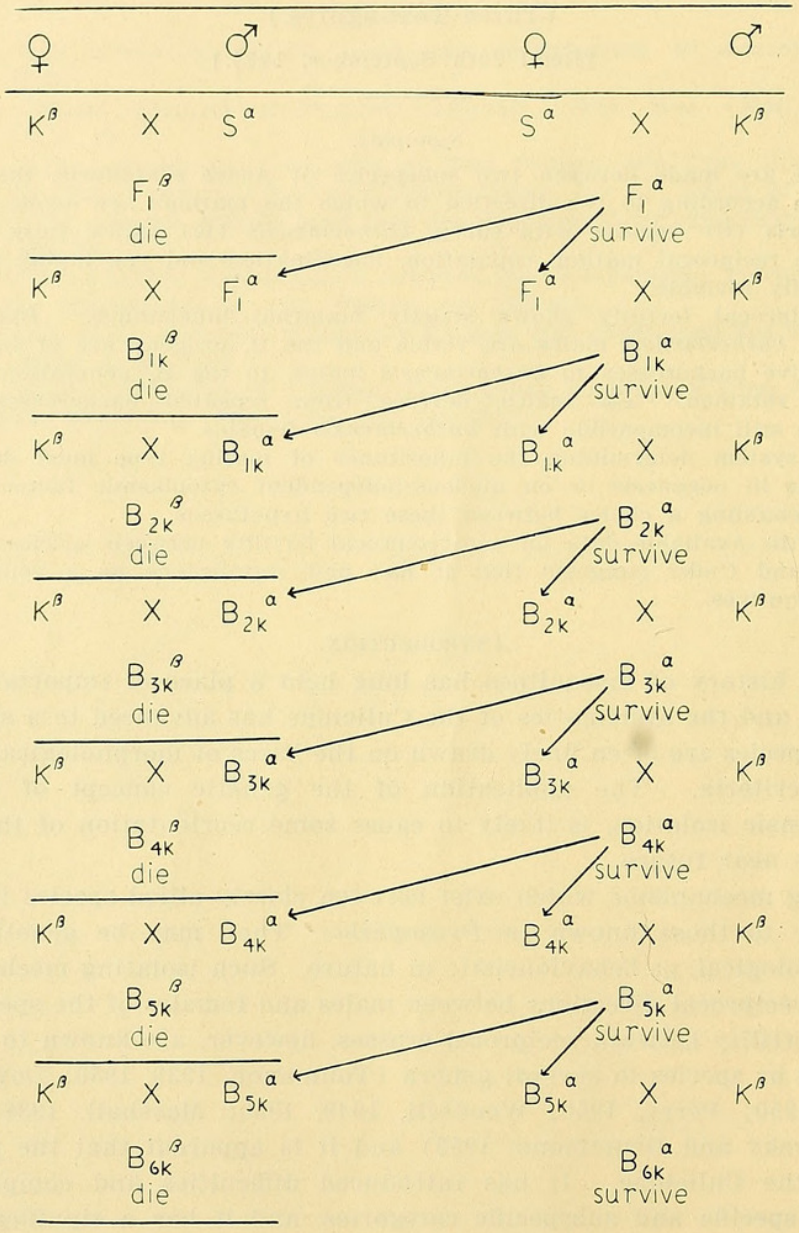
The isolating mechanisms which exist between closely allied species include a range of types similar to those known in *Drosophila*. They may be genetic, mechanical, ecological, physiological, or behaviouristic in nature. Such isolating mechanisms usually operate in both reciprocal directions between males and females of the species concerned. Differences in fertility between reciprocal crosses, however, are known to occur between races, subspecies or species in several genera (Toumanoff, 1939, 1950; Downs and Baker, 1949; Bonnet, 1950; Perry, 1950; Woodhill, 1949, 1950; Marshall, 1938; Laven, 1951, 1953; Dobrotworsky and Drummond, 1953) and it is apparent that the phenomenon is widespread in the Culicidae. It has introduced difficulties and complexities in the appreciation of specific and subspecific categories, and it has a significant bearing on problems of medical entomology. It is also significant to genetical and evolutionary theory. Its mechanics must depend on a uniparental genetic system, and its role in the origin of intrinsic species barriers is problematical.

The Material.

Aëdes scutellaris Walker belongs to a species-complex which includes a number of species, subspecies and geographical races of doubtful rank. Many of the forms show complete intrinsic isolation, and undoubtedly deserve specific status. A peculiar non-reciprocal fertility was described by Woodhill (1949, 1950) in crosses between two subspecies, *A. scutellaris scutellaris* Walker and *A. scutellaris katherinensis* Woodhill. Complete fertility was found when subsp. *scutellaris* females and subsp. *katherinensis* males were mated, but hybrid eggs from the reciprocal cross were totally inviable. This

mating incompatibility was found to extend to the backcrosses to *katherinensis*. Smith-White (1950) drew attention to the possible genetic and evolutionary significance of the phenomenon, and suggested a backcross program which might clarify its nature.

The original isolation of subsp. *katherinensis* was obtained from Katherine, in the Northern Territory, in January, 1948, and consisted of a batch of eggs from an unknown number of females. This isolation has been maintained in the laboratory under the culture designation "K". An isolation of subsp. *scutellaris* was obtained from New Guinea at about the same time, and has been maintained as culture "S".



Text-figure 1.—*The Backcross Program.*

S = *A. scutellaris scutellaris*; K = *A. scutellaris katherinensis*; *α* and *β* represent maternally inherited factors, affecting survival. They may be carried either in the cytoplasm or in the nuclei.

In April, 1953, Mr. K. O'Gower obtained eggs from eleven females of subsp. *katherinensis* at Batchelor, 126 miles north-west of Katherine. This new isolation, designated culture "B", is morphologically identical with the Katherine material, and shows an identical behaviour when crossed with subsp. *scutellaris*. In the cross S × B, 3200 F_1 eggs yielded 2495 larvae (77·9% hatch), but in the reciprocal cross B × S, 2514 eggs were totally inviable. *A. scutellaris katherinensis* possesses a wide geographical distribution in northern Australia, and it can be distinguished from the type subspecies by morphological criteria.

Aëdes scutellaris is a less satisfactory laboratory subject than is *Culex molestus*. It requires warm temperatures and high humidity, which necessitate special culture rooms. Blood-feeding is necessary before egg-laying, and matings are only successful when large numbers of the two sexes are confined in the breeding cages. The eggs are laid singly, and the progeny of individual females cannot easily be isolated from the mass mating cages.

The Breeding Program.

The main part of the breeding program has consisted of a series of backcrosses to test for permanence or breakdown of incompatibility with K females. Data on sex ratio in cultures, and of F_2 segregation for a morphological character "white line", are reported. An inbreeding program with culture S was commenced for the isolation of possible recessive genes, but has been abandoned temporarily.

All larval cultures were maintained in incubators at 27°C. and matings were made in muslin cages measuring 10″ × 10″ × 12″, in a warm room at 27°C. and 75–80% relative humidity.

The backcross data. Three series of backcrosses have been made. The first series commenced with the F_1 hybrids produced by Woodhill (1950), and was continued to the fifth backcross generation (Text-figure 1 and Table 1). In the Text-figure, and in Tables

Table 1.
The First Backcross Experiment.

	Cross.	Progeny Designation.	Eggs Laid.	Eggs Hatched.	Percentage Hatch.
1	$K^{\beta} \times S^{\alpha}$	F_1^{β}	—	—	0
2	$S^{\alpha} \times K^{\beta}$	F_1^{α}	—	—	high
3	$F_1^{\alpha} \times S^{\alpha}$	B_{1s}^{α}	650	normal	high
4	$S^{\alpha} \times F_1^{\alpha}$	B_{1s}^{α}	1150	normal	high
5	$F_1^{\alpha} \times K^{\beta}$	B_{1k}^{α}	480	normal	high
6	$K^{\beta} \times F_1^{\alpha}$	B_{1k}^{β}	1220	0	0
7	$B_{1k}^{\alpha} \times K^{\beta}$	B_{2k}^{α}	normal	normal	*ca* 90
8	$K^{\beta} \times B_{1k}^{\alpha}$	B_{2k}^{β}	2000	13	0·65
9	$B_{2k}^{\alpha} \times K^{\beta}$	B_{3k}^{α}	normal	normal	*ca* 90
10	$K^{\beta} \times B_{2k}^{\alpha}$	B_{3k}^{β}	4800	12	0·25
11	$B_{3k}^{\alpha} \times K^{\beta}$	B_{4k}^{α}	normal	normal	*ca* 90
12	$K^{\beta} \times B_{3k}^{\alpha}$	B_{4k}^{β}	4200	58	1·38
13	$B_{4k}^{\alpha} \times K^{\beta}$	B_{5k}^{α}	normal	normal	*ca* 90
14	$K^{\beta} \times B_{4k}^{\alpha}$	B_{5k}^{β}	4600	84	1·87
15	$B_{3k}^{\beta} \times K^{\beta}$	B_{4k}^{β}	not recorded		over 90
16	$K^{\beta} \times B_{3k}^{\beta}$	B_{4k}^{β}	not recorded		over 90

1–3, the backcross formulae require brief explanation. The subscripts indicate the generation, B_{3k} being the third backcross to K after the F_1, and the superscripts indicate the "maternal line" of each family, and are significant for any cytoplasmic or nuclear factors having a strictly maternal inheritance. $B_{3k}{}^{\alpha}$ is the third backcross to K, tracing its maternal ancestry back to the original S stock. $B_{3k}{}^{\beta}$ has had the K maternal line introduced.

TABLE 2.

The Second Series of Backcrossing.

Serial No.	Cross.	Progeny Designation.	Numbers Mated.		Eggs Laid.		Hatch.	
					Total.	%	Total.	%
1	S^{α} selfed	S^{α}	48	100	2022	42·1	1450	71·1
2	K^{β} selfed	K^{β}	104	76	2104	20·2	1583	75·3
3	$S^{\alpha} \times K^{\beta}$	$F_1{}^{\alpha}$	56	62	2482	44·3	1499	60·4
4	$K^{\beta} \times S^{\alpha}$	$F_1{}^{\beta}$	126	202	3390	27·0	0	0·0
5	$F_1{}^{\alpha} \times F_1{}^{\alpha}$	$F_2{}^{\alpha}$	135	205	3534	26·2	863/1254	68·7
6	$F_1{}^{\alpha} \times S^{\alpha}$	$B_{1s}{}^{\alpha}$	86	126	2006	23·3	320/573	55·8
7	$S^{\alpha} \times F_1{}^{\alpha}$	$B_{1s}{}^{\alpha}$	164	150	9218	56·2	686/748	91·7
8	$F_1{}^{\alpha} \times K^{\beta}$	$B_{1k}{}^{\alpha}$	112	126	2589	23·1	1647	63·6
9	$K^{\beta} \times F_1{}^{\alpha}$	$B_{1k}{}^{\beta}$	91	103	1767	19·4	0	0·0
10	$B_{1k}{}^{\alpha} \times K^{\beta}$	$B_{2k}{}^{\alpha}$	82	68	1892	23·1	1340	70·7
11	$K^{\beta} \times B_{1k}{}^{\alpha}$	$B_{2k}{}^{\beta}$	51	113	1408	27·6	0	0·0
12	$B_{2k}{}^{\alpha} \times K^{\beta}$	$B_{3k}{}^{\alpha}$	59	73	1618	27·2	1051	64·8
13	$K^{\beta} \times B_{2k}{}^{\alpha}$	$B_{3k}{}^{\beta}$	88	56	1254	14·3	0	0·0
14	$B_{3k}{}^{\alpha} \times K^{\beta}$	$B_{4k}{}^{\alpha}$	77	91	1831	23·8	1072	58·6
15	$K^{\beta} \times B_{3k}{}^{\alpha}$	$B_{4k}{}^{\beta}$	92	92	1567	17·0	0	0·0
16	$B_{4k}{}^{\alpha} \times K^{\beta}$	$B_{5k}{}^{\alpha}$	71	98	1573	22·2	1083	68·7
17	$K^{\beta} \times B_{4k}{}^{\alpha}$	$B_{5k}{}^{\beta}$	75	102	1564	20·8	0	0·0
18	$B_{5k}{}^{\alpha} \times K^{\beta}$	$B_{6k}{}^{\alpha}$	78	86	1654	21·2	989	60·5
19	$K^{\beta} \times B_{5k}{}^{\alpha}$	$B_{6k}{}^{\beta}$	69	68	1372	19·9	0	0·0

The first series of backcrosses show a slight breakdown in the inviability of K-line hybrid eggs, in the $B_{2k}\beta$ generation, and there is a slight but very doubtfully significant increase in breakdown in the later generations. Breakdown individuals possessed complete fertility with the K parental stock in both reciprocal directions, but were not tested against the S stock. These results parallel those obtained by Laven (1953). Since

the successive backcrosses have increasing dosages of the K genotype, a more rapid breakdown might have been expected.

The second series of backcrosses was carried out to collect additional data, to test for the occurrence of breakdown, and to obtain breakdown individuals for a compatibility test with S stock. There was a complete absence of breakdown up to and including the $B_{6k}\beta$ generation (Table 2). A third series of backcrosses was made solely to check the occurrence of breakdown, and the result was also negative. It seems probable that the breakdowns in the first experiment may have been due to an accidental contamination of the $F_1 \times K$ mating with a K female. The complete inviability of eggs

TABLE 3.
Repeat Experiment to Test for "Breakdown".
(Each mating of approx. 130 and 150.)

Serial No.	Cross.	Progeny Designation.	No. of Eggs.	Hatch.	Percentage Hatch.
1	$F_1^{\alpha} \times K^{\beta}$	B_{1k}^{α}	normal	normal	high
2	$K^{\beta} \times F_1^{\alpha}$	B_{1k}^{β}	5600	0	0·0
3	$B_{1k}^{\alpha} \times K^{\beta}$	B_{2k}^{α}	normal	normal	high
4	$K^{\beta} \times B_{1k}^{\alpha}$	B_{2k}^{β}	6894	0	0·0
5	$B_{2k}^{\alpha} \times K^{\beta}$	B_{3k}^{α}	normal	normal	high
6	$K^{\beta} \times B_{2k}^{\alpha}$	B_{3k}^{β}	6962	0	0·0
7	$B_{3k}^{\alpha} \times K^{\beta}$	B_{4k}^{α}	normal	normal	high
8	$K^{\beta} \times B_{3k}^{\alpha}$	B_{4k}^{β}	5368	0	0·0
9	$B_{4k}^{\alpha} \times K^{\beta}$	$B_{5k}\alpha$	normal	normal	high
10	$K^{\beta} \times B_{4k}^{\alpha}$	B_{5k}^{β}	5054	0	0·0

derived from K females and S maternal line males is maintained up to the $B_{6k}{}^{\alpha}$ generation, and probably indefinitely. $B_{5k}{}^{\alpha}$ individuals, predominantly K in genotype, however, show full fertility and high egg viability when crossed to S stock in either direction. The strictly maternal inheritance of some determinant, either on a chromosome or in the cytoplasm, is demanded.

Sex ratio. Data on sex ratios in S and K stocks, and in F_1 hybrids are presented in Tables 4 and 5. In all cases there is a significant surplus of males, approximating to a ratio of 1·25:1. The most probable cause of this inequality is differential mortality. Males mature and emerge several days earlier than females, and the last larvae to pupate in any culture are invariably female. In the larval and pupal cultures, the death rate increases gradually with time, and there is no reason to assume other than a normal genetic sex determination.

Segregation in the F_2. The two subspecies differ in a small but distinctive character. In K there is a line of white scales on the anterior surface of the femur of the mid-leg, and this line is absent in the S race. In the F_1 there is a thin broken line, indicating an absence of dominance. In the F_2 there is segregation for the character indicating a monofactorial mendelian inheritance independent of sex (Table 6). Actually the genetic control of the character is complex, and probably modifier genes influence its degree of

development. The significance of the segregation for the present purpose lies in the independence of a major "line" gene and the sex chromosome. Normal meiotic segregation is indicated for two of the three chromosome pairs present in *Aëdes*.

The inbreeding program. In the *Aëdes scutellaris* group, mating seems to be conditional on the formation of swarm flights of males, and multiple copulations may occur for each female entering the swarm. Such a system seems adapted to the maintenance of genetic heterogeneity.

TABLE 4.

Sex Ratios.

Culture.	Females.	Males.	Total.	Sex Ratio.
S. 52/5B	176	330	516	1 : 1·87
52/11–B	92	214	306	1 : 2·36
52/7–B	215	201	416	1 : 0·94
Total S	483	745	1238	1 : 1·54
K. 52/1–B	39	45	84	1 : 1·15
52/4–B	239	312	551	1 : 1·31
52/10–B	105	84	189	1 : 0·80
Total K	383	441	824	1 : 1·15
S × K (F_1)*	546	672	1218	1 : 1·23

* See analysis in Table 5.

A need was felt for the isolation of genes with a marked phenotypic effect for use as markers of chromosome segregation. An inbreeding program was commenced with the laboratory stock S, and based on the plan outlined by Spencer (1947). Mass matings were made, and were given two blood feedings within three days. Gravid females were then immediately isolated in 8″ × 1″ tubes, each with a pad of wet filter paper, and the

TABLE 5.

Sex Ratio in S × K Progeny.

Egg Batch.	Date Laid.	Date Sown.	Number of Eggs.	Hatch.		Adults Reared.			% Survival.	Sex Ratio.
				No.	%	♀	♂	Total.		
1	25–26/2/52	3/3/52	39	19	48·7	5	11	16	84·2	1 : 2·22
2	26–27/2/52	,,	139	96	69·1	24	31	55	57·3	1 : 1·39
3	27–29/2/52	,,	167	152	91·0	69	74	143	94·1	1 : 1·07
4	29/2–3/3/52	,,	506	501	99·0	177	217	393	78·4	1 : 1·22
5	3–4/3/52	10/3/52	86	7	8·1	2	2	4	57·1	1 : 1·00
6	4–7/3/52	,,	465	308	66·2	105	132	237	74·5	1 : 1·35
7	7–10/3/52	,,	655	315	48·1	113	108	221	70·1	1 : 0·95
8	10–17/3/52	17/3/52	425	201	47·3	60	105	165	79·0	1 : 1·70
Total			2482	1499	60·4	546	672	1218	81·2	1 : 1·23

tubes were sealed with cotton plugs. Egg laying commenced and continued for 5 to 14 days without further blood feeding. From each family of progenies, sib mass matings were made, and females isolated as before. On the assumption that females copulate repeatedly, each female initially isolated would carry a sample of the sperm produced by the available males. Should any initially isolated female be heterozygous for an uncommon recessive gene *a*, 50% of its progeny would be *Aa*. In the subsequent sib mass mating, 25% of the sperm would be *a*. If three females are isolated from the sib mating there would be an 82½% chance that at least one would be *Aa*, and its progeny should show 12½% of homozygous recessives.

The results of this program are given in Tables 7A and 7B. No success was achieved in the isolation of recessive genes. Possibly the laboratory stock had lost genetic heterogeneity over four years and perhaps 25 generations of artificial culture. Probably the mutant characters likely to be most frequent would be inconspicuous to casual inspection. Nevertheless the results are of considerable interest.

TABLE 6.

F_2 Segregation for White Scales on Midfemur.

	"White."	"Intermed."	"Black."	Total.
Females ..	52	90	51	193
Males ..	33	91	48	172
Total ..	85	181	99	365

Total data: Fit to 1 : 2 : 1 ratio. $\chi^2 = 1 \cdot 08$. 2d.f. $P = 0 \cdot 6$.
Independence of sex linkage: $\chi^2 = 5 \cdot 21$. 5d.f. $P > 0 \cdot 3$.

Reference to Table 2 shows that, under the artificial breeding conditions of the laboratory, there is a considerable difference in fecundity between S and K females. There is no significant difference between self and cross matings, and the lower fecundity of the K females appears to be dominant in the F_1.

TABLE 7A.

Egg Laying of Isolated Females.

Individual Female.	Eggs Laid.	Hatch.	Hatch Percentage.	Means for Inbred Daughters.	
				Eggs Laid.	Percentage Hatch.
52/11·1	24	20	83·3	38·5	31·6
52/11·2	58	34	58·6	24·7	37·4
52/11·3	50	36	72·6	27·2	59·5
52/11·4	29	19	65·5	17·3	33·3
52/11·5	85	59	69·4	29·7	39·3
52/11·6	96	50	52·1	32·0	8·7
52/11·7	56	34	60·7	46·2	33·0
52/11·8	50	30	60·0	26·8	66·7
52/11·9	30	19	63·3	1·0	0·0
52/11·10	35	30	85·7	19·0	67·5
52/11·11	29	24	82·7	56·6	54·1
52/11·13	32	26	81·2	38·5	51·9
52/11·14	80	60	75·0	13·3	22·6
52/11·15	42	37	88·1	18·5	55·7
52/11·16	11	0	0·0	—	—
52/11·17	19	17	89·5	—	—
52/11·18	31	0	0·0	—	—
52/11·19	14	14	100·0	26·0	23·1
52/11·20	53	43	81·1	29·6	40·6
52/11·21	48	42	87·5	39·0	52·1
52/11·22	19	0	0·0	—	—
52/11·23	139	131	94·2	16·3	39·1
Total	1030	725	70·4	—	41·7
Mean	46·8	—	—	24·7	—

TABLE 7B.

Egg Laying of Inbred Females.

Individual.	Eggs Laid.	Hatch.	Hatch %
52/11· 1– 1	42	20	
2	32	3	
3	35	20	
4	49	7	
M.	*39·5*	—	*31·6*
52/11· 2– 1	11	7	
2	25	20	
3	4	0	
4	59	10	
M.	*24·7*	—	*37·4*
52/11– 3– 1	45	30	
2	39	27	
3	7	0	
4	3	2	
5	61	37	
6	8	1	
M.	*27·2*	—	*59·5*
52/11· 4– 1	23	8	
2	0	0	
3	17	4	
4	29	11	
M.	*17·3*	—	*33·3*
52/11· 5– 1	51	21	
2	22	8	
3	16	6	
M.	*29·7*	—	*39·3*
52/11· 6– 1	59	0	
2	1	0	
3	2	0	
4	1	0	
5	97	14	
M.	*32·0*	—	*8·7*
52/11· 7– 1	40	0	
2	44	0	
3	43	38	
4	33	26	
5	53	0	
6	34	7	
7	76	39	
8	47	12	
M.	*46·3*	—	*54·1*
52/11· 8– 1	58	48	
2	0	—	
3	0	—	
4	38	Lost	
5	38	16	
M.	*6 8*	—	*66·7*
52/11· 9– 1	0	—	
2	1	0	
3	3	0	
4	0	—	
M.	*1·0*	—	*0·0*
52/11·10– 1	46	37	
2	1	0	
3	0	—	
4	49	40	
5	0	—	
6	18	0	
M.	*19·0*	—	*67·5*
52/11·11– 1	62	35	
2	53	44	
3	53	12	
M.	*56·0*	—	*54·1*
52/11·13– 1	70	37	
2	7	3	
M.	*38·5*	—	*51·9*
52/11·14– 1	0	—	
2	6	1	
3	26	17	
4	38	0	
5	22	3	
6	0	—	
7	1	0	
M.	*13·3*	—	*22·6*
52/11·15– 1	17	17	
2	41	0	
3	1	0	
4	0	—	
5	0	—	
6	21	21	
7	0	—	
8	1	0	
9	1	0	
10	41	31	
11	71	39	
M.	*18·5*	—	*55·7*
52/11·19– 1	0	—	
2	52	12	
M.	*26·0*	—	*23·1*
52/11·20– 1	0	—	
2	39	29	
3	89	25	
4	10	2	
5	0	—	
M.	*29·6*	—	*40·6*
52/11·21– 1	59	35	
2	58	26	
3	0	—	
M.	*39·0*	—	*52·1*
52/11·23– 1	0	—	
2	29	6	
3	0	—	
4	28	19	
5	49	24	
6	49	5	
7	43	16	
8	3	0	
9	10	5	
10	9	4	
11	16	12	
12	18	9	
13	2	0	
14	0	—	
15	0	—	
16	5	2	
M.	*16·3*	—	*39·1*
Total ..	2422	1010	41·7
General mean	24·7	—	—

Isolated females of race S show considerable variation in fecundity, but the mean, 46·8 eggs per female, is comparable with the figure obtained for S females in mass culture (Table 7A). The mean hatching, as a percentage of total eggs, was 70·4% in the isolations, and 71·1% in the mass culture. Among the inbred daughters, many failed to oviposit, and the means, eggs per female (24·7) and hatching (41·7%), were much lower. The figures suggest a marked degree of inbreeding depression.

The time of action of lethality. In a considerable proportion of eggs derived from K × S and B × S matings, sperm heads have been seen in the egg cytoplasm if the eggs are crushed in aceto-orcein within half an hour of oviposition. Similar observation of 6-hour eggs indicates that some at least undergo early embryonic development. Inviability is not due to any failure of sperm to penetrate the eggs, but rather to an incompatibility between the sperm, or the hybrid embryo, and the egg cytoplasm.

In viable eggs of *Aëdes scutellaris*, and in S × K hybrids, development is very rapid, and an almost fully developed embryo is produced in 48 hours. In the inviable K × S eggs, death ensues at an early stage, and after 24 hours they show a definite collapse. Lethality is effective either before or during the cleavage divisions, or in the early blastoderm—i.e., in the stage 1 of Hadorn's (1948) classification of *Drosophila* lethals. In contrast, lethality in Laven's *Culex molestus* hybrids (Laven, 1953) is effective much later, during late embryonic stages, or during or shortly after hatching. The *Culex molestus* lethality is also characterized by a much lower penetrance.

Discussion.

It is neither desirable nor possible to attempt an adequate discussion of the data presented in the present paper without a consideration of the main features of non-reciprocal fertility in other groups of the Culicidae. In particular, the results obtained by Laven (*l.c.*) in *Culex* and by us in *Aëdes* show such a close parallelism that they must be dependent on similar genetic mechanisms.

1. *Possible genetic mechanisms.*

At the present stage, several possible genetic mechanisms must be considered, if only for the summary rejection of some. Parthenogenesis and pseudogamy can be dismissed on the basis of the F_2 segregations given in Table 6. Laven (1953) and Toumanoff (1950) also give evidence eliminating parthenogenesis, but Downs and Baker (1949), Bonnet (1950), and Perry (1950) find some support for its assumption.

Hypotheses of predetermination, of the *Limnaea* type, are incompetent to explain the behaviour. A possible system of multiple incompatibility (*s*) genes affecting the survival of S or of hybrid sperm in K egg cytoplasm, suggested by Smith-White (1950), is denied by Laven's and our own results. Such a system should give a uniformly increasing rate of breakdown in the later backcross generations, the actual rate depending on the number of *s* genes involved, and on their linkage relationships. In our material, breakdown is absent or extremely rare, and in Laven's there was a constant rate of breakdown up to the 11th backcross generation. Both cases are characterized by their permanence, and neither is affected by the building up of K or O* genoms, respectively, in the backcrosses.

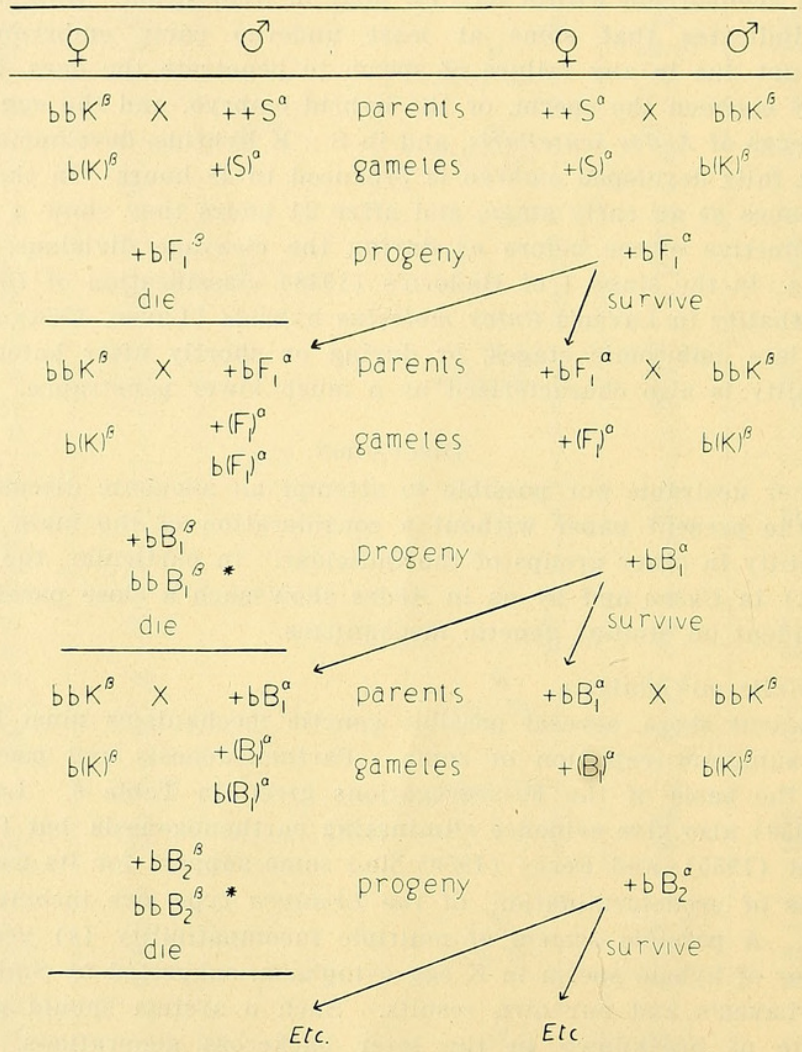
In a female-heterozygous sex system, a sex-linked incompatibility-lethal could explain both the *Culex* and *Aëdes* systems, with breakdown due to crossing over between the lethal and sex genes. However, Gilchrist and Haldane (1947) have demonstrated a male-heterozygous sex-system in *Culex molestus*, and this type of sex determination is characteristic of the Nematocera (White, 1949).

Two kinds of genetic mechanisms remain. One is chromosomal, and is dependent on the uniparental inheritance of chromosomes or of chromosome segments, and involves anomalies of the meiotic cycle. The other is nucleus-independent and cytoplasmic.

Very peculiar meiotic cycles are known to exist in other families of the Nematocera. In *Sciara* the chromosomes of paternal origin are eliminated during spermatogenesis

* O is the symbol used by Laven for his Oggelshausen strain of *Culex molestus*.

(Metz, 1938). In *Miastor* and other genera of the Cecidomyidae, many chromosomes are eliminated in spermatogenesis, and are transmitted only in the female line. In the Culicidae, limited chromosomes are not present. In *Culex* (Callan and Montalenti, 1947) and in *Aëdes* chiasmata are formed in all three bivalents in male meiosis. Moreover, in *Aëdes scutellaris*, the approximately normal sex ratio, and the sex independent segregation of "line" provide genetic evidence for the normal meiotic separation of two of the three chromosome bivalents.



Text-figure 2.—Gene control of non-reciprocal fertility, dependent on the elimination of a paternal chromosome segment in oogenesis.

The symbols K, S, F_1, B_1, etc., refer to the *unlimited* gametic genoms. *b* and + are alleles which condition the cytoplasm, and are carried on limited (maternally-inherited) chromosome segments.

Note.—Sperm $b(F_1)^{\alpha}$ from *a* males are unadapted and hybrids derived from unadapted sperm and $b(K)^{\beta}$ eggs die.

It is possible to devise chromosomal systems of permanent non-reciprocal fertility on the basis of two assumptions. There must be a strictly polarized segregation of a chromosome segment in oogenesis, with the elimination of the segment of paternal origin in hybrids. It is not necessary to assume any similar elimination in spermatogenesis, and the sperm wastage which would result from such behaviour is not evidenced. In oogenesis, the paternal segment must be directed into the polar bodies. The second necessary assumption is the existence of a cytoplasm-conditioning gene, carried on the polarized chromatin, in one or other of the two species or races involved. An example of this type of hypothesis is offered in Text-figure 2. Race K is there considered to be homozygous for the cytoplasm-conditioning gene "bar" or "*b*", carried on polarized