living specimens, is that in *fatigans* the fourth and fifth segments are held approximately at right angles to the shaft; in *australicus* the fifth segment is bent backwards (Text-fig. 1).

The male hypopygium is also intermediate between those of *pipiens* and *fatigans* but it is sharply distinct from both (Text-fig. 2). The dorsal processes of the mesosome are directed outwards, are thickened distally and are slightly excavated at the tip. In *fatigans* these processes are upright, i.e. are almost parallel and are pointed. The ventral processes in *australicus* are leaf-like distally and are thus unlike the narrow sickle-shaped processes of *pipiens* (and *molestus*).

With regard to North American *pipiens*, however, the position is not clear. The mesosome of the Baltimore *pipiens* studied by Sundararaman (1941) and Rozeboom (1951) is distinctly different from that of European *pipiens*. This is shown by

TABLE 3.

Characteristics of the Male Palps of Members of the pipiens *Complex. Measurements are expressed in Millimetres. Measurements of European* pipiens *and* molestus *are taken from Christophers* (1951).

	Number of Specimens.	Length of Proboscis.	Dimensions and Proportions of Palps.						Number of Hairs on Shaft of Palp.			
			Segments 1–3.	Segments 1–4.	Total.	Segs. 1–4/ Proboscis.	Segs. 1–3/ Seg. 4.	Segs. 1–3/ Seg 5.	Number of Specimens.	Minimum.	Maximum.	Mean.
pipiens (Europe)	20	2·54	1·90	2·60	3·42	1·02	2·71	2·32	8	19	27	24
australicus	100	2·40	1·80	2·43	3·13	1·01	2·86	2·57	50	29	50	35
fatigans (Victoria)	100	2·06	1·58	2·05	2·55	0·99	3·36	3·16	50	6	14	10
molestus (Europe)	20	2·40	1·80	2·36	2·95	0·98	3·21	3·05	7	11	18	16
molestus (Victoria)	100	2·50	1·79	2·37	3·00	0·94	3·14	2·78	50	12	21	15

Rozeboom's illustration (Mattingly *et al.*, 1951, p. 347) and by his statement that it "closely resembles" the mesosome of the type specimen of *C. comitatus* from California for, according to Edwards (1931) and Freeborn (1926), *comitatus* is identical with *C. pipiens pallens* from the Orient. Edwards recognized *pallens* as a separate subspecies because of its distinctive mesosome.

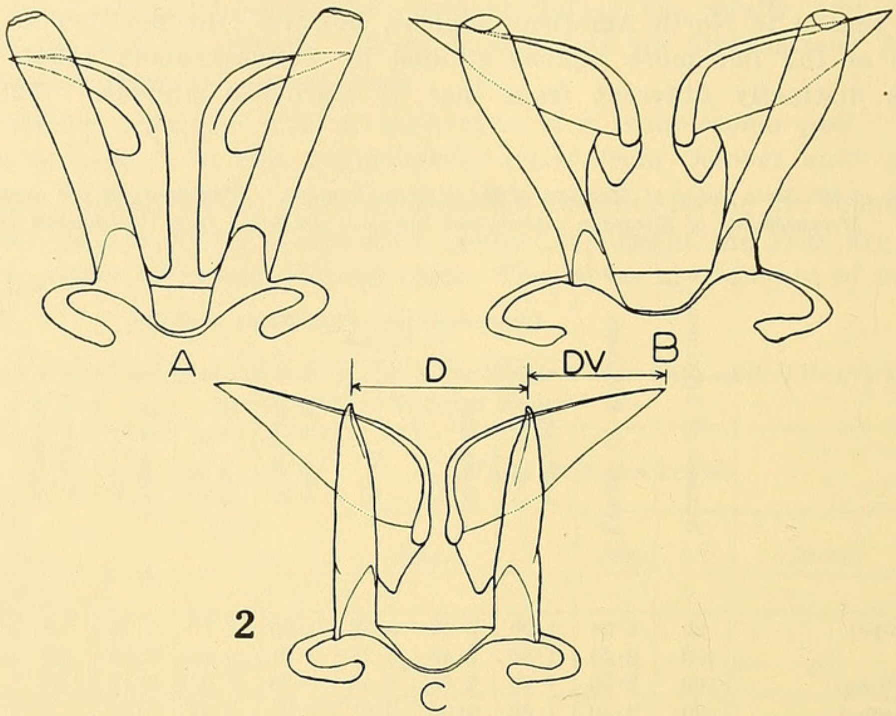
Further evidence that the mesosome of Baltimore *pipiens* is different from that of the European is given by the data of Sundararaman (1949) and Barr (Rozeboom, 1951) on the DV/D ratio. Both these workers found that the ratio was zero or positive. Christophers (1951) pointed out that in his strains of *pipiens* and *molestus* the ratio was negative and this was generally true of the Cairo *molestus* studied by Knight and Malek (1951), where the ratio varied from minus 0·14 to plus 0·02. There is then reason to doubt Sundararaman's identification of his material as *C. pipiens pipiens*.*

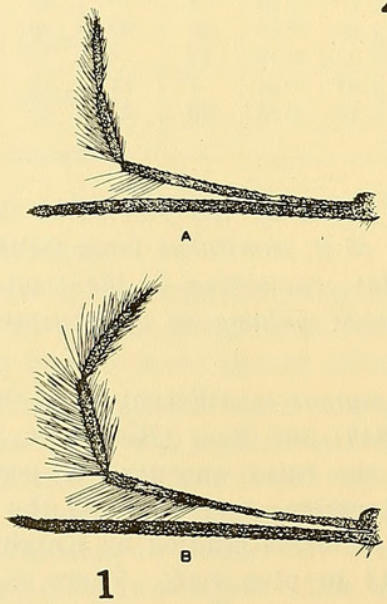
In respect of the structure of the mesosome, *australicus* approaches *pipiens pallens* and the Baltimore *pipiens*, but it is distinct from both these forms. Little information is available on *pipiens pallens*, but the observations of Feng (1938) indicate that it is a typical domestic mosquito. In their biology and morphology *pallens* and *comitatus*, in contrast to *australicus*, are closer to *fatigans* than to *pipiens*. It is, indeed, not clear why *pallens* is not regarded as a subspecies of *fatigans* rather than of *pipiens*. *australicus* and Baltimore *pipiens* differ in their biology, e.g. Baltimore *pipiens* will

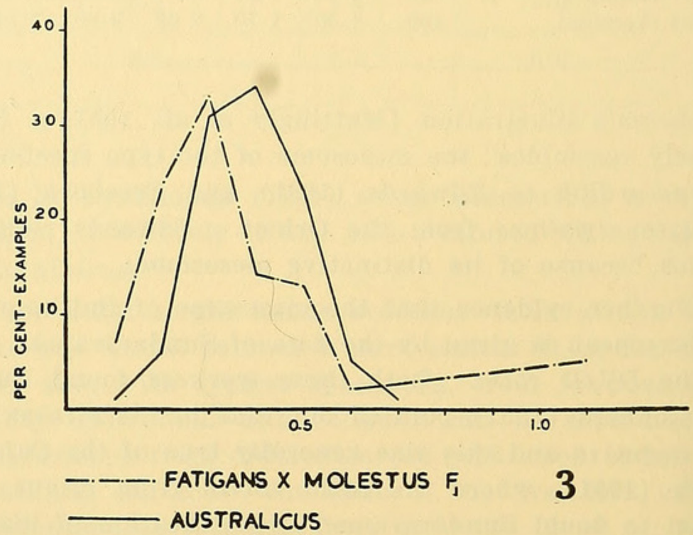
* The position is further complicated by the fact that in specimens of Baltimore *pipiens* sent to us by Professor Rozeboom the mesosome is identical with that of typical *pipiens*. The siphon index of larvae varied from 3·9 to 4·7, with a mean of 4·2; these values correspond to those of *molestus* and *fatigans*.

mate in a space of one cubic foot whereas *australicus* is eurygamous, and also in the structure of the mesosome. This is evident from a comparison of the published figures of the two forms and from the DV/D ratio. In *australicus* the ratio is higher and scarcely overlaps that of Baltimore *pipiens*.

As is shown below, *molestus* and *fatigans* will interbreed readily in the laboratory. The mesosome of the hybrids is intermediate between those of the parent forms; the ventral arms are long and broad; the dorsal arms are sometimes pointed but are







Text-fig. 1.—Structure of the male palp. A. *fatigans*; B. *australicus*.
Text-fig. 2.—Structure of the male mesosome. A. *molestus*; B. *australicus*; C. *fatigans*.
Text-fig. 3.—Distribution of DV/D in *australicus* and in *molestus* × *fatigans* hybrids.

usually of uniform thickness with a slight hollowing at the tip. The position of the dorsal arms is very variable; sometimes they are almost parallel, as in *fatigans*, but generally are directed more or less outwardly towards the tips of the ventral processes. Through the courtesy of Professor Rozeboom we have been able to examine specimens of the "Alabama *quinquefasciatus*". The range of morphological variation of the mesosome seems to be the same as in our *molestus* × *fatigans* hybrids. This observation supports the contention of Sundararaman (1949) and Rozeboom (1951) that the "Alabama *quinquefasciatus*" is a hybrid between *pipiens* (or *molestus*) and *fatigans*.

The DV/D ratio of this American form, like that of our laboratory hybrids, is very similar to that of *australicus* (Text-fig. 3); the mesosome of *australicus*, however, is morphologically distinct.

In several morphological characters *australicus* approaches *fatigans*; biologically it is almost identical with *pipiens*.

It is anautogenous. It is not a man-biting mosquito; adults caught in houses were never freshly engorged and further, when fed, in the laboratory, on human blood, the egg rafts deposited were only about one-third the size of those found in nature (Table 4). Although chickens and canaries were not attacked in the laboratory, birds are evidently normal hosts. Many adults were caught in a chicken house (chickens and ducks) in Melbourne; ten freshly engorged ones had bird blood in the gut; others laid rafts of normal size (Table 4).

Unpublished observations of Mr. D. J. Lee show that *australicus* also attacks rabbits.

TABLE 4.

Size of Egg Rafts of australicus. *The Measurements were made along the Axes of Greatest Length and Greatest Breadth.*

	Number of Rafts.	Size in mm.			Number of Eggs.		
		Min.	Max.	Mean.	Min.	Max.	Mean.
From natural breeding places	51	2·9×1·4	5·6×2·1	4·7×1·4	136	503	256
From females caught in chicken house ..	18	3·0×1·0	6·5×1·3	4·9×1·4	113	380	247
From females fed on human blood ..	25	1·6×0·6	3·0×1·2	2·3×1·0	30	126	73

australicus is eurygamous and in the laboratory we have not been able to get it to mate regularly. Mating never occurred in cages of 2400 cubic inches and only rarely in cages of 40 cubic feet. It was no more frequent when several hundred adults were liberated in a room (500 cubic feet). The temperature was maintained at different levels between 20°C. and 25°C., the humidity and intensity of illumination (white and blue lighting) were varied, but over a period of a fortnight only three females out of a hundred examined were fertilized.

Judging from the results of cross breeding experiments between members of the *pipiens* group, the failure to obtain free mating of *australicus* is due to a disability of the males rather than of the females.

Swarming of males in the field has been observed on many occasions. It occurs shortly after sunset in the vicinity of breeding grounds. Swarms consist of 100–150 males which move rhythmically in a vertical direction some five to six feet above the ground.

australicus is heterodynamic. Oviposition seems to cease early in April. Adults collected later in this month refused to feed and could only be induced to do so by exposure to artificial lighting for about ten days. Feeding was followed by oviposition. In the field, neither adults nor larvae were found during the winter. A few advanced larvae were present late in August but the numbers were far too small to account for the abundance of adults in early spring. It appears that some females are active in August but that the majority remain in hibernation until late in September.

In Melbourne, *australicus* continues to breed throughout the summer, but some observations at Mildura suggest that in northern Victoria reproduction is interrupted during mid-summer. In early December *australicus* was found to be the dominant *Culex*; adults were abundant in chicken houses and larvae were numerous. In early February it was rare except for first stage larvae. Two months later, in mid-April, all the larvae were at the third and fourth stages; few adults were found in chicken houses;

presumably they had entered hibernation. These observations, though limited, suggest that in Mildura, *australicus* has a peak of abundance in spring and early summer and a second one in early autumn. On the other hand, *fatigans*, after starting rather later than *australicus*, breeds continuously throughout the summer and autumn.

TABLE 5.

Breeding Sites of fatigans *and* australicus *at Merbein.*

Breeding Sites.	Number of Males Examined.	*fatigans.*	*australicus.*
Goose pond (foul, muddy water)	70	97 per cent.	3 per cent.
Rain water tanks ..	56	94 ,,	6 ,,
Horse trough	50	100 ,,	0 ,,
Marsh	56	18 ,,	82 ,,
Flooded pasture ..	35	5 ,,	95 ,,

Larval Ecology.—Larvae of *australicus* are found in a variety of habitats both urban and rural. They may be present in artificial containers and occasionally in polluted water. The favoured breeding sites, however, are pools, swamps or channels characterized by stationary or slowly moving, clean water. The contrast between *australicus* and *fatigans* in relation to breeding sites is shown by observations made at Merbein (Table 5). Table 5 was compiled by counting males, identified by their hypopygia, which emerged from collections of larvae from the various sites. It will be seen that *fatigans* predominated in polluted water and artificial containers; *australicus* was predominant in natural ground water.

TABLE 6.

Siphon Index and Length of Siphon of Larvae of australicus *from Various Localities. Measurements are in Microns.*

	No.	Siphon Index.			Siphon Length.		
		Max.	Min.	Mean.	Max.	Min.	Mean.
Williamstown	37	6·4	5·2	5·6	1710	1386	1512
Gunbower	19	5·8	4·7	5·3	1854	1458	1620
Undera	25	6·3	5·2	5·7	1908	1476	1674
Inglewood	25	6·3	5·3	5·5	1710	1350	1530
Melbourne suburbs ..	100	6·3	4·4	5·3	1692	1260	1386
	206	6·4	4·4	5·5	1908	1260	1494

australicus is a rural or semi-rural mosquito; in this, as in other important biological characters, it is different from *fatigans* but similar to *pipiens*.

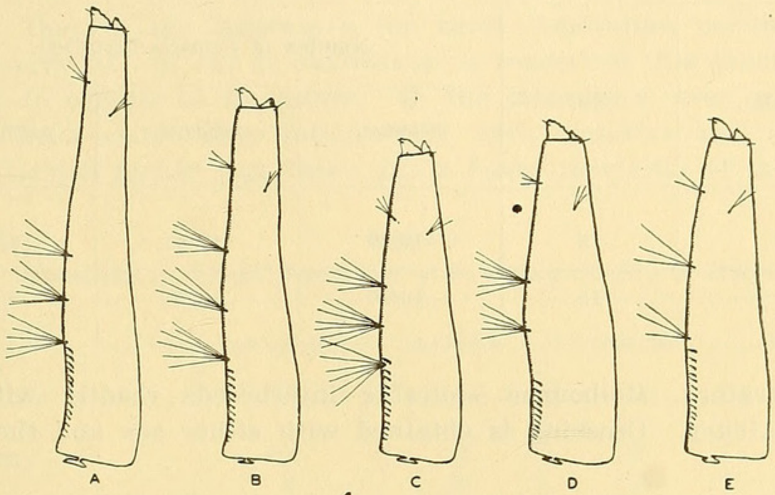
The larvae of *australicus* are morphologically similar to those of *fatigans* and *molestus* but can be distinguished by the siphon index (Table 6; Text-fig. 4). The average value of the index in the three forms is: *australicus*, 5·5; *fatigans*, 4·2; *molestus*, 4·3. As can be seen from Text-figure 4, there is only a small overlap between *australicus* and *fatigans*. The siphon is slightly curved while in *fatigans* it is straight (Text-fig. 5).

The pupa of *australicus* can be distinguished from those of *molestus* and *fatigans* by the trumpet, which in *australicus* is almost cylindrical and at least five times as long as its greatest width. The paddle is oval and more narrow than in *molestus* or *fatigans*.

B. CROSS-BREEDING WITHIN THE PIPIENS COMPLEX.

a. Laboratory Experiments.

For cross-breeding experiments we have used (1) *australicus* from natural populations in the suburbs of Melbourne; (2) *molestus* from a laboratory colony established from females caught in Melbourne and maintained autogenously; (3) *fatigans* from a laboratory colony derived from egg rafts collected at Albury. Examination of male genitalia showed that the laboratory colonies were pure strains. Some additional experiments were made with *C. globocoxitus* which were obtained from natural populations in Melbourne. All the adult mosquitoes used in these experiments had emerged from pupae reared singly in separate tubes.



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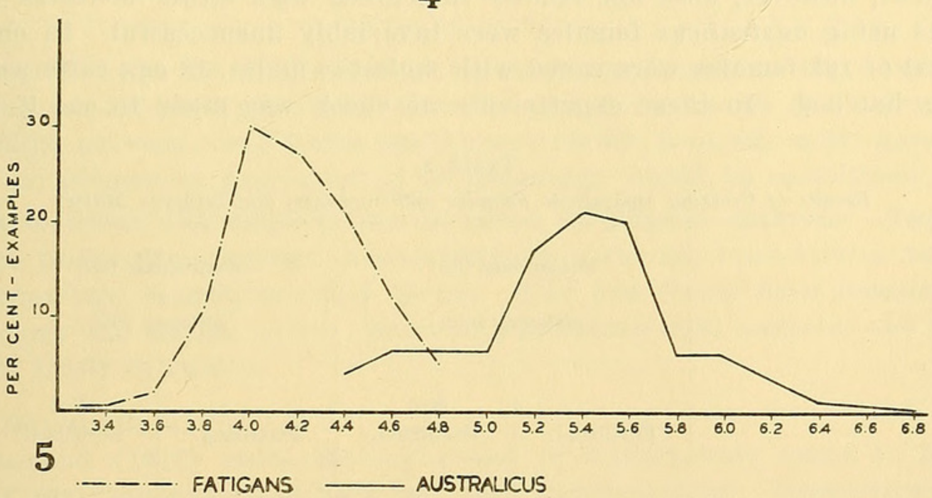

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Text-fig. 4.—Siphon of the fourth-stage larva. A, B. *australicus*; C, D. *fatigans*; E. *molestus*.

Text-fig. 5.—Distribution of the siphon index in fourth-stage larvae of *australicus* and *fatigans*.

The object of the first experiments was to test the mating preferences among the *pipiens* complex. Females of *molestus*, *fatigans* and *australicus* were caged together with either *molestus* or *fatigans* males and after twenty-four hours were dissected and their spermathecae examined. For *molestus* males the cage had a capacity of a thousand cubic inches; for *fatigans* males it was a cubic foot in size. The temperature was 23°–24°C.

These experiments showed that *molestus* and *fatigans* males did not distinguish between their respective females (Table 7). Mating with *australicus* was less frequent. In the two experiments only 20 per cent. of these were fertilized as against 80 per cent. of the other two forms.

In another experiment of this kind the *fatigans* females were replaced by *globocoxitus* females. A group of sixty females, twenty of each form, were caged with forty *molestus* males for four days at 18°–20°C. Fertilization occurred in twelve *molestus*, five *australicus* and four *globocoxitus*.

The infrequent mating of *australicus* females with *molestus* and *fatigans* males, and this was also observed in direct cross-breeding experiments, may possibly be due to the existence of some mechanical barrier to copulation. However, as will be shown later, *globocoxitus* males, whose distinctive genitalia might be expected to prove a bar to mating with members of the *pipiens* complex, mate freely with *molestus* and *fatigans*. A more probable explanation lies in the fact that *australicus* is eurygamous whereas the others are stenogamous.

TABLE 7.

Preferential Mating within the pipiens *Complex.*

Males.	No.	Number of Females Fertilized.		
		molestus.	*fatigans.*	*australicus.*
molestus ..	15	17/20	12/20	3/20
fatigans ..	15	16/20	18/20	5/20

In the laboratory, Melbourne *molestus* interbreeds readily with *fatigans* from Melbourne and Albury. Crossing is obtained with either sex and the F1 are vigorous and fertile.

australicus, however, does not readily interbreed with either *molestus* or *fatigans*. Experiments using *australicus* females were invariably unsuccessful. In one series, in which a total of 101 females were caged with *molestus* males, 18 egg rafts were obtained but no eggs hatched. In these experiments no check was made to see if the females

TABLE 8.

Results of Crossing australicus *Females with* molestus *and* fatigans *Males.*

	australicus (38) × *molestus* (60)		*australicus* (30) × *fatigans* (50)	
	Fertilized.	Not Fertilized.	Fertilized.	Not Fertilized.
Refused to feed ..	1	1	2	9
Fed :				
Eggs not laid ..	2	19	1	13
Rafts laid ..	11	3	4	1
Eggs hatched ..	0	0	0	0

laying the egg rafts had been fertilized. In a later experiment each female, after laying, or after death if no eggs were laid, was dissected and the spermatheca examined. Thirty-eight *australicus* females were caged with 60 *molestus* males for two days. After a blood meal the females were placed separately in tubes with water for oviposition. It will be seen from Table 8 that 11 of the 14 females which deposited eggs had been fertilized. None of the eggs hatched. Similar results were obtained in crosses between female *australicus* and male *fatigans* (Table 8). Four egg rafts were obtained from fertilized females, but again none hatched.

Reciprocal matings were not often successful because, as pointed out above, *australicus* males rarely mate in the laboratory. Only a few *molestus* and *fatigans* females were fertilized even when caged with large numbers of *australicus* males for periods of two to three weeks. However, in contrast to the previous experiments, all the egg rafts deposited were fertile to some degree. In *molestus* × *australicus* crosses the hatch in different rafts varied from 21 per cent. to 95 per cent.; in *fatigans* × *australicus* crosses, hatching averaged about 80 per cent. In both crosses the F1 larvae appeared to develop normally but there was a heavy mortality in the pupal stage. The viability of the F2 eggs was low; there was never more than a 50 per cent. hatch.

Thus crosses between female *australicus* and male *molestus* or *fatigans* were sterile but the reciprocal crosses were fertile. This phenomenon has been observed in various species and subspecies of *Aëdes* (Woodhill, 1949, 1950; Perry, 1950; Downs and Baker, 1949) and also between different races of *molestus* (Laven, 1951*a*).

It is clear that in the laboratory the three Australian members of the *pipiens* complex can interbreed. As far as *australicus* is concerned this conclusion probably has little relevance to conditions in nature. In the laboratory, even when no choice was possible, *australicus* mated only infrequently with *molestus* and *fatigans*, and when these matings yielded fertile eggs there was a heavy mortality of the F1 pupae. These

TABLE 9.

Composition of Natural Populations of the pipiens *Complex in Melbourne.*

	australicus.	*molestus.*	*fatigans.*	Hybrids.
February	62	19	17	2
May	8	20	42	30

facts, coupled with the differences in larval ecology and mating behaviour between *australicus* on the one hand and *molestus* and *fatigans* on the other, suggest that interbreeding between these forms would occur rarely, if at all, under natural conditions and that no permanent population of intermediates would be established.

With *molestus* and *fatigans* the situation is entirely different. These two forms exhibit no preferential mating, crosses between them are fully fertile, and the hybrids are vigorous and themselves fully fertile. The two forms have essentially the same larval ecology and mating habits. One would anticipate that *molestus* and *fatigans* would interbreed freely in nature.

b. Field Observations.

Drummond (1951) noted the occurrence of intermediate forms in Melbourne and suggested that *molestus* and *fatigans* were interbreeding. Supporting evidence has come from observations on the mosquito population of a water butt at the Zoology Department. Two large samples of late larvae and pupae were taken, one in February and one in May. From each sample 100 males were reared and classified on their hypopygia (Table 9).

Both *australicus* and *molestus* had been established in the water butt for several months prior to taking the first sample, but *fatigans* which, as stated earlier, is common in Melbourne only during late summer and autumn, was a recent arrival. Only two of the hundred males of the February sample were hybrids. By the end of May, however, the *australicus* population had declined, *fatigans* had become numerous and there were 30 hybrids.

Hybrids obtained in the laboratory between members of the *pipiens* complex are very similar morphologically and caution must be exercised when assigning the parentage of natural hybrids. However, of the 32 hybrids recorded above, 30 fell within the range of variation found in *molestus* × *fatigans* laboratory hybrids. The remaining two were different but were also different from any of the *australicus* × *molestus* or *australicus* × *fatigans* laboratory hybrids. Their origin remains in doubt.

Apart from these two specimens we have found no others which could be regarded as *australicus* × *molestus* hybrids, although the two forms are found breeding in close proximity to one another over a wide area in southern Victoria.

Melbourne does not provide adequate material for investigating natural hybridization between *australicus* and *fatigans*. *fatigans* does not become numerous until autumn, by which time *australicus* is declining. However, in northern Victoria the two forms are found together for a large part of the year. Of 300 males of the *pipiens* complex collected at several localities at Merbein, and classified on their hypopygia, 207 were definitely *fatigans* and 92 definitely *australicus*. The remaining specimen was possibly a hybrid.

Our general conclusion from these laboratory and field observations is that *australicus* is reproductively isolated from both *molestus* and *fatigans* but that the two latter forms interbreed where they come into contact. A permanent population of intermediates has not been found in Melbourne but may become established in the northern part of the State.

As already indicated, *C. globocoxitus*, the fourth member of the *pipiens* group in Australia, will interbreed freely in the laboratory with both *molestus* and *fatigans*. The crosses were fully fertile and the larvae developed normally to give a fertile F1. In crosses with *australicus* no adult hybrids were obtained. About 80 per cent. of the eggs hatched but the larvae failed to develop.

Crossing between *globocoxitus* and *molestus* occurs occasionally in nature. Three specimens have been collected in suburbs of Melbourne which are indistinguishable from laboratory hybrids between these forms.

C. Taxonomic Status of the Members of the C. pipiens Complex.

a. molestus.

The discussion on the *C. pipiens* complex (Mattingly *et al.*, 1951) revealed a wide divergence of opinion on the status of *molestus*. Christophers and Shute believe that the morphological and biological differences between *pipiens* and *molestus* warrant both being regarded as distinct species. On the other hand, Laven and Mattingly were of the opinion that "in the *pipiens-molestus* complex we are faced with an assemblage of diverse genetical potentialities, the expression of which is conditioned by the selective action of the environment rather than by any limitation to cross breeding".

The gene concerned with autogeny is not restricted to *molestus* and is not necessarily of high frequency in all *molestus* populations. Similarly the other biological characteristics of *molestus* are not necessarily associated; there are forms known which are eurygamous and man-biting, stenogamous and non-man-biting. For these reasons Mattingly (1951, 1951*a*) concluded that the occurrence of "typical" *molestus* is a local phenomenon, and, since it had been recorded mainly in large cities, he suggested that it should be considered an urban biotype and called, if a name were necessary, form *molestus*.

In Australia the range of *molestus* extends from the south coast of Victoria and northern Tasmania to Mildura, some 400 miles to the north. Throughout this range the combination of characters which typify *molestus* are preserved. It appears, therefore, that either the environmental differences within this area are too small to have any appreciable selective action or we are dealing with a pure *molestus* stock. All our observations indicate that in south-eastern Australia we have a mosquito which presents constantly the morphological and biological characters of *molestus* as defined by Marshall and Staley.

We cannot accept Mattingly's contention that *molestus* is a strictly urban biotype. In Australia it is associated with dwellings, but it breeds in water butts, ditches and drainage pits, and in such situations larvae are found in rural areas.

Our conclusion is that *molestus* should be distinguished from *pipiens* and called *C. pipiens* L. form *molestus*, using the term "form" as it is used by Knight and Malek (1951) to indicate that its relationship to other members of the complex has yet to be determined. As Mattingly (1951*a*) has pointed out, future work may show that *molestus* has its closest affinities with *fatigans* rather than *pipiens*.

b. fatigans.

The status of *fatigans* as a species has been questioned because of its ability to interbreed with other members of the *pipiens* complex. However, the statement in several recent publications that it interbreeds with *pipiens* requires qualification.

In laboratory crosses Weyer (1936) found that *molestus* and *fatigans* were interfertile but that when *pipiens* and *fatigans* were crossed no eggs were produced. In similar experiments Roubaud (1941) obtained eggs from both crosses, but those resulting from *pipiens* × *fatigans* matings yielded no fertile hybrids. Farid (1949), Sundararaman (1949) and Rozeboom (1951) have reported complete interfertility in crosses between laboratory strains of *pipiens* and *fatigans* but, as pointed out above, their *pipiens* was not typical.

The position seems to be that *fatigans* will not interbreed with *pipiens* but will interbreed freely with *molestus* and with a North American form of *pipiens* which may itself be a hybrid. Until the status of these latter forms has been determined, it is premature to treat *C. fatigans* as a subspecies of *C. pipiens*.

c. australicus.

This is primarily a rural mosquito. It is widely distributed in Australia but, as far as is known, does not occur elsewhere. This suggests that it is a relatively ancient member of the Australian fauna. The other two members of the *pipiens* complex appear to be recent introductions. Mackerras (1950) suggests that *fatigans* was brought in by the early white settlers; *molestus* has been found here only during the last ten years.

australicus has thus been isolated for a long period from other members of the complex and, as shown by laboratory and field observations, is reproductively isolated from *molestus* and *fatigans*. In Victoria it exists side by side with *molestus* without the production of an intermediate population; in New South Wales, Queensland and Western Australia it is in contact with *fatigans* but the two forms remain distinct. Whether *australicus* and *pipiens* would be interfertile is not known; there would be no ethological barrier to mating.

If *fatigans* and *molestus* were definitely accepted as subspecies of *C. pipiens*, *australicus* could be regarded as a distinct species. As Mayr (1942, p. 179) has written, "owing to range expansion two formerly allopatric forms begin to overlap and to prove thereby to be good species. If no overlap existed and if we had to classify these forms merely on the basis of their morpohological distinctness, we would probably decide, in most cases, that they were subspecies. But overlap without interbreeding shows that they have attained species rank." The status of *molestus* and *fatigans*, however, is not settled, and to describe *australicus* as a distinct species would ignore its very close relationship to *pipiens*. The status of *australicus* should be determined by this relationship rather than by reference to *molestus* and *fatigans*.

Within the *pipiens* complex there seem to be two major evolutionary lines: one, represented by *molestus* and *fatigans*, leading to domestic, stenogamous, man-biting and homodynamic mosquitoes, the other, represented by *pipiens* and *australicus*, leading to rural, non-man-biting, eurygamous and heterodynamic mosquitoes. The two lines tend to be isolated ethologically; genetic isolation between them seems to have been largely achieved except as between *molestus* and *pipiens*.

For these reasons we propose to describe *australicus* as a new subspecies of *Culex pipiens* L. A formal description is given below.

CULEX PIPIENS AUSTRALICUS, n. subsp.

Adult.

The male differs from *C. pipiens* L. as follows. The general colour is darker, almost black. The upper surfaces of the proboscis, palps and legs, the tergites and the median and lateral patches on the sternites are black-scaled. The shaft of the palp is more hairy than in *C. pipiens* L. The pleurae, in addition to the usual patches of white scales,