Direct Evidence for the Specific Distinctness of Forms A, B and C of the Anopheles gambiae complex.

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It is now rather generally accepted that the two salt-water-breeding members of the Anopheles gambiae complex are distinct species, Anopheles melas Theobald and A. merus Dönitz (Paterson, 1962 and 1963a; Coluzzi, in press; Davidson, 1964). Less agreement has up to now been obtained on the status of each of the three freshwater-breeding members of the complex, the forms A, B and C. Paterson (1963), Paterson (in press), Paterson, et al (1963) have argued that these forms are good species mainly because they coexist at various places. This coexistence is interpreted as evidence for the existence of intrinsic isolating mechanisms which strongly limit, or prevent altogether, gene exchange between the forms in nature (see Mayr, et al, 1953). This basic argument was supported by evidence of partial sterility between the forms (Davidson & Jackson, 1962; Paterson et al, 1963) and by some evidence for behavioural differences, at least between form C and the other two forms. (Paterson, et al, 1963). Paterson (in press) has also shown that, on simple population genetical grounds, it is improbable that two forms could coexist for long if assortative mating did not prevail.

This evidence is valid and acceptable, indirect though it is. In this contribution direct evidence for assortative mating in

nature between forms A, B and C is reported in support of the earlier conclusions.

Paterson, et al (1963) reported forms A, B and C from Chirundu, N. Rhodesia, a virtually unsprayed village on the bank of the Zambesi River. Here breeding occurs all the year round. In the dry season the only breeding places available are sandy pools at the edge of the river. Due to the prevalence of Glossina morsitans the only domestic animals kept in the village are some sheep and goats, a few dogs and chickens. Wild mammals may also act as hosts for form C, at least.

Since December 1962 sporadic identifications of wild-caught females have been made by means of crossing experiments. These results are presented in table 1.

TABLE 1.

Because the three forms coexist at Chirundu under circumstances which apparently preclude the possibility of any kind of extrinsic isolation occurring between them, it was clear that this village was ideal for an investigation of the extent to which natural hybridization occurs between these forms.

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Methods:

Laboratory crosses between forms A, B and C (Davidson and Jackson, 1962; Paterson, et al, 1963) have shown that in most of the possible crosses the F_1 hybrid males are sterile. The one exception is the cross C♀♀ x A♂♂ which yields an F_1 generation grossly deficient in females but with males which are at least partially fertile.

In planning the search for natural hybridisation it was decided to attempt to detect only females which had mated heterogamically in nature. The methods available were considered inadequate for detecting backcross matings. The method adopted was to capture naturally inseminated females, and to obtain an egg batch from each. The sex ratio of the adults obtained from each egg batch was recorded and the males were checked for fertility. In assessing fertility the male internal genitalia were dissected out and examined under a phase-contrast microscope. If any doubt existed the internal genitalia were stained in aceto-orcein before examination. If the sex ratio of a brood was found to approach normality and it was found to contain one or more fertile males, as judged by the presence of sperm in the vasa deferentia, the female which had produced the brood was considered to have mated homogamically. A female would have been judged to have mated heterogamically if her brood had contained only sterile males, or if the sex ratio had shown a gross deficiency in females even though the male internal genitalia had shown signs

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of fertility. Towards the end of this study another criterion was used. It had been found that inter-form hybrid larvae yielded salivary gland chromosomes which had rather extensive areas of asynapsis. Asynaptic areas of this sort have not been noted within pure stocks. Since they are very obvious they may be useful additional criteria for hybridity.

Results:

During this investigation 174 broods have been examined and not one mating was judged to have been heterogamic on the criteria given above. Table 2 presents these results in more detail.

TABLE 2.

An occasional male with abnormal testes was found within broods containing normal males. These were usually males with unusually small testes most of which actually contained sperm. These abnormal males were not thought to be hybrids since similar individuals occur within pure colonies, though at low frequencies.

Discussion:

It might be as well to examine the method used in this study, and to decide on its validity. I have in the past (e.g. 1963a) criticized it and my original objections are probably valid when hybridization is at a very low frequency so that it may not be possible to distinguish sterility due to hybridization from the cases of male

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sterility due to rare genetic causes, which probably occur within all populations. However, I believe that the method is quite acceptable as a tool for deciding whether hybridization occurs frequently or not, and it is for this limited purpose that I have employed it here. If use is made of the salivary gland chromosomes as suggested in this paper, my original objections fall away completely.

Paterson (1963a) has given reasons why the forms of A. gambiae complex cannot be considered as polymorphs or individual variants, two intrapopulation categories. If extensive hybridization could be shown to occur between coexisting populations of the different forms, they would have to be regarded as subspecies and their co-existence would be an ephemeral state.

However, if they are separate biological species they will be able to coexist indefinitely due to the presence of intrinsic reproductive isolating mechanisms. The ability to coexist in nature without significant hybridization is the definitive evidence for two populations belonging to two separate biological species. For an extended discussion of these points see Mayr (1963).

Although the results presented in table 1 cannot be taken to indicate the relative abundance of the three forms at Chirundu, it is believed that it is fair to conclude from these figures that all three forms are regularly present and that none of them is rare. In reaching these conclusions consideration was taken of the small size of the samples taken, and the sources of the samples (sheep shed

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Summary.

This contribution is a report on a search for natural hybrids in an area where forms A, B and C of the Anopheles gambiae complex coexist. It was decided to limit the study to a search for females which had mated heterogamically in nature. The criterion for fertility of a male was the presence of sperm in the vasa deferentia. The discovery of at least one fertile male in a brood of which the sex-ratio was approximately normal was taken to indicate that the female had mated homogamically. A female which had mated heterogamically would have been recognized by all the males in a brood being sterile, or, if they seemed semifertile, by a sex-ratio which was grossly deficient in females.

Broods from 174 wild caught females from Chirundu were examined but no evidence was found for heterogamic mating. It was therefore concluded that at Chirundu mating by forms A, B and C was at least mainly assortative. This is, therefore, direct evidence in support of the view that forms A, B and C are distinct species.

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TABLE 1.

Details of mosquitoes which have been firmly identified from Chirundu.

Date of Sample	Source	Numbers of each form		
		A	B	C
XII.1962	Huts	1	0	0
II.1963	Huts	1	1	0
VI.1963	Sheep Shed	0	2	3
III.1964	Huts	1	4	0
IV.1964	Sheep Shed	0	0	2
IV.1964	Larvae	1	0	0
V.1964	Huts	0	1	0
TOTALS:		4	8	5

TABLE 2.

Details of broods from females of the Anopheles gambiae complex which had been inseminated in nature at Chirundu, N. Rhodesia. Presence of fertile males in a brood together with a normal sex ratio was evidence for homogamic mating.

Month of Sample	Source	Broods examined	Broods with fertile males	Heterogamic matings
I.1964	Huts	31	31	0
III.1964	Huts	67	67	0
IV.1964	Sheep Shed	33	33	0
V.1964	Huts	35	35	0
VIII.1964	Sheep Pen	8	8	0
TOTAL:		174	174	0