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Genetic observations on the taxon *Anopheles (Cellia) pharoensis* Theobald (Diptera: Culicidae)

S. J. Miles[1]*, C. A. Green[2] and R. H. Hunt[2]

[1]*Department of Entomology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT and* [2]*Botha de Meillon Laboratory, Department of Medical Entomology, South African Institute for Medical Research, PO Box 1038, Johannesburg 2000, South Africa*

Summary

A photomap of the ovarian polytene chromosomes from *Anopheles pharoensis* is presented. The arrangement shown is that found in populations from Zululand, South Africa, and represents the arbitrary standard for the taxon. Two X-chromosome arrangements exist in samples from allopatric natural populations. Crosses between females homozygous for the inverted arrangement, Xa, and males carrying the standard arrangement, $X+^{a}$, give F_1 males that are sterile. Females from this cross, and both males and females from the reciprocal cross, are fertile. The simplest hypothesis is that the two X-chromosome arrangements mark two species within the taxon *An. pharoensis* Theobald. The practical implications are briefly discussed.

Introduction

Analysis of chromosomal rearrangements visible in polytene chromosomes from ovarian nurse cells of wild-caught females assigned to specific taxa in the anopheline subgenus *Cellia* are in progress (see Green 1982) in an exploration of the evolutionary history of this group of mosquitoes. A consequence of this work with implications for epidemiological studies of malaria is an answer to the question: do individuals assigned to the same vector species taxon represent the same field for gene recombination? Already this question has been answered in the negative for individuals assigned to the taxon *An. culicifacies* Giles (Green & Miles 1980). We report here our observations on genetic differences between individuals assigned to the vector species taxon *An. pharoensis* Theobald, and the possible interpretations of such differences.

Materials and methods

Natural populations of *An. pharoensis* were sampled at the following localities: Taveta, Kenya (3° 40´S, 38° 20´E); Adama, Ethiopia (8° 48´N, 39° 38´E); El Hosh Council, Gezira, Sudan (14° 20´N, 33° 30´E); Zinder, Niger (11° 15´N, 9° 3´E); Wallikunda, The Gambia (13° 33´N, 14° 55´W); Kavango, Namibia (18° 3´S, 21° 39´E); Pelindaba, Zululand, South Africa (27° 5´S, 32° 30´E). In addition a colony derived from one that had been maintained in the Institute of Medical Entomology, Ministry of Public Health, Cairo, for more than 15 years, was sampled. The details of the founding of the original colony are not known to us except that material was collected from the Kharga Oasis area (25° 25´N, 30° 40´E), Egypt. All populations except two were sampled as half-gravid females. The Ethiopian population was sampled as egg batches from wild-caught females. Each oviposition was reared separately in the insectary and the parental polytene chromosome arrangements inferred from those in the F_1 females. The population from Sudan was sampled as wild-caught larvae, and the polytene chromosome arrangements examined in females reared from them.

Ovarian polytene chromosomes were prepared, processed and photographed according to the methods detailed by Green & Hunt

*Present address: C/ — Students' Centre, Royal Free Hospital School of Medicine, Rowland Hil Street, London NW3 2PF.

(1980). Since that publication, however, the manufacturers have discontinued production of Scientia film. We now use Kodak pan film 2415 (ESTAR-AH base) developed as recommended for normal contrast.

Reciprocal crosses, and determination of the existence of any F_1 male and/or female sterility, were carried out according to the methods outlined by Miles (1981).

Results

An extensive search of the literature revealed that, although a map of the polytene chromosomes of individuals assigned to the taxon *An. pharoensis* did exist, it had been prepared from larval salivary gland chromosomes (Sidky & Riad 1976). Since larval polytenes are usually of poor quality a photomap of the ovarian polytene chromosomes was constructed (Figure 1), using as the arbitrary standard arrangement that found in females sampled from Zululand, South Africa. The autosomal arm designation is that proposed by Green & Hunt (1980). It should be noted that *An. pharoensis*, and other close relatives so far studied, i.e. *An. argenteolobatus*, *An. cydippis* and *An. squamous* (also belonging to the series *Cellia*) have the same arm association as that found in the *An. gambiae* group of species. Furthermore the extensive heterochromatin at the centromeric end of the X-chromosome is typical of taxa belonging to this series.

The frequencies of the standard arrangement of the inversions shown in Figure 1 detected in our samples are given in Table 1. This table does not include data from the Kharga Oasis colony. Ten females from this colony were in fact scored for inversion genotype, and each had the chromosomal arrangement Xa, 2, 3, 4 and 5. An interesting aspect of the data is the absence of heterozygotes for the two X-chromosome arrangements in those populations for which the sampling might be considered adequate, i.e. The Gambia and Zululand, South Africa.

Crosses between individuals carrying different X-chromosome arrangements were carried out to determine whether other genetic differences existed between the two forms. The results are summarized in Table 2. Crosses in the direction Xa/Xa female $\times$ X+^{a} male gave F_1

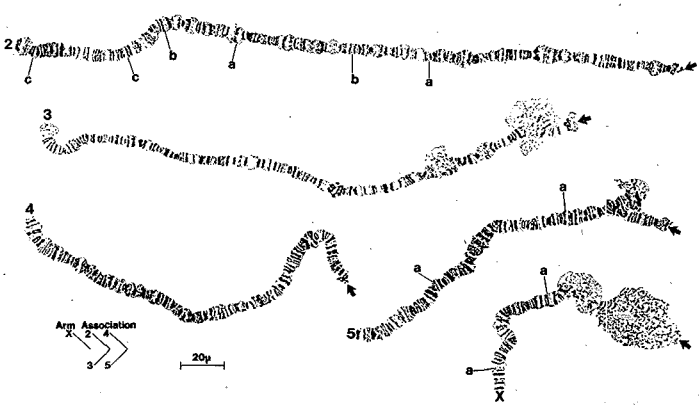

Figure 1. A photomap of the ovarian polytene chromosomes of *Anopheles pharoensis* Theobald compiled from females caught at Pelindaba, Zululand, South Africa. The arm association is the same as that found in the *Anopheles gambiae* group of species. The breakpoints of inversions are indicated by lower case letters.

Table 1. Frequencies of the standard arrangements for inversions on the X-chromosome, and autosome arms 2 and 5, in natural populations of the taxon *Anopheles pharoensis*

Locality	n†	Arrangement				
		$2+^a$	$2+^b$	$2+^c$	$5+^a$	$X+^a$
Zululand/South Africa, Pelindaba	177	1.00	1.00	1.00	1.00	1.00
Kenya, Taveta	8	1.00	1.00	1.00	1.00	1.00
Ethiopia, Adama	4	1.00	1.00	1.00	0.00	1.00
Niger, Zinder	2*	1.00	1.00	1.00	n.s.	0.00
The Gambia, Wallikunda	55	0.52	0.54	0.63	0.98	0.00
Namibia, Kavango	3	1.00	1.00	1.00	0.00	0.00
Sudan, El Hosh Council	13	1.00	1.00	1.00	0.00	0.00

*One individual was heterozygous for a fourth inversion on arm 2 (2d) for which the breakpoints have not yet been confirmed.
†Number of genomes sampled.
n.s. Not scored.

Table 2. Summary of the results of crosses between individuals carrying different X-chromosome arrangements and assigned to the taxon *Anopheles pharoensis*

♀ \ ♂	$X+^a$ Zululand	Xa Egypt	Xa Namibia
$X+^a/X+^a$ Zululand	—	males fertile, females fertile $n=1$	n.d.
Xa/Xa Egypt	males sterile, females fertile $n=2$	—	n.d.
Xa/Xa Namibia	males sterile, females fertile $n=4$	n.d.	—

n is the number of inseminated females from whom an F_1 generation was obtained.
n.d. = cross not done.

males whose testes contained mostly primary spermatocytes and a few apparently mature spermatozoa. The *vasa deferentia* were reduced in length relative to those of Xa and $X+^a$ males. The accessory glands appeared normal. No egg hatch was obtained from backcrosses of these hybrid males to either Xa/Xa or $X+^a/X+^a$ females. Crosses in the direction $X+^a/X+^a$ female × Xa male gave males with apparently normal gonads. Eggs from backcrosses of these males to either Xa/Xa or $X+^a/X+^a$ females hatched and gave viable offspring. No sterility was detected either visually or by backcrosses in F_1 females from either of the reciprocal crosses. Sex ratio and larval viability were not scored in any of these crossbreeding experiments.

We failed to establish colonies from either of the two Southern African populations sampled despite intensive efforts to do so. All experimental material therefore originated from the families of wild-caught females that had been chromosomally identified. Consequently the numbers available for the crosses were limited.

Asynapsis of the X-chromosomes was almost complete in the laboratory-produced $X+^a/Xa$ hybrids, and extensive in the autosomes. Very little, if any, asynapsis was seen in wild-caught material, or in the laboratory produced homozygotes for either X-chromosome arrangement.

Discussion

The existence and apparent fixation of two

different X-chromosome arrangements in geographically-separated populations of the taxon *An. pharoensis* in nature represents either geographic variation within a single species, or at least two genetic species within the taxon. The ideal situation for testing these hypotheses is the coexistence in nature of individuals carrying different X-chromosomes with a significant deficiency, or in the extreme case absence, of $X+^{a}/Xa$ heterozygotes. Such an approach is well-founded in the extensive literature on *Drosophila* (e.g. Carson 1954). Clearly, an interesting area to sample would be that between Kenya (Xa) and Zululand, South Africa ($X+^{a}$), i.e. Tanzania and Mozambique.

In the absence of this direct evidence we attempted to decide between the hypothesis by means of crossbreeding experiments. It must be emphasized that data from such experiments can be misleading. For example, in the case of the *Drosophila bocainensis* complex Carson (1954) found that interspecific hybrids were fully fertile. Infertility can also be an intraspecific phenomenon, as seen in several plants (Oka 1978) and at least one species of *Drosophila* (Prakash 1972). However, the data from anopheline mosquitoes show that infertility of F_1 hybrids, or some other expression of genetic differences, is frequently found in interspecific crosses. Such information is still inferior to that obtained from natural populations in studies of gene flow. The conclusion we draw from our data is that there is a strong possibility of two genetic species at least within the taxon *An. pharoensis*.

The existence of more than one species under the name *pharoensis* may be of some practical significance. Individuals assigned to this taxon have been implicated as vectors of malaria in Egypt (Barber & Rice 1937). Sporozoites (presumed to be of human origin) have been reported in *An. pharoensis* from other parts of Africa (Gillies & De Meillon 1968, Molineux & Gramiccia 1980). Members of this taxon have also been shown experimentally to be able to transmit *Wuchereria bancrofti* (Mosha & Magayuka 1977). Some observations suggest to us that it is the Xa form that is a potential vector. Zahar (1974) reports *An. pharoensis* in the southern part of the Nile Delta to bite man (and animals) both indoors and outdoors. One of us (C.A.G) noted that, in The Gambia, *An. pharoensis* (Xa form) was very common resting during the day in bed-nets. In contrast, in Zululand *An. pharoensis* ($X+^{a}$ form) has never been seen in association with man other than biting cattle in kraals well away from villages.

We are not in a position to continue our sampling of *An. pharoensis* populations in Africa. However, as we expect any further studies to support our conclusion that the two X-chromosomes in fact mark two different species within the taxon, chromosomally-identified females, and link-bred specimens from those females, have been deposited as voucher specimens (marked 'Chrom. det. C.A. Green') in the insect collection of the South African Institute for Medical Research.

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