MONANDRY (MONOGAMY) IN NATURAL POPULATIONS OF ANOPHELINE MOSQUITOES

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ABSTRACT. Polymorphic Y chromosomes within two species of anopheline mosquitoes provide markers for testing if females are inseminated by one or more males in nature. Wild-caught females of *Anopheles dirus* (sp. A) and *An. maculatus* s.s. produced 291 and 55 families, respectively, which showed a single type of Y chromosome. One family of the former species showed two types of Y chromosomes. These field data support the idea, established from laboratory studies, that female mosquitoes are largely monandrous (monogamous). Such information is important in interpretation of population biological data and, practically, in attempts to control insect pests by use of genetically designed males.

INTRODUCTION

Studies in population biology require an understanding of the breeding systems of the particular organisms being investigated. An important element in the breeding systems of most arthropods is the presence of organs for the storage of sperm within the female. The facility is thereby provided for the fertilization of several broods of progeny, over a period of time, from a single insemination. The question arises as to whether one or more males contribute to a single store of sperm in individual females. Anopheline mosquitoes have a single receptacle (spermatheca) for storage of sperm. Little is known about the mating behavior of anophelines in nature; however, males of some species form swarms into which individual females are attracted and where copulation occurs (Gillies and de Meillon 1968; Reid 1968, p. 408; Rao 1984, pp 32–34). Therefore, the physical opportunity for multiple insemination probably exists in anophelines. Both swarming and multiple copulations do occur in some species of culicine mosquitoes under natural conditions (Miles 1976). He showed that multiple copulations occur after finding females *in copulo* with heterospecific males, though fertilization was shown subsequently to be due to one or more conspecific males.

The possibility and mechanism of monandry in culicine and anopheline mosquitoes has been reviewed (Kitzmiller 1976). An example of laboratory evidence for monandry (Bryan 1968) in *Anopheles* involved the force-mating of sterile, interspecific hybrid males of the *An. gambiae* group with fertile females which subsequently were induced to copulate with conspecific males. Sterility of all eggs laid by these females indicated effective fertilization from the first mating, i.e., monandry.

Anopheles dirus Peyton and Harrison and *An. maculatus* s.l. include important vectors of human plasmodia in southeast Asia. The two taxa are related distantly within the subgenus *Cellia* (Reid 1968). Cytological data recently provided evidence for four biological species within each taxon (Baimai et al. 1984b). Species A of the *An. dirus* group (Baimai et al. 1984a) and *An. maculatus* s.s. (Green et al. 1985) are polymorphic for heterochromatic segments of Y chromosomes. In anopheline mosquitoes (usually 2 n = 6), males are the heterogametic sex with X/Y; females being X/X. The polymorphic Y chromosomes thus provide a marker for testing for possible monandry in wild-caught females, judged from their larval, male progeny.

In the following report, the laboratory findings that females are monandrous are extended and supported by information from natural populations of these two species of anophelines.

MATERIALS AND METHODS

Collection localities (except the Malaysian site) are indicated in Fig. 1, and site names are listed in Table 1. Table 1 also shows the sample sizes from *An. dirus* (sp. A) and those from *An. maculatus* s.s.

The nominate taxon, *Anopheles maculatus* sensu lato, currently includes four chromosomally discovered species; A, B (forms E and F), C and G (Green et al. 1985). Rattanarithikul and Green (1987) have made an extensive study of the morphology of all these species and formally named them using two synonyms and describing two species as new to science. Species B and its forms E and F, reported in Green et al. (1985), have been referred to *An. maculatus* Theobald 1903, sensu stricto. This is the species referred to in this report.

Individual wild-caught females were captured biting man, water buffalo or cattle and allowed to blood-feed. They were isolated for egg laying and each egg batch reared separately in the laboratory. Mitotic chromosome preparations were made from brain cells of fourth instar larvae using a slight modification of the method of Baimai (1975). The sex of larvae is determined from the mitotic sex chromosomes. At

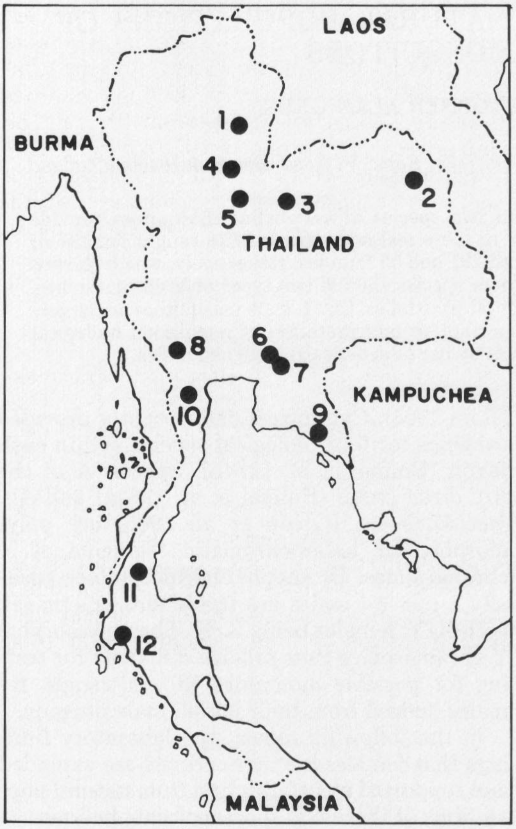

Fig. 1. Localities in Thailand from which females of species A of the *Anopheles dirus* complex (1 through 10) and *An. maculatus* s.s. (6, 11 and 12) were collected. The numbers appear, in brackets, with locality names in Table 1.

least three male larvae from each family were scored for types of Y chromosome.

RESULTS AND DISCUSSION

Male progeny from 291 females of *Anopheles dirus* (sp. A), and 55 females of *An. maculatus* s.s. (see Table 1) showed only one or the other of two polymorphic Y chromosomes known from each species (shown in Fig. 2). Only one *An. dirus* family showed two types of Y chromosome present in this species. Therefore, the present data support the view that female mosquitoes are generally monandrous.

Jones (1973) argued that the use of the terms monogamy and, by inference, polygamy in mosquito literature, is semantically ambiguous in that the intended meaning appears to be that females are inseminated either by a single male or by more than one before their first oviposition. Jones did not suggest alternative terms; and yet, such terms seem useful in order to avoid the repetition of, for example, clauses such as "inseminated by a single male". We suggest monandry/polyandry as clear substitutes. Monandry is defined as the "custom of having only one husband at a time" (Fowler and Fowler 1964) and so refers specifically to the female

Table 1. Collection sites in Thailand and sample sizes of females and their families scored for types of Y chromosome in species A, *Anopheles dirus*, and *An. maculatus* s.s. Numbers in brackets are those used to indicate localities in Fig. 1.

Locality	Females/ Families	Y chromosome types		
Anopheles dirus species A		Y1	Y2	Y1 + Y2
(1) Phrae	8	2	6	0
(2) Sakon Nakhon	52	17	35	0
(3) Nam Nao	28	8	20	0
(4) Uttaradit	10	1	9	0
(5) Phitsanulok	133	35	98	1
(6) Nakhon Nayok[1]	4	2	2	0
(7) Prachinburi[1]	17	8	9	0
(8) Kanchanaburi[1]	7	3	4	0
(9) Chantaburi[1]	16	7	9	0
(10) Phetchaburi[1]	17	8	9	0
Anopheles maculatus s.s.[2]		Y3	Y4	Y3 + Y4
(6) Nakhon Nayok	18	17	1	0
(11) Phato	21	13	8	0
(12) Aoluk	8	2	6	0
Genting Highlands, near Kuala Lumpur	8	5	3	0

[1] Baimai et al. 1984a.
[2] Green et al. 1985.

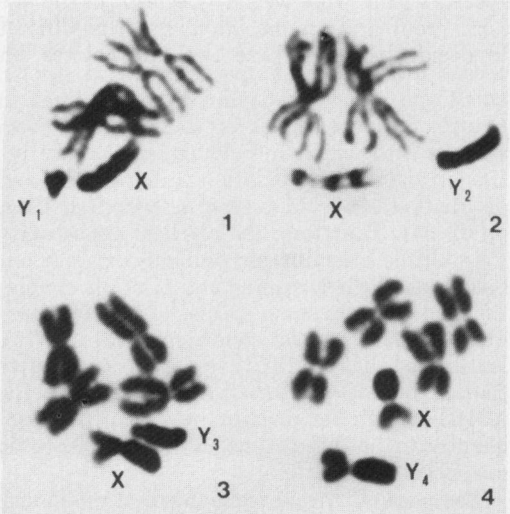

Fig. 2. Metaphase figures of mitotic chromosomes from the larval neuroblasts of males showing the polymorphic Y chromosomes in species A of the *Anopheles dirus* complex, 1 and 2, and *An. maculatus* s.s., 3 and 4. Designations of the Y chromosomes in these two taxa bear no relationship to each other; a Y1 and Y2, specific to *maculatus* s.l. occur in other members of that group (Green et al. 1985).

sex; furthermore, the possibility is not precluded that a female mosquito refreshes her depleted store of sperm from a male different from that which first inseminated her. Two of Jones' objections to "monogamy" are thus eliminated. An inseminated female may copulate with a second male even though insemination does not occur a second time. For instance, Miles (1976) captured wild females of the *Culex pipiens* group copulating with males of other species. However, the progeny of these females showed that they had been inseminated previously by conspecific males. Such a second male would fall loosely under the terms husband and mate and so indicate polyandry rather than monandry as these terms are applied to humans since the terms, strictly speaking, refer to pair-bonding (copulation?) rather than fertilization. Our use here refers to fertilization by one or more males.

The results of this study are interpreted as showing monandry in female mosquitoes under natural conditions but this interpretation depends on the fact that the Y chromosome variation represents polymorphism within species. There is an alternative possibility that the Y chromosomes might mark as yet unrecognized species within what we call species A of the *An. dirus* group and *An. maculatus* s.s. It is difficult to decide between these two alternatives. In some *Drosophila* there is evidence that alternative Y chromosomes within a species do not coexist in single, natural populations (Miller and Roy 1964). However, published cytogenetic evidence and cross breeding data (Baimai et al. 1981, 1984a; Green et al. 1985) on both the anopheline species are consistent with the idea that each is a single species with polymorphic Y chromosomes as outlined in the following paragraph.

Species A, *An. dirus* shows quantitative variation in heterochromatin in the mitotic X chromosome and *An. maculatus* s.s. is polymorphic for various paracentric inversions seen in polytene chromosomes. Population genetic data give no evidence of 'linkage disequilibria' or heterozygote deficiencies (Wahlund Effect) for these polymorphisms that might be expected if two or more species are included in our samples. In cross breeding experiments between families having different Y chromosomes, no infertility was noticed in progeny nor did asynapsis of polytene chromosomes occur in the case of progeny from the *An. maculatus* crosses. These are common phenomena associated with interspecific hybrids in anophelines. This evidence thus supports the view that the Y chromosome variation is intraspecific in nature rather than interspecific. However, this evidence does not unequivocally exclude the possibility of cryptic species within our samples.

Given that the different Y chromosomes are polymorphic within the two species, the data strongly support the laboratory findings that female anophelines are very likely monandrous in nature. The single family from Phitsanulok which showed two kinds of Y chromosome may represent a rare case of polyandry. However, it might have resulted from contamination within the laboratory; a not-unheard of occurrence in the best run insectaries.

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