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Natural Mortality in Two Filarial Vectors *

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Little has been written about the survival of filarial mosquitos in nature, although methods are available for determining natural mortality in the field. Every filarial infection in a mosquito provides some information about the number of days it has survived after infection. This can be used to determine the probability of survival of an infected mosquito population in the field. The parous rate of the vector provides another method for estimating natural mortality. Several estimates of natural mortality in two mosquitos, Culex fatigans *and* Anopheles peditaeniatus, *in South India have shown a daily mortality of from 14% to 24% during a season favourable for survival. Information on natural mortality can be obtained during routine dissections of mosquitos for filarial larvae, but a more positive approach to the problem of the identification of filarial infections in mosquitos is needed.*

Filarial mosquitos are dissected as a routine in many parts of the world, yet little information is available about the natural mortality of these vectors. The results of dissections are recorded as the percentage of female mosquitos containing filarial larvae at all stages of development—the infection rate—and the percentage of females containing mature infective filarial larvae—the infectivity rate. The proportion of mosquitos carrying infective parasites can be used, as Macdonald (1957) has shown, to estimate mosquito survival. For example, in *Culex fatigans*, if females carrying infective larvae of *Wuchereria bancrofti* can be assumed to have survived at least ten days, which is about the minimum period from ingestion of the microfilariae to the development of the infective stage, then the probability of survival over one day is given by $\sqrt[10]{}$ of the ratio of the number of mosquitos with infective larvae to the total number of infected mosquitos. But few mosquitos survive long enough for the complete development of the parasite, or infective larvae may be lost when the mosquito feeds, and the number of mosquitos collected with infective filarial larvae is usually low, often too low to provide reliable estimates of mortality by this method alone. Other methods of determining mortality in filarial vectors exist. It is possible to divide the period of filarial development within the mosquito into more stages than just the infective and pre-infective stages. It takes some time for microfilariae to thicken and shorten to the "sausage stage" within the mosquito. A further interval must elapse before the sausage stage begins to elongate rapidly and moult to the second-stage larva. If these early stages of filarial development can be linked with the number of days a mosquito must survive after infection before each stage of parasite development is reached, then more than one estimate of mosquito mortality can be obtained from the filarial dissections alone.

It is also possible to estimate mortality from the proportion of mosquitos that have survived long enough to lay eggs. There are objections to the use of the parous rate as a method for determining mortality in filarial mosquitos. Wharton (1959) showed for forest-dwelling *Mansonia* in Malaya that ovary development appeared to be unrelated to filarial parasite development and attributed this discrepancy to the greater length of time between blood meal and oviposition in the wild compared with that observed in the laboratory. A second objection to the parous rate as a measure of the survival of mosquitos infected with filariae is that the parous rate is more easily determined from collections of both uninfected and infected mosquitos and uninfected mosquitos may survive for longer than those infected by parasites.

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The present paper compares estimates of natural mortality based on both filarial and ovary development in two species of mosquito, *Culex pipiens fatigans* Wied. and *Anopheles* " *hyrcanus* " (mainly *A. peditaeniatus* Leicester) from Vellore in Madras State, South India. The purpose of this paper is to show that the developmental stages of filariae in mosquitos, as well as the parous rate, can be used to estimate mosquito mortality, and that it may well be possible and profitable to derive these estimates from routine dissections.

METHODS

The mosquitos were collected daily for six days in each week, between October and December 1961, by the field assistants of the Virus Research Centre Field Station based at Vellore. All dissections were carried out by the author himself.

Culex fatigans

Resting females were collected from houses in Vellore and were dissected daily in saline. The ovaries were first removed, the stage of ovary development was recorded and, if the ovaries had not developed too far, they were then squashed under a small, 6-mm square cover-slip and examined for the presence of follicular relics. In *C. fatigans* usually some follicles do not develop, even after the first blood meal, and the degenerated follicles are conspicuous, as noticed by Colless (1958), and have the appearance of pocket watches. Only occasionally was it difficult to determine the parity of females with small follicular relics. Some nulliparous females were newly emerged and had very small ovaries and a meconium in the gut. The head, thorax and abdomen of the female were then dissected and the saline searched for developing filariae. Any mosquitos that were not dissected immediately were preserved in 80% alcohol and dissected for filariae later in London after staining with Mayer's acid haemalum (Laurence & Pester, 1961). The length and breadth of any developing filarial larvae discovered were measured with a micrometer eye-piece. The infective stages of *Wuchereria bancrofti* were recovered from 18 females of *C. fatigans* but one female contained infective larvae resembling those of *Setaria equina* (Nelson, 1959, 1960). This observation requires confirmation. A laboratory culture of *C. fatigans*, derived from egg masses laid by females captured in Vellore, was fed at midnight on a human carrier of *W. bancrofti* from Vellore. The infected mosquitos were then kept in a cage, one end of which was stood in water, at room temperature in Vellore; females were killed daily, dissected and the developing filariae measured.

Anopheles peditaeniatus

Recently fed, resting females were collected from bullock-baited Magoon-type traps permanently situated at three villages around Vellore. A selection of 64 pinned "*hyrcanus*" from the traps were sent to Dr J. A. Reid for identification; 56 specimens were identified as *A. peditaeniatus* Leic. and eight specimens as *A. nigerrimus* Giles. Only 30 females out of 472 specimens preserved in alcohol had the humeral vein covered by scales and hence referable to *A. nigerrimus*: the rest appeared to be *A. peditaeniatus*. Filarial infection was found only in females identified as *A. peditaeniatus*. As few *A. nigerrimus* were found in the traps, the name *A. peditaeniatus* is used in this paper.

The same dissection procedure was followed, the ovaries being dissected out first and examined for follicular relics, the rest of the mosquito then being examined for developing filariae. The follicular relics were distinct but not so conspicuous as in *C. fatigans*, but the coiling of the ovarian tracheoles was of great value in confirming the identity of females classified as nulliparous. Any mosquitos not dissected immediately were preserved in alcohol and dissected after staining in London. The length and breadth of any developing filarial larvae were measured with a micrometer eye-piece. The infective stages of a species of *Setaria* were recovered from six mosquitos. These are similar to the infective stages of *S. cervi* obtained by the Filariasis Training Centre, Malaria Institute of India, and to the descriptions of *S. digitata* recorded by Ono (1960) and *S. labiatopapillosa* recorded by Nelson (1962)

RESULTS

Culex fatigans—filarial infection

The experimental infection in the laboratory provided a method for timing the growth of *W. bancrofti* in the vector. During the first and second nights after the night of infection, development had not progressed much beyond the microfilarial stage, or a very thin sausage stage less than 16μ in breadth. By the third night and following morning most larvae had thickened to 20-25μ in breadth and 140-170μ (mean 157μ) long, and were typical sausage-stage larvae. Twenty-four hours later the filarial larvae were still short, 176-283μ (mean 213μ) long,

TABLE 1

FILARIAL INFECTION IN *CULEX FATIGANS* AT VELLORE, SOUTH INDIA

Method of dissection	No. dissected	No. infected	Number in filarial stage shown:			
			Microfilariae: <16μ wide	Sausage stage: <250μ long	Sausage stage: >250μ-stage II	Infective stage
Saline	376	65	24	13	22	6
After staining	275	36 (+ 1 not measured)	13	9	10	4
Total	651	102	37	22	32	10
	Infection rate = 15.7 %.			Infectivity rate = 1.5 %.		

and 24-31μ broad, and the development of the gut was not yet complete. But on the fifth and sixth nights the larvae were markedly elongating, 253-329μ (mean 293μ) long, with the gut complete anteriorly; this elongation continued until the tenth night, when the first infective larvae were found.

The relatively slow growth period over the first four days after infection can be divided into two stages—the first two days following infection when microfilariae or very thin sausage stages (shortened microfilariae) were still present, and the next two days when typical, thickened, but not markedly elongated sausage stages were found. From the laboratory results, at room temperature in Vellore, it has been assumed (1) that any wild-caught mosquito containing filarial larvae that had developed beyond the microfilarial or very thin sausage stage had survived for at least two days, (2) that any wild-caught female with a sausage stage longer than 250μ or with filarial larvae at a later stage of development had survived for at least four days, and (3) that any female containing infective-stage larvae had survived for ten or more days.

The results of the filarial dissections of the wild-caught *C. fatigans* are given in Table 1. It will be seen that 64 out of 101 infected mosquitos survived for at least two days, 42 out of 101 survived for at least four days and that 10 females with infective larvae survived for at least ten days. The probability of survival over one day (p) is then given by $p^2 = \frac{64}{101} = 0.634$, $p^4 = \frac{42}{101} = 0.416$, and $p^{10} = \frac{10}{101} = 0.099$, which give $p = 0.796$, 0.803 and 0.794 with estimates of daily mortality, 100 $(1 - p)$, of 20%, 20% and 21% respectively.

Culex fatigans—parous rate and ovary development

Out of 200 females of *C. fatigans*, 108 showed follicular relics on dissection and 92 were nulliparous. The parous rate was 0.540. There was little change in the parous rate from October, at 0.516, to November-December, at 0.583. The minimum period between a nocturnal blood meal and a nocturnal oviposition in a laboratory cage at room temperature was found to be 72 hours. Egg rafts were laid during the third and fourth nights following the blood meal. Several females failed to develop eggs when they were confined in a cage at a high humidity immediately after a blood meal in the laboratory, but there was no evidence of any significant gonotrophic dissociation in wild-caught females. Out of 316 females, 77% had developing ovaries at stage II or beyond, and of the females with undeveloped ovaries 36 were nulliparous and 37 parous, and most of these were without blood meals.

Parous females collected during the day in houses have been assumed to be three to four or more days old. This assumption is supported by a comparison of the growth stages of filarial larvae found in nulliparous and parous females. The latest stage of growth found in a female classified as nulliparous was a single sausage stage 247μ long. Three parous females, with undeveloped ovaries, one of which had just fed again and had taken up microfilariae, contained sausage stages 234μ, 254μ and 255μ long, and these must have been taken up at the previous blood meal. This stage of filarial development was reached during the fourth night following the experimental infection in the laboratory. The probability of a mosquito surviving through one

TABLE 2

FILARIAL INFECTION IN *ANOPHELES PEDITAENIATUS* AT VELLORE, SOUTH INDIA

Method of dissection	No. dissected	No. infected	Number in filarial stage shown:			
			Microfilaria or sausage stage: <110μ long	Sausage stage: >100μ-<200μ long	Sausage stage: >200μ- stage II	Infective stage
Saline	201	16	4	1	7	4
After staining	548	38	11	12	13	2
Total	749	54	15	13	20	6
	Infection rate = 7.2 %.			Infectivity rate = 0.8 %.		

day (p), when $p^4 = 0.540$, or $p^3 = 0.540$, is 0.857, or 0.814, with a daily mortality of 14%, or 19%.

Of the 92 females classified as nulliparous, 76 had just emerged or had fed only the previous night, with ovaries at stage I or II. The rest were semi-gravid with ovaries at stage III, contained a partially digested blood meal, and had fed earlier. In addition 36 other semi-gravid and 80 gravid mosquitos, not classified for parity, were collected, as well as the 108 parous females, all of which had fed at some time before the previous night. Thus 240 out of 316 females were known from their ovary development to have survived for more than one night and day, having fed earlier. From this the ratio 240:316 gives $p^1 = 0.760$ with a daily mortality of 24%.

Anopheles peditaeniatus—filarial infection

The filarial infection of mosquitos trapped in the bullock traps presented a different problem. None of the bait animals was known to be infected; consequently all the infected mosquitos collected in the traps had taken up the infection at a previous blood meal and were parous (with the exception of one nulliparous female which had ovaries with follicles at different stages of development and contained microfilariae possibly ingested with a previous incomplete blood meal). The mosquitos were biting between gonotrophic cycles and, as there is little delay between oviposition and the next blood meal (see below), any distinct stages of filarial development in the trapped mosquitos are separated in time by the length of the gonotrophic cycle. It was possible to recognize three stages of growth during the early development of the filarial larvae: (1) very short sausage stage, less than 110μ long, with the gut scarcely differentiated; (2) short sausage stage, 110-200μ long, with differentiating but incomplete gut (corresponding to four-day-old larvae of *W. bancrofti* in *C. fatigans*); and (3) long sausage stage, more than 200μ long, with gut complete anteriorly. It is believed that these stages represented the development attained at the first, second and third blood meals taken by the vector after infection. The third stage of development merged with later stages of growth as the filarial larvae had then entered the phase of rapid elongation up to the distinctive infective stage. The period between blood meals in *A. peditaeniatus* is believed to be two days (see below), so that any infected mosquito containing filariae at the second or third of the above stages of development or at subsequent stages has been assumed to have survived for at least two days (from the first stage collected in the traps); similarly, mosquitos with infections at the third of the above stages or later have survived for at least four days from the first stage. Lastly, mosquitos carrying infective larvae are assumed to have survived for at least ten days from infection, at least eight days from the first stage of the infection collected in the traps. It is possible that this last group is underestimated as some females might lose their infection when they fed in the traps.

The results of the dissections of *A. peditaeniatus* for filarial larvae are given in Table 2. It will be seen that 39 out of 54 infected parous mosquitos had survived for at least two days, 26 out of 54 had survived for at least four days, and 6 out of 54 for eight or more days. These proportions give for parous *A. peditaeniatus* $p^2 = 0.722$, $p^4 = 0.482$ and $p^8 = 0.111$, which give $p = 0.850$, 0.833 and 0.760, with daily mortalities of 15%, 17% and 24% respectively.

Anopheles peditaeniatus—parous rate

The bullock trap collections provided females which had recently fed and, except for an occasional gravid female which had entered the trap one day before, all could be classified for parity. Out of 181 females dissected, 110 showed follicular relics and 71 were nulliparous, giving a parous rate of 0.608. In the laboratory, blood-fed females laid batches of eggs, under optimum conditions, on the second night after feeding. In the field, parous females were collected as they fed on bullocks between 21.00 and 06.00 hours at night, and these females had the ovariole wall still distended and sac-like after recent ovipositions. As the rice-field breeding-places are immediately adjacent to the villages where cattle are kept at night out-of-doors, there is little delay between oviposition and the next blood meal. There was little evidence of a significant pregravid stage in this species. Of the females classified as nulliparous, 9 out of 71 had not developed eggs to late stage II-III, compared with 4 out of 110 parous mosquitos.

Parous females collected in the traps are assumed to be two or more days old. The probability of survival over one day (p) is given by the square root of the parous rate, as $p^2 = 0.608$. From this, $p = 0.780$ with a daily mortality of 22%. The results from the three villages, which were well separated and lay in different directions from Vellore, are here pooled, but the daily mortality in these villages was very similar—20%, 23% and 23% respectively—and there was little change from October, at 21%, to November-December, at 22%.

DISCUSSION

Estimates of mosquito survival at Vellore, South India, have given daily mortalities of between 14% and 24% in *Culex fatigans* and 15% and 24% in *Anopheles peditaeniatus*. *C. fatigans* is a mosquito that rests indoors whereas *A. peditaeniatus* is seldom found in houses. Despite this difference in adult resting behaviour the natural mortality in the two species is outstandingly similar. Both act as the vectors of filarial worms. The differences between the estimates of the probability of survival through one day are from small samples and of little significance, the most notable being the differences between estimates of mortality obtained by dissection of the ovaries of *C. fatigans*. This may well be due to the heterogeneous physiological condition of the sample of this species taken by indoor collections. Females in certain stages of ovary development may be easier to collect than others. There were no demonstrable differences in natural mortality between infected and mainly uninfected mosquitos. The parous rate of a population of filarial mosquitos which have been collected in a homogeneous physiological condition (for instance, by trapping as they come to bite) would appear to be a valid method for estimating the natural mortality of the vector. The main difficulty is the need to establish the length of life of the gonotrophic cycle in the field. Laboratory observations may not apply to some mosquitos, such as *Mansonia* in Malaya, where host and breeding-place may be far apart but, as in the two species discussed here, appear to apply where host and breeding-place are close together (see also Samarawickrema, 1962). In addition, it now seems to be a practicable proposition to estimate natural mortality from the stages of filarial development within the mosquito. Much of the present labour of filarial dissection could provide this additional information on the mortality of the vector.

Also it should be noticed that daily mortality remains more or less constant with age; estimates of survival from mosquitos of different ages fall within the same range. This supports the theoretical pattern of mortality suggested by Macdonald (1957); female mosquitos die too rapidly from other causes for ageing changes postulated from laboratory observations to be important. As Gillies (1961) has suggested recently, under tropical conditions predation pressure may be the most important factor in the natural control of mosquito populations. The spectacular increases in populations of *C. fatigans* following recent insecticide campaigns in many parts of the world may be due to the removal, by the insecticides, of the natural mechanisms which regulate the population size of this species.

The daily mortality of around 20% in both filarial vectors at Vellore was recorded during months of the year which were favourable to mosquito survival. The relative humidity during the night was 70%-100% and decreased to 40%-70% during the day. Temperatures, in an out-door screen, ranged from minima of 16°C-24°C at night to maxima of 27°-36°C during the day. The climate during other months at Vellore is more severe. The possibility exists that filarial transmission is interrupted during the dry, hot months of the year. Whether intermittent exposure to infection affects the clinical manifestations of the disease in man is a matter for speculation at present, but the presence

of 102 infected mosquitos in a sample of 651 specimens of *C. fatigans* at Vellore indicates a surprisingly high number of active microfilarial carriers in a human population which was not obviously filarious.

Lastly, it is pertinent to inquire if the labour of filarial dissection by present methods is worth while. The infection rate tells us how many mosquitos are infected with mainly unidentifiable filarial larvae. The infectivity rate tells us how many of the mosquitos, often 1% or less of the total dissected, have survived for long enough for the filarial larvae to develop to the infective stage and also, as the infective stages tend to be distinctive, what species of filaria is present in this proportion of the mosquito population. As the majority of the mosquitos discovered on dissection to be infected contain young unidentifiable filarial larvae, it might be more profitable to protect the vector from the hazards of natural mortality in laboratory cages for about eight days after capture, and thus permit the majority of the parasites present to develop to the identifiable infective stage.

This delayed dissection method was tested at Vellore, where a batch of 86 wild-caught *C. fatigans* were kept in laboratory cages for seven to nine days and then dissected. This dissection gave an infection rate of 15.1%, compared with a rate of 15.7% from other methods, and the additional information that filarial infections in this batch of mosquitos were of *Wuchereria bancrofti* alone and not of some other species of filaria. This method, combined with a technique for establishing the natural mortality of the population by the filarial or parous rate, the latter being the easier to apply, would give more information, for possibly less labour, about the infection and survival of filarial vectors than the present routine survey methods provide.

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RÉSUMÉ

La mortalité naturelle des moustiques vecteurs de filaires a été jusqu'à présent l'objet de publications peu nombreuses. En pratique cependant, la dissection systématique permet déjà de connaître d'une manière approchée le pourcentage des individus femelles porteurs de larves infectieuses du dernier stade. A propos de chaque moustique, il est alors possible de déduire le temps de survie naturelle à partir du moment où il a été infecté en déterminant soit le stade infectieux ou pré-infectieux des larves, soit leurs stades intermédiaires. C'est là une notion qui peut servir sur le terrain à connaître la probabilité de survie de l'ensemble d'une population de moustiques infectés. Par ailleurs, et bien que la méthode soulève des objections, il est encore possible de déterminer la mortalité par le taux des femelles pares, c'est-à-dire par le pourcentage des femelles qui ont survécu jusqu'au moment de la ponte.

Pour sa part, l'auteur a procédé à l'évaluation de la mortalité naturelle chez *Culex fatigans* et *Anopheles peditaeniatus* en recherchant à la fois le stade de développement des larves et la présence ou l'absence d'œufs. Effectuée en Inde méridionale, l'enquête a montré que la mortalité quotidienne varie entre 14% et 24% à la saison la plus favorable. Il ressort de cette étude que la double méthode indiquée permet d'être informé de la mortalité naturelle des moustiques à l'occasion de dissections de routine effectuées pour rechercher les larves de filaires.

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