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Crypto-periodicity in *Mansonella ozzardi*

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Summary

Studying nycthemeral microfilarial density in eight carriers of *Mansonella ozzardi*, Nathan *et al.* concluded that there is an absence of periodicity in *M. ozzardi* in Trinidad.

Re-examination of the results obtained shows evidence of crypto-periodicity. Two of the eight patients showed highly significant periodicity but the biorhythms appear to be almost out of phase, the respective peaks being at 18·00 hours and 02·00 hours. The six other patients showed no obvious periodicity; the calculated acrophases (peak hours), instead of being randomly spaced, regrouped with the preceding ones: late afternoon in two subjects and in the second half of the night for the other four.

It is concluded that the apparent non-periodicity is due to the co-existence of two periodic forms, but these are markedly out of phase.

Introduction and Methods

Different families of Diptera transmit the filaria *Mansonella ozzardi* Manson, 1897: in the Amazon region, *Simulium amazonicum* was suspected by CERQUEIRA (1959) and by SHELLEY & SHELLEY (1976), observations confirmed by SHELLEY *et al.* (1980) and by TIDWELL *et al.* (1980). In the West Indies, BUCKLEY (1933, 1934) incriminated *Culicoides furens* in St Vincent, and NATHAN (1981) incriminated *C. phlebotomus* in Trinidad.

Similarly there appear to be discrepancies in the microfilarial periodicity of this parasite: it was generally considered as a non-periodic form, until RACHOU & LACERDA (1954) and MORAES (1959) reported a slight early morning increase in the number of microfilariae in capillary blood of carriers in Brazil. With the same data, SASA & TANAKA (1974) found a peak hour at 11.06 hours. Finally, studying eight carriers from Trinidad, NATHAN *et al.* (1978) concluded periodicity was absent in this filarial species.

Effectively, if one pools the three-hourly figures from the study by NATHAN *et al.* (1978) and considers the totals (or the means) of all the subjects for each hour-section, as recommended by MATTINGLY (1962) or by SASA & TANAKA (1972, 1974), one finds a very low periodicity-index (ratio of standard deviation on the average of the counts): 12% (Table I). The relative amplitude (5·7%) is not significantly different from zero. The estimated peak hour (acrophase) would be close to midnight (23.07 hours).

HAWKING (1975) pointed out that the calculations should be based on the data from single individuals: if the calculations are made on the means of a group of individuals who are not synchronous, the curve may be much flattened. Actually, by this "individual" method, PICHON *et al.* (1979) and PICHON (1981) were able to demonstrate a consequential diversity among the biorhythms of the sub-periodic forms of *Wuchereria bancrofti* and *Brugia malayi.*

Using the data from NATHAN *et al.* (1978), instead of grouping the three-hourly counts we have fitted every series of counts to a sinusoidal function, using the least-squares method (KENNEY & KEEPING, 1951). This methodology (cf. Annex) was first applied

Table I—Fit of *Mansonella ozzardi* biorhythms to a sinusoidal curve and calculations of the parameters

	No. mf/25 mm^3				%		Hour
Patient No. and Sex	Mean (m)	Standard Deviation (s)	Amplitude (a)	Test F^2_5	Periodicity Index D = s/m	Relative Amplitude a/m	Acrophase (k)
1—M	145·125	51·285	14·758	0·248	35·3	10·2	05·43
2—M	15·000	4·660	3·638	2·672	31·1	24·3	03·76
3—F	13·125	6·896	7·548	10·853*	52·5	57·5	17·42
4—F	40·375	15·602	2·146	0·055	38·6	5·3	02·49
5—M	11·500	8·767	5·659	1·562	76·2	49·2	18·47
6—M	138·900	31·791	24·719	2·639	22·9	17·8	17·64
7—F	13·125	6·770	2·249	0·336	51·6	17·1	03·56
8—M	135·250	24·616	28·461	16·177**	18·2	21·0	02·04
Total	512·375	62·372	29·075	0·709	12·2	5·7	23·68

*significant P = 0·025 (F = 5·79) **highly significant = P = 0·01 (F = 13·3) to microfilariae by AIKAT & DAS (1976), and to individual counts by PICHON *et al.* (1979). Without the aid of a computer, it enables one to calculate the amplitude *a* (in fact, half-amplitude), to test whether it is statistically different from zero (i.e., whether in fact there is periodicity) and to calculate the acrophase *k* (peak hour).

Results

The results are assembled in Table I. Whereas the over-all periodicity index is very low (12·2%), one finds that the individual indices are much higher (range: 18·2 to 76·2%, average: 40·8%). This suggests that, from one subject to the other, the oscillations tend to average out.

Table I shows clearly that the "periodicity index" of SASA & TANAKA is an unreliable indication on the existence of periodicity: subject No. 5, who has the highest index (76·2) is not significant while subject No. 8 (with index of 18·2) is highly significant. As long as periodicity has not been proven (by the F. test), the periodicity index is no more than, and should be called, a coefficient of variation, i.e., a relative standard deviation, which expresses random, or at least non-circadian fluctuations.

Two subjects show a highly significant periodicity:

(a) Subject No. 3: the relative amplitude is 57·5%, and the acrophase is 17.42 hours, i.e., about 18.00 hours.

(b) Subject No. 8: the relative amplitude is 21·0%, and the acrophase is 02.04 hours, i.e., about 02.00 hours.

As Fig. 1 illustrates, these two subjects show an evident periodicity. Nevertheless, contrary to what occurs in other filarial species, the biorhythms of these two subjects are completely out of phase: the first acrophase is located in the late afternoon, whereas the second one is located in the second half of the night.

Among the six remaining subjects, there is no statistically significant periodicity. That could be due, at least in part, to the small volume of capillary blood collected (25 mm^3) and to the small number of counts per nycthemere (8 rather than 12). Anyway, if the fluctuations observed in these subjects occurred absolutely randomly, their calculated acrophases would be evenly distributed throughout the daily period. In fact, one observes that the six calculated acrophases all assemble in two groups, corresponding to the acrophases of the two subjects with significant periodicity:

(a) in the late afternoon for subjects Nos. 5 and 6 (18·47 and 17·64 hours) as for subject No. 3 (17·42 hours).

(b) in the late night for the other subjects: Nos. 1, 2, 4 and 7 (05·43, 03·76, 02·49, and 03·56 hours) as for subject No. 8 (02·04 hours).

The acrophases of both groups have respectively an average ± standard deviation of 17·8 ± 0·6 hours and 3·5 ± 1·3 hours.

This finding seems to rule out the possibility that the non-synchronization between subjects Nos. 3 and 8 could be due to the fact that one of them was a "night worker", e.g., a night fisherman.

When one looks at the chronograms of the six subjects without evident periodicity, one finds that among at least two of them (Nos. 1 and 4) the lack of fit to a 24-hour sinusoidal curve could be due to the superimposition of two out-of-phase oscillations, presenting as a bimodal curve, with one peak in the late afternoon and one peak in the late night.

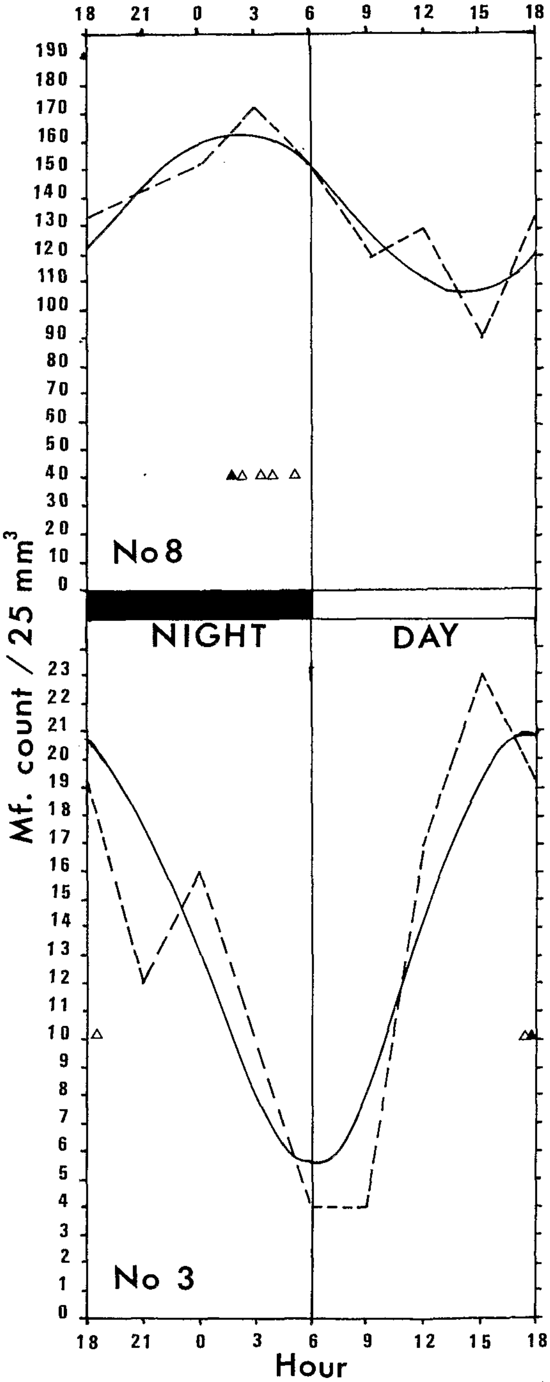

Fig. 1. Comparison of the observed (broken line) and theoretical (solid line) periodicity curves of two subjects carrying *Mansonella ozzardi* (Data: NATHAN *et al.*, 1978). Triangles represent the calculated acrophases for these two subjects (dark), and for the six other cases (light) who do not show statistically significant periodicity.

Conclusions

Our scrutiny of the results published by NATHAN *et al.* (1978) does not allow us to conclude, along with these authors, that there is an absence of periodicity in *Mansonella ozzardi* microfilariae from Trinidad. Rather we find in this filarial parasite a phenomenon of crypto-periodicity. This is ascribed to the sympatric existence of two out-of-phase, periodic fluctuations, the acrophases of which are respectively located in the late afternoon and in the second half of the night. Apparently, both may co-exist in the same individual.

Crypto-periodicity is different from sub-periodicity of low amplitude in the sense that the last is due to synchronized fluctuations. In such a case, the over-all analysis of a group of subjects could produce evidence of an even weak, periodic fluctuation. In a crypto-periodic situation, fluctuations may be obviously periodic at the level of some individuals, but, being out-of-phase, their mixture tends to produce non-periodicity in other subjects or at the community level.

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Annex (from AIKAT & DAS, 1976)

The microfilarial density (y) is assumed to follow a sinusoïdal function of hour of day (h):

$$y = m + a\cos 15\,(h-k)^\circ \qquad (1)$$

Where m, a and k are the mean, amplitude and peak hour respectively. The hours 0 to 24 are multiplied by 15 to correspond to angle 0° to 360°.

To simplify the computations, equation (1) can be modified as follows:

$$y = m + b\cos 15\,\mathrm{h}^\circ + c\sin 15\,\mathrm{h}^\circ \qquad (2)$$

$$\text{where } b = a\cos 15\,\mathrm{k}^\circ \qquad (3)$$

$$c = a\sin 15\,\mathrm{k}^\circ \qquad (4)$$

$$\text{so that } a^2 = b^2 + c^2$$

$$\text{or} \quad a = \sqrt{b^2 + c^2} \qquad (5)$$

$$\text{and } \tan 15\,\mathrm{k} = c/b \qquad (6)$$

The least squares estimates (KENNEY & KEEPING, 1951) of m, b and c of equation (2) are as follows =

$$m = (\Sigma y)/n \qquad (7)$$

$$b = 2(\Sigma y\cos 15\,h)/n \qquad (8)$$

$$c = 2(\Sigma y\sin 15\,h)/n \qquad (9)$$

Where n is the number of evenly spaced observations; n must be even, at least equal to 4.

The test of significance of a (i.e. a^2 not equal to zero) is given by:

$$F = \frac{n}{2}a^2 \Big/ \frac{1}{n-3}\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n} - \frac{n}{2}a^2\right] \qquad (10)$$

The value of F should be greater than the theoretical 5 percent F-value with 2 and (n - 3) degrees of freedom.