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OBSERVATION OF THE CIRCADIAN BIORHYTHM IN SOME INSECTS GROUPS

Abstract - The Circadian rhythms of several flyers' insects, Hymenoptera, Lepidoptera and Diptera, were monitored in the field by means of a Malaise trap, modified to capture separately every 3 hours intervals. The analyzed data are the insects number observed during each time interval and accumulated over an averaged trap operation of a few days. The Gaussian statistical distribution fit satisfactory the observed data, thus improving the precision in the determination of peak activity and the time-span of biorhythms. From the peak value and Gaussian width, the daily rhythm of each group can be accurately investigated. As a general result, all studied diurnal insects are active with the maximum in the early afternoon (about 14.00 hours after midnight), for duration of about 6-8 hours.

Key words - Circadian rhythm, Chrysididae, Mutillidae, Sphecidae, Rhopalocera, Noctuidae, Tipulidae, Tabanidae, Tettigoniidae, Ichneumonidae

Riassunto - *Osservazione dei ritmi circadiani in alcuni gruppi di insetti*. I ritmi circadiani di alcuni gruppi d'insetti volatori sono stati investigati nella Tenuta di San Rossore (Pisa) nella stagione 2010, dal mese di maggio ad ottobre. L'attività degli insetti è stata misurata in continuazione contando il numero d'insetti catturati in intervalli successivi di tre ore. In questo modo si ottiene il vero valore medio dei conteggi indipendentemente da fluttuazioni metereologiche della durata di qualche giorno. Per questo scopo è stata modificata una trappola a intercettazione di tipo Malaise. Un motore comandato da un temporizzatore sostituisce la bottiglia di raccolta ogni tre ore per cui ogni giorno si hanno otto intervalli di cattura a partire dalla mezzanotte. La trappola funziona autonomamente per alcuni giorni, permettendo di accumulare un numero sufficiente di catture per la successiva interpolazione dei dati con una funzione gaussiana, che permette di individuare l'ora del massimo di catture e la durata temporale del periodo di attività. Per alcuni gruppi (Chrysididae, Sphecidae, Rhopalocera, Tabanidae) si osserva una distribuzione delle catture semplice, ben interpolata da una sola curva gaussiana, con il massimo nel primo pomeriggio e una larghezza di circa sei ore. Per altri gruppi (Mutillidi, Noctuidae, Geometridae) la distribuzione è più complessa, interpolabile come la somma di due gaussiane. In particolare i Macrolepidotteri notturni mostrano una prima fase di attività serale, col Massimo prima della mezzanotte, e una seconda, inferiore per numero, prima dell'alba. Sono evidenti le possibili implicazioni per un più efficiente uso dei fitofarmaci.

Parole chiave - Bioritmi, Chrysididae, Mutillidae, Sphecidae, Rhopalocera, Noctuidae, Tipulidae, Tettigoniidae, Tabanidae, Ichneumonidae

Introduction

The Circadian (Circa-diem) rhythm is: «A biological rhythm having a periodicity of about 1 day length (24 h); diurnal rhythm» (Cambridge dictionary of ecology. Cambridge, 1982).
The principal activities of living insect are: «feeding», «reproduction» and «dispersion». All require the capacity of moving and wander in their biotope.
Thus the number of flayer's insects can be assumed directly proportional to the biological activity.
Useful data are obtained by mean of an interception, non-attractive, trap.
The effectiveness of this method was tested on the field by means of a purposely-modified Malaise trap. The captures were counted in successive and equal time intervals and the data interpolated to a Gaussian function, that, according to the distribution suggested by the central limit theorem. The interpolation gives more accurately the peak of the biological activity and the time-span.

Materials and method

A Malaise Trap of the design proposed by Townes (Townes, 1962; 1972; Matthews & Matthews, 1983) was purposely modified.
In order to count the flying insects as in the time intervals, the fabric of a commercial Malaise trap was retained and only the exit from the trap modified.
Eight bottles were appended to a metal disc driven by a DC electric motor that can rotate and position successively each bottle at the trap exit. A timer switch on the motor every 3 hours and a contact switch shut of the motor when the next bottle is properly positioned (Fig. 1). A commercial battery provides energy for a continuous operation in the field for about 3 weeks. The apparatus is enclosed in a box to prevent contamination and reduce alcohol evaporation in the collecting bottles.
In 2010 summer season the modified trap was positioned in «San Rossore» reserve near Pisa town. A sandy and sunny ground, at border of an oak wood, was chosen because favourable to Hymenoptera nesting. A nearby water pool make the location favourable also to other insects during the dry months of July and August. The day-time was divided in 3 hours intervals ad the continuous operation for a few days allow to capture the number of individuals necessary for data interpolation. The 3 hours period is a compromise between the need to accumulate numbers sufficiently large and to obtain the good time resolution allowed by the Gaussian interpolation.

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Fig. 1 - Modified Malaise trap: detail of the rotating disc with the suspended 8 bottles positioned every 3 hours under the trap output.

Nevertheless the captures for each single species were to small, even with an accumulation time of about a week, and it turned out convenient to consider cumulatively not a single species but insect groups with similar behaviours, and, possibly, similar biorhythms. The collected material was counted and arranged in the selected groups. In any case, the results are at least the average over the group.

Results

An example of the typical counts observed in the interval 16.06-28.06.2010 is given in Table 1. Of the 244 counts, 213 belong to truly diurnal insects; wile only 31 refers to known nocturnal species. The counts for some interesting groups (Chrysididae, Mutillidae, Tettigoniidae, Asilidae, Tipulidae,) resulted scarce and accumulation over longer period proved to be necessary. By assuming that the catching probability around maximum is purely statistic, we can perform the data interpolation by using a Gaussian function, defined as:
$N = A*EXP(-0.5*((X-M)/SD)^2)$
Were M is the Mean, time of maximum count, X the time interval, SD the standard deviation of the distribution, approximately the half width of the curve.
In Figure 2 the fit to all diurnal insect counted in Table 1 is shown. The Interpolation goodness is indicated by the coefficient of determination R^2, a number ranging from 0 to 1 ($R^2 = 1$ means that the curve fit well all the data points with no residual variance). A second bell shaped curve, the Lorentzian distribution, was also tested, as shown in Figure 2. As results the Gaussian perform better ($R^2 = 0.9733$ for the Gaussian and $R^2 = 0.9204$ for the Lorentzian). As a consequence of the small number of counts the interpolation is essential to increase the resolution and precision in the data analysis.
In spite of the small number of captured individuals a difference is observed in the interpolations of different groups of diurnal insect, as shown in Figures 4 and 5. The corresponding values of maximum hour and of the

Tab. 1 - Example of insects count in the interval 14 -28 June 2010.

Family or Group	**0.00-3.00**	**3.00-6.00**	**6.00-9.00**	**9.00-12.00**	**12.00- 15.00**	**15.00- 18.00**	**18.00- 21.00**	**21.00- 24.00**	**All**
Acrididae	2	0	0	2	1	1	1	1	8
Apoidea	0	0	0	12	19	12	2	0	45
Asilidae	0	0	0	1	2	10	2	0	15
Bombyliidae	0	0	0	2	0	1	0	0	3
Chrysididae	0	0	0	0	3	0	0	0	3
Ichneumonidae	0	1	3	0	2	7	4	3	20
Mutillidae	0	0	0	1	0	0	2	0	3
Myrmeleontidae	0	0	0	0	0	0	0	1	1
Myzininae	0	0	0	6	8	0	0	0	14
Noctuoidea	0	4	1	1	0	0	0	2	8
Pompilidae	0	1	0	7	9	5	0	0	22
Rhopalocera	0	0	1	6	18	13	4	0	42
Sphecidae	0	0	1	3	7	7	0	0	18
Tabanidae	0	0	1	11	10	16	1	0	39
Tettigoniidae	0	0	0	1	1	1	1	0	4
Tipulidae	0	0	0	0	0	1	0	0	1
All	**2**	**6**	**7**	**53**	**81**	**72**	**16**	**7**	**244**
Diurnal	2	1	3	51	77	65	12	2	213

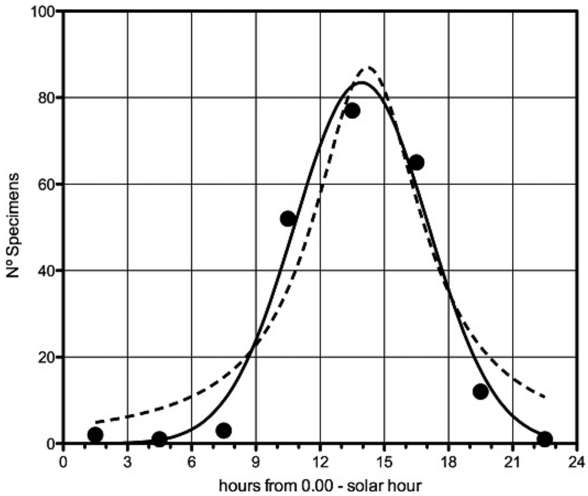

Fig.2 - non-linear interpolation for all diurnal insects counted in Table 1. The continuous and the broken lines are respectively the best fit with a Gaussian function and a Lorentzian function. The abscissa shows the legal hours from midnight.

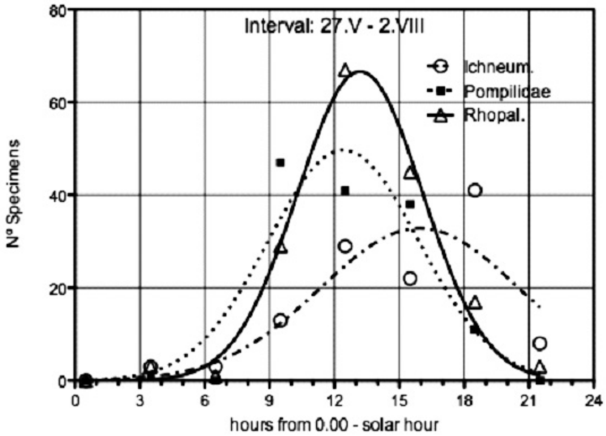

Fig. 4 - Biorhythms observed for the Hymenoptera Ichneumonidae and Pompilidae and for the Lepidoptera Rhopalocera (Butterfly) in summer 2010 during the interval 27.V-2.VII.

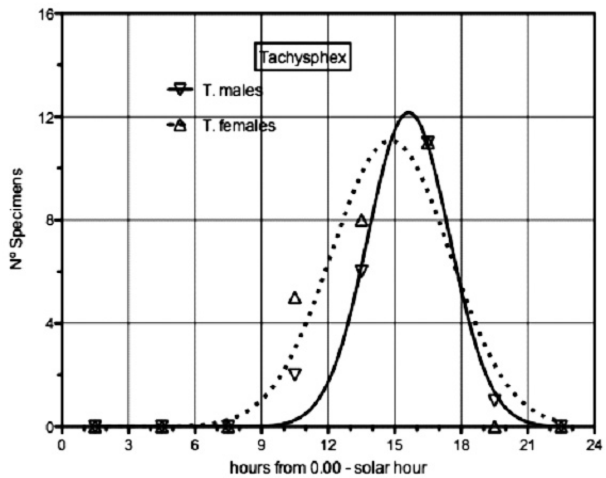

Fig. 3 - Nonlinear interpolation of data for 20 males and 24 females of the genus Tachysphex captured in the time interval 28.VI-1.IX.

Tab. 2 - Peak hour, SD and R^2 for the Mutillidae males in the interval 27.V-12.VIII, and for males and females of Hymenoptera of genus Tachysphex in the cumulated intervals 28.VI-1.IX.

Males of Mutillidae			
Mutillidae	**M**	**SD**	**R^2**
Gaussian 1	10,05	1,802	1
Gaussian 2	15,91	2,43	1
Tachysphex			
Males	15,63	1,837	0,97
Females	14,82	2,672	0,88

Tab. 3 - Mean peak hour, Standard deviation and coefficient of determination obtained for several insect groups.

Group	**Mean**	**SD**	**R^2**
Noctuidae I	0,80	1,29	0,89
Noctuidae II	5,40	1,74	0,89
Pompilidae	13,06	2,75	0,96
Apoidea	13,58	2,83	0,98
Tabanidae	13,89	3,60	0,92
Diurnal all	13,93	3,12	0,97
Rhopalocera	14,39	2,70	0,99
Sphecidae	14,52	2,60	0,94
Asilidae	16,05	2,24	0,98
Geometridae	21,00	2,06	0,98
Tipulidae	21,98	2,63	0,81
Tettigoniidae	22,16	3,22	0,96

SD are summarized in Tables 2 and 3. All Hymenoptera show a peak in the early afternoon (Mean value 12.02 after midnight) and are active for about 6 hours. Only Mutillidae and Ichneumonidae appear later (peak after 14.00), and remain in flight longer (SD = 4.278 hours). In looking for a possible difference between males and females, we considered the Sphecidae of genus Tachysphex. This is a cosmopolite genus, whose species shows a similar behaviour, nest in the ground and hunt mostly small Orthoptera. In the interval 28.VI-1.IX the trap captured 24 females and 20 males of the following species: *Tachysphex costae* De Stefani, 1882, *T. obscuripennis* (Schenck, 1857), *T. mediterraneus* Kohl, 1883,

T. *fulvitarsis* A. Costa, 1867. The result is shown in Figure 3, were it appears that the males are active early and for a longer time interval (Tab. 2). In Figures 5a and 5b the results observed for some Hymenoptera and for the frequently captured Diptera Tabanidae are shown.

The Circadian rhythms usually show a single peak in the day length. These are called monophasic rhythms (Tremblay, 1996). In some cases the data may appear more complex with an additional maximum (biphasic rhythm). Interesting examples could be observed. In the family Mutillidae the females are wingless and only males are capture by the Malaise trap. In the period 8.VI-2.VIII, 39 males were captured and the distribution is shown in Figure 6 and table III. We obtain the best interpolation with a sum of two Gaussian functions (R^2 = 1.00). Apparently the male appears in flight two times a day, and this behaviour is, possibly, related to the strong influence of soil temperature on female's activity (Nonvellier, 1963).

A more interesting result was observed for the nocturnal Macrolepidoptera. At first we consider the cumulative count of all families (mainly Noctuidae, Geometridae and Arctiidae). The result shown in Fig.7 is clearly biphasic with a maximum around 5.00 and a second in late afternoon before midnight. Only the captures of Noctuidae and Geometridae resulted sufficiently large to allow disjunctive interpolations.

The results are shown in Figures 8 and 9. The Noctuidae are clearly biphasic with the similar behaviours as in Figure 7. The data of Geometridae confirm the activity peak of late afternoon, before midnight, but the counts are insufficient to unambiguously fit the data of late night data, even there is some evidence.

An after sunset biorhythm was also observed in two weak flyers groups: Orthoptera Tettigoniidae (Ensifera) and Diptera Tipulidae. The results are shown in Fig-

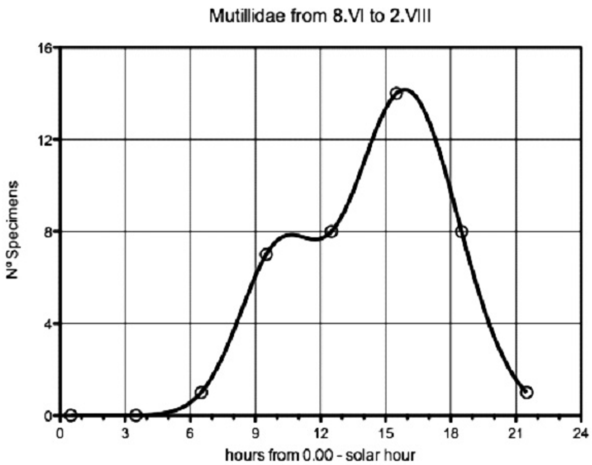

Fig. 6 - Biphasic biorhythm observed for all Mutillidae males captured in the interval.

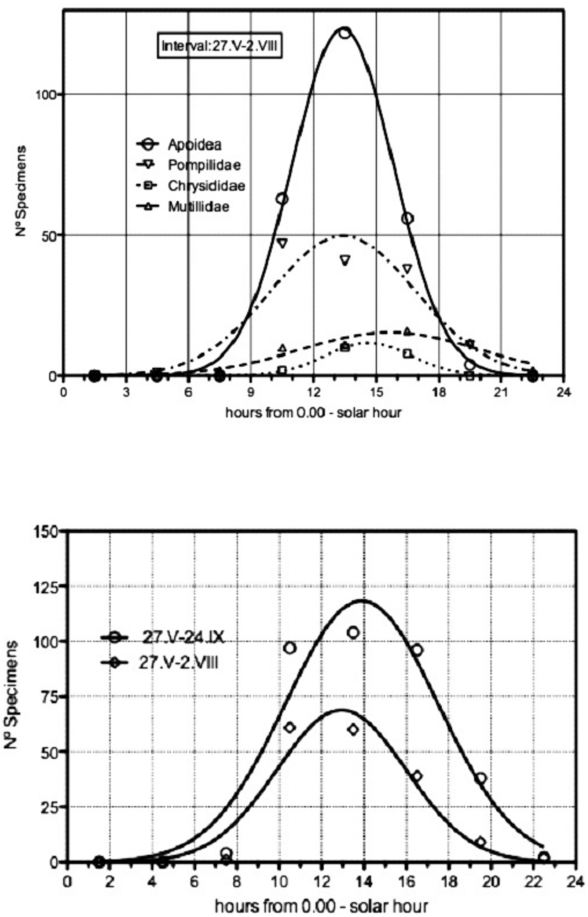

Fig. 5 - a) Diurnal Biorhythms observed for four Hymenoptera group; b) Diurnal Biorhythms of Diptera Tabanidae captured in the interval 27.V-24.IX (341 specimens) and 27.V-2.VIII (172 specimens).

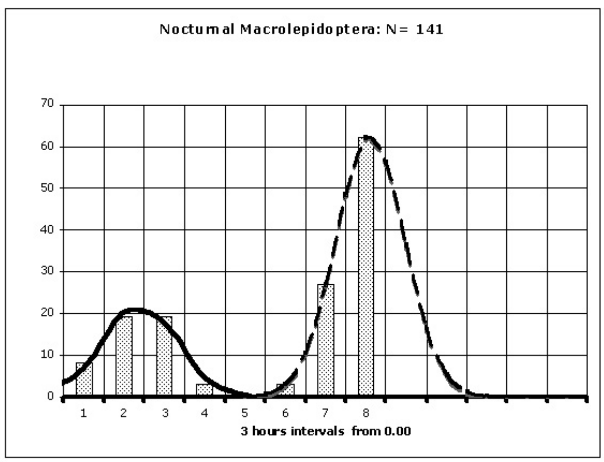

Fig. 7 - Biphasic byorhithm of the 141 nocturnal Macrolepidoptera captured in the range VII-IX.2010. The last bar corresponds to midnight interval. The horizontal axis is extended to show the full interpolation.

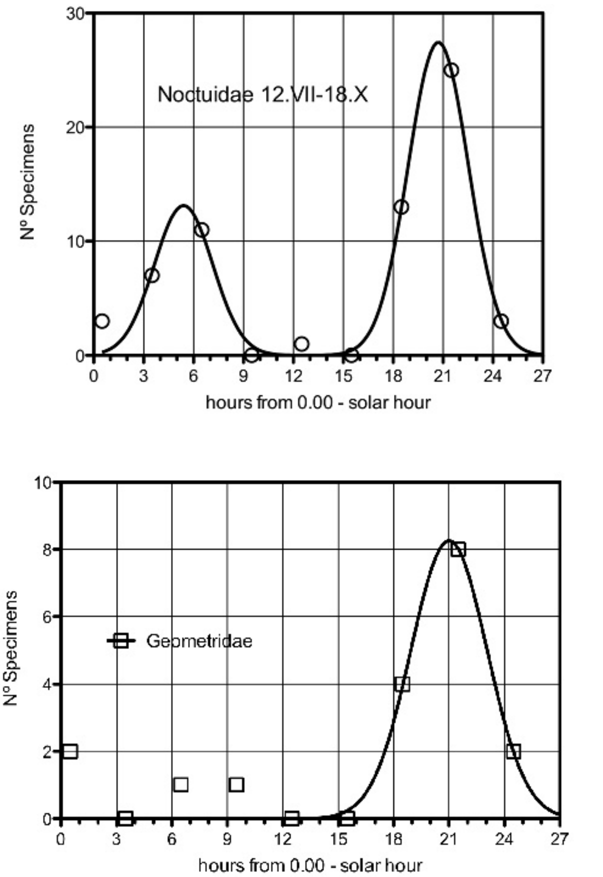

Fig. 8 - Biphasic biorhythms observed for the separate counting of the Lepidoptera Noctuidae and Geometridae. Experimental data are obtained as a periodic function of 24 hours and can be extended beyond 24.00.

ures 9 and 10. In both groups the captures start in late afternoon with the maximum after the sunset and a decrease after midnight.

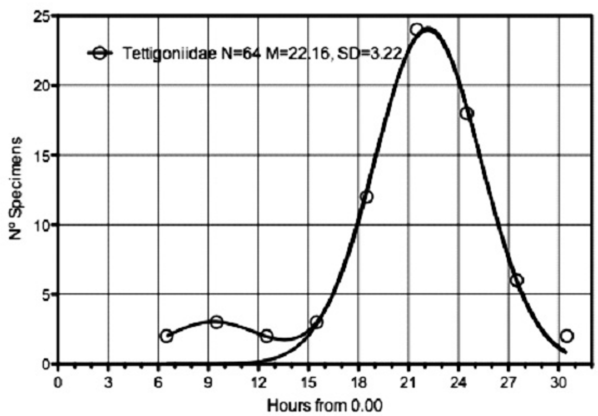

Fig. 9 - Biorhythm observed on 64 individuals of Orthoptera Tettigoniidae captured in the period 12.VIII-15.IX.

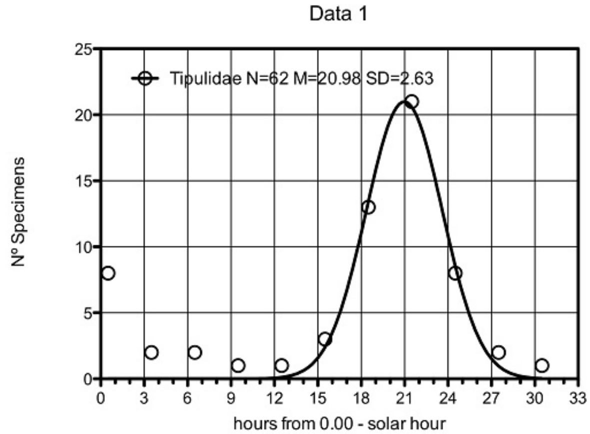

Fig. 10 - Biphasic biorhythm observed in the case of 62 Diptera Tipulidae captured in the period 1.IX-5.X.

Conclusion

The modified Malaise trap described in the present paper results successful for study the biorhythms of flyers insects. The trap was tested on the field providing quantitative data, not already available in the literature (Wilson 1971, Price 1975, Borror *et al.* 1976, Huffaker *et al.* 1984, Ricklefs 1990, Chapman 1998).
Precise and reliable data on insect pest circadian rhythm may have practical interest allowing a correct timing in the release of insecticide. We can envisage the introduction of fast decay compounds, thus better preserving the environment and better selecting between pest and beneficial insects.

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