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Worker honeybee hemolymph lipid composition and synodic lunar cycle periodicities

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Abstract

The aim of the present investigation was to extend a previous study, showing a correlation of the variations of hemolymph carbohydrates with synodic lunar-like cycle and its circaseptan harmonics to worker honeybee hemolymph lipids. Hemolymph lipid concentrations of emerging worker imagos were analyzed in terms of one ideal synodic lunar cycle and processed by the cosinor method testing the null hypothesis versus the presence of 29.5-, 14.8- or 7.4-day periods in the data. A rhythmicity statistically compatible with a 29.5-day rhythm was observed for triacylglycerols and steroids as well as for body weight. A circadiseptan rhythm was determined for 1,3 diacylglycerols, while fatty acids and phospholipids exhibited a circaseptan rhythm. An agreement of peaks for triacylglycerols, steroids and body weight at the new moon, but not at the full moon, was noted with respect to trehalose and glucose circadiseptan rhythms. The latter moon-phase timing of peaks and nadirs, compared with that previously determined for trehalose and glucose, appeared to be identical to the circadiseptan rhythm and reciprocal for the circaseptan rhythms of 1,3 diacylglycerols. Reciprocal tendencies in circaseptans of trehalose and glucose on the one hand, and fatty acids and phospholipids on the other are indicated. The underlying causal nexus of these relationships is unknown.

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Key words
- *Apis mellifera*
- Body weight
- Fatty acids
- Diacylglycerols
- Triacylglycerols
- Phospholipids
- Steroids
- Hemolymph
- Synodic lunar cycle
- Circa(di)septan subcycle

Introduction

It has been shown that the flight activity of honeybees is correlated with the phases of the moon (1). This observation is supported by the finding of a rhythmicity compatible with the synodic lunar circadiseptan and circaseptan subcycles for the hemolymph concentrations of trehalose and glucose, respectively, the major circulating reserve and direct fuel supply for the insect's muscular energy (2). Close relationships have been shown between the hormonal control of bee lipids and carbohydrates (3,4), and lunar variations in the activity of both aminergic and cholinergic compounds involved in the regulatory processes which control the energy supply and muscular activity (5).

These findings prompted us to further examine the corollary hypothesis that lipid

composition could also exhibit lunar phase-correlated fluctuations. An important variability has been observed during a full-year experiment on lipid hormonal control. The experiments were done using the same colony, without changing the artificially inseminated queen during the study period. This means that all bees had the same genes as is the case for identical twins. Accordingly, differences would not be expected for such a homogeneous population, except in the case of some other environmentally driven variations. The present report presents the results of the chronobiological study of worker honeybee hemolymph lipid composition.

Material and Methods

Sampling procedure

Apis mellifera, mellifera L. emerging workers were gathered from still operculated alveoli. They were immediately weighed, placed on glass dishes and kept in darkness in chambers with controlled temperature (25 ± 1°C) and relative humidity (60 ± 10%), where they remained quiet and protected from stress-induced biochemical changes. Hemolymph was collected by puncturing the dorsal aorta with a 25- or 50-µl Hamilton syringe. The same volume of chloroform was added, and the samples were stirred to homogeneous emulsion and then centrifuged at 2000 *g* for 5 min. The lower (chloroform) phase was recovered and its volume adjusted to a known value corresponding to 1 to 5 µl of chloroform extract per µl hemolymph. The hemolymph levels of fatty acids (FAc), 1,2 and 1,3 diacylglycerols (1,2 and 1,3 DiG), triacylglycerols (TriG), phospholipids (PhL) and steroids (Ste) were measured, at least in triplicate. Data are reported as the average value of 57 days starting April 29, 1981, with the sampling days corresponding to days 25, 1, 2, 8, 9, 14, 15, 16, 17, 21, 22 and 23 of the synodic lunar cycle.

Chromatographic procedure

The equivalent of 1 to 10 µl hemolymph was spotted on TLC-precoated silicagel sheets (Merck-Darmstadt, Ref. 5748) using a Desaga automatic micro-applicator and chromatographed as previously described (6). A calibration curve was constructed for each lipid class using phosphatidylinositol, cholesterol, oleic acid, diolein or triolein as standards. Curves were linearized by semi-logarithmic transformation, and the final accuracy of the method, after quantitative recording of the chromatograms using a Shimadzu CS930 spectro-densitometer, was better than ± 5%. Results are reported as means ± SD for three determinations.

Biomathematical and statistical treatment of the data

Each day of measurement was expressed in terms of synodic lunar cycle (SLC) with the new moon as day zero. Thus, for each substance, a synodic lunar plexogram was constructed with 12 averaged data, as shown in Figures 1 to 3. Halberg's cosinor analysis (7) modified by Mikulecky (8) and Valach et al. (9) was applied. The approximating regression function has been written in the form:

$$y = a + A.\cos(360^{o}.t/\tau + \phi)$$

where y = estimate of the approximating function at time (t), a = mesor of the rhythm: a constant concentration value, A = amplitude of the rhythm, in concentration units, τ = period length of the rhythm, in days, i.e.: $\tau 1$ = 29.5 days (lunar rhythm), $\tau 2$ = 14.8 days (semi-lunar or approximately circadiseptan rhythm), or $\tau 3$ = 7.4 days (quarter-lunar or approximately circaseptan rhythm), ϕ = acrophase in negative angle degrees, indicating the time of peaking after the new moon (t = 0), which is, for example, for $\tau 2$: -360° = the first peak at time t = 14.8 days, -270° = the first peak at time t = 11.1 days, and -180°

= the first peak at time t = 7.4 days.

The regression calculation includes the estimation of the 95% confidence intervals and the determination of the statistical significance of the amplitude of the corresponding rhythm. The coefficients of determination are also given since they represent the proportion of the variance of the data which is explained by the regression equation. In order to avoid spurious significance due to the three-fold testing with the three different τ values, the corresponding significances were adjusted by Bonferroni modification (10). The level of significance was set at α = 0.05 in all tests.

All averaged data ± SD are available on request at the E-mail address of the senior author: mikuleky@upkm.sanet.sk.

Results

Biological parameters

Figure 1A presents the average weights of the bees during the experimental period. A maximum was observed near new moon. The data did not indicate the presence of subcycles.

Biochemical parameters

One of the three tested rhythms was statistically significant for each class of lipids except 1,2 diacylglycerols. As assessed by the corresponding significance levels given in Table 1 two cases of lunar rhythmicity were found (for triacylglycerols and steroids), one case of semi-lunar rhythmicity (for 1,3 diacylglycerols), and two other cases of quarter-lunar rhythmicity (for fatty acids and phospholipids).

The experimental data are plotted in Figures 1B and 2A, B and C, showing the 95% confidence regression estimates. The weights and concentrations indicate the following rhythms: 29.5 days (lunar rhythm) around the new moon, 14.8 days at new moon and full moon, and 7.4 days (quarter-lunar rhythm) at the 2nd-3rd days, 9th-10th days, 17th-18th days, 24th-25th days. In other words, the minimal values were observed and predicted shortly before the new and full moon.

The statistical treatment of the data are summarized in Table 1.

Discussion

It should be pointed out that the observed data sets were tested against the basic lunar-like rhythm of 29.5 days, which corresponds to a circaseptan period of 7.4 days. Of course, the fact that the null hypothesis was rejected with error type I probability α = 0.05 does not prove the direct influence of the moon. Moreover, no direct proof can be provided since it is not possible to reproduce these experiments under "moonless" conditions. Sea tides could be also examined as a possible index which would allow comparison of biochemical rhythms in colonies situated

Table 1 - Statistical parameters (stat.) of moon phase dependence of body weight and hemolymph concentrations of various lipid classes in worker honeybees.

CD = Coefficient of determination, i.e., the fraction of the total variance of the data explained by the regression equation; PBon = P value by Bonferroni modification. Period length indicates lunar (29.5 days), semi-lunar (14.8 days), and quarter-lunar (7.4 days) periodicities. FAc = Fatty acids; 1,2DiG = 1,2 diacylglycerols; 1,3DiG = 1,3 diacylglycerols; TriG = triacylglycerols; PhL = phospholipids; Ste = steroids. Asterisks (*) indicate the P≤0.05 level of significance. Underlined asterisks (*) denote limit cases with 0.05<P<0.06.

Period length (days)	Stat.	Body weight	Lipid classes					
			FAc	1,2 DiG	1,3 DiG	TriG	PhL	Ste
29.5	CD	0.648	0.402	0.202	0.070	0.423	0.217	0.449
	PBon	0.007*	0.054*	0.249	0.645	0.046*	0.223	0.036*
14.8	CD	0.092	0.082	0.200	0.500	0.042	0.074	0.185
	PBon	0.739	0.688	0.281	0.036*	0.910	0.745	0.421
7.4	CD	0.281	0.483	0.431	0.093	0.289	0.692	0.274
	PBon	0.173	0.036*	0.058*	0.546	0.164	0.002*	0.180

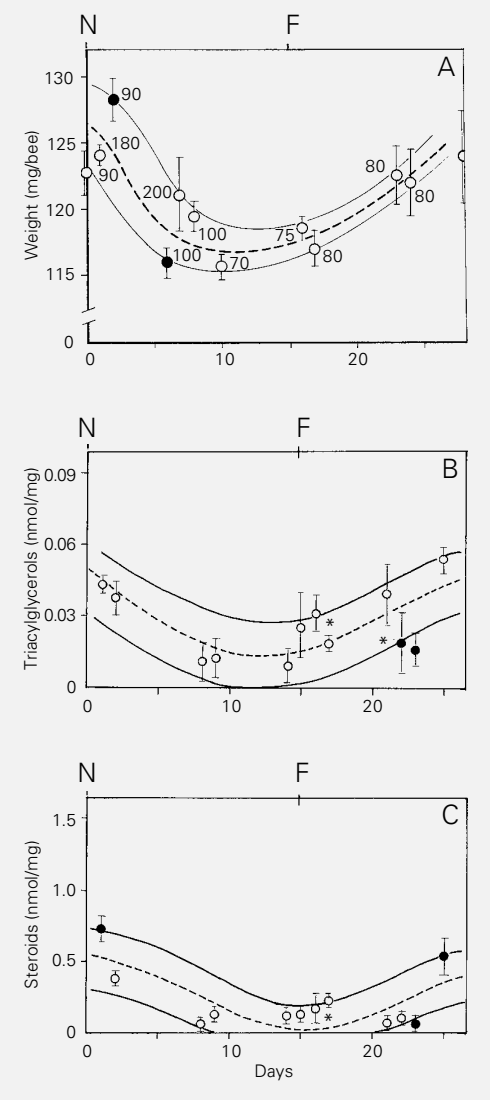

Figure 1 - Correlation of emerging worker bee weight (A) and honeybee hemolymph triacylglycerol content (B) and hemolymph steroid content (C) with the synodic lunar cycle. N = New moon data; F = full moon data. The approximating regression function is indicated by the broken line in each panel together with the 95% confidence limits. The data within the 95% limits are indicated by *open circles* and those outside these limits are indicated by *filled circles*. *A*, Emerging honeybee weight is reported as means ± SD, mg/bee, and the number of bees is given next to each symbol. *B*, Triacylglycerol content is reported as means ± SD, nmol/mg bee fresh weight, for N = 3, except for the two points indicated by an asterisk for which N = 6. *C*, The data for hemolymph steroids are reported as described in the legend to panel B.

close to or far from climatic sea influence. This is one of the suggestions for further research raised by the data presented here.

The most pronounced periodicity (coefficient of determination, CD = 0.692) was circaseptan, found for phospholipids. In contrast, no significant rhythmicity was demonstrated for 1,2 diacylglycerols, although a circaseptan rhythm was at the borderline of being statistically significant. Chronobiological similarities were noted for two groups of lipid classes: first, triacylglycerols and steroids share a common 29.5-day rhythm, while fatty acids and phospholipids display the 7.4-day rhythm, with almost identical timing of peaks. The latter peaks of lunar and semilunar rhythms exhibit similarities with those previously characterized for trehalose and glucose (2). The agreement is however better for new moon than for full moon.

In both circaseptan lipid periodicities, a reciprocity with those of carbohydrates was observed. As to the partial agreement between the carbohydrate and lipid chronobiology timing, it should be pointed out that the data for carbohydrates and lipids were obtained in different and independent experiments. The data were available only for 12 nonequidistant classes of days of SLC, but over a two-month period of time. In spite of i) these constraints inherent in the homogeneity of the assays, and ii) the careful statistical treatment to avoid spurious significance, a number of statistically significant results were obtained. Indeed, further complementary experiments would be needed in order to verify the phenomena in a more detailed manner and to strengthen the conclusions. However, honeybee biochemistry already appears to be very closely connected to the time-course of lunar phases. In this respect, it should be added that the statistical significances obtained are corroborated by the fact that averaged values for almost identical sample sizes and not individual measurements were used for the calculations.

The causality of the observed effects remains far from being interpretable in terms of fundamental physical forces. For instance, one could expect a discrimination between strongly and weakly charged molecules from a geomagnetic influence oscillating during the synodic lunar cycle. This obviously is not the case if one considers the paired behavior of steroids and the less polar triacylglycerols, or that of phospholipids, more polar than fatty acids. However, this distinction might not be relevant at the level of the various enzyme systems involved in the corresponding metabolic pathways. Since the kinetic properties of enzymic proteins are

likely to be modified by fields (11,12), these possibilities should be examined with respect to moon phases. On the other hand, gravitational influences (tidal variations) have not been really studied, except in the case of the well-known phenomenon of gravitropism (13). In this case, too, we suggest that the hypothesis of a possible moon phase-related rhythmicity should be examined. The significance of the results indeed will depend on the sensitivity of the methods.

It should be recalled that the general biological concept of circaseptan (5-9 days) rhythms extends from human beings (14) to animals, including insects and even unicellular organisms (15). All these observations do not necessarily involve a direct causality. The observed phenomena might rather be due to some indirect influences mediated by unknown common periodic factors operating within a complicated causal nexus. This remains a major question which deserves further investigation, not only at the experimental but also at the theoretical level.

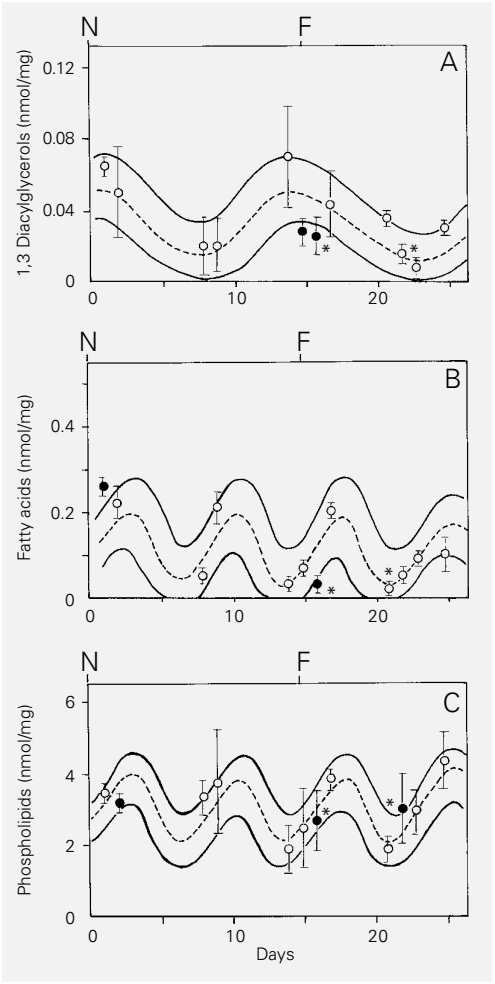

Figure 2 - Correlation of honeybee hemolymph 1,3 diacylglycerol (A), fatty acids (B) and phospholipids (C) with days of the synodic lunar cycle. Data are reported as described in the legend to Figure 1 (means ± SD, nmol/mg bee fresh weight).

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