

pollen placement by twisting their nectar spurs and illustrates how evolution sometimes comes up with unexpected solutions.

Supplemental Information

Supplemental Information includes one supplemental movie and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.07.056>.

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Reply to Cordi *et al.*

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In their paper on the influence of the moon on sleep, Cordi *et al.* [1] have analyzed a large number of subjects and found no significant effects, as opposed to our positive study findings with a smaller cohort [2]. More is not necessarily better. There are two main reasons why we think the comparison of these two data sets is not just comparing a small with a big sample size, since increasing the number of study volunteers in a sleep study does not automatically increase data quality.

Several factors and processes influence the quality and structure of human sleep, primarily duration of prior wakefulness, circadian phase and the environmental light–dark cycle (for a review, see [3]). To thoroughly investigate and quantify the contribution of each of these influences on sleep, studies need to be carefully designed with the aim of controlling for each factor. Thus, to investigate a potential rhythmic influence on sleep retrospectively, it is essential that the examined cohort (study volunteers) was studied under very controlled conditions. For instance, light affects our circadian rhythms and in turn our sleep more powerfully than any drug. Consequently, synchronizing study volunteers to the 24-hour light–dark cycle according to their own preferred sleep–wake timing is a requirement for any sleep study which aims at quantifying sleep measures. Chronobiologists call this ‘enforcing circadian entrainment’, which is a *sine qua non* for proper quantification of the influence of the circadian process and prior wakefulness on sleep. We also consider this a necessary prerequisite when carrying out post-hoc analyses of the potential impact of rhythmic phenomena (such as the lunar cycle) on sleep.

To explain this phenomenon with a more allegorical approach, imagine an orchestra with a certain number of musicians. You are trying to recognize a rhythmic characteristic of the music

played by the musicians, but you can only listen to them every 10 minutes for a very short time retrospectively. If the orchestra was not precisely synchronized to the conductor, you will not recognize the melody, even if the number of musicians is massively increased, which does not augment signal quality but instead leads to cacophony. However, if the players (regardless of their number) play tuned synchronously to the conductor (i.e., circadian entrainment), the chance of recognising a melody or a superimposed weaker melody is much greater, even if you sample only every 10 minutes. In chronobiological experiments, therefore, we synchronize study volunteers to the 24-hour light–dark cycle with respect to their own natural sleep timing, to unmask as well as possible the influence of circadian rhythmicity on any variable of interest. If this is ignored, one may probably miss very different rhythmic influences, such as that of the moon, because the signal to noise ratio becomes very weak. That could be the main reason that, although part of folklore, it has up until now been difficult to detect the rather weak influence of the moon on human sleep, since it cannot be revealed just by pooling non-synchronized sleep data.

For future studies, we therefore suggest to carefully control the following variables, and to perform a power analysis to estimate the sample size for a targeted statistical effect: circadian phase, light–dark cycle, circadian entrainment, prior duration of wakefulness, menstrual cycle and age.

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