



Fig. 2. The ranges of free-running periods (abscissa) of 22 species as compiled from the literature (ordered according to sequence in Table). The curve at the bottom summarizes the data (as % of all species) with which each portion of the period regime (20 min classes) is included within the several ranges of free-running periods. For example, the period ranges of 11 species overlap 23.3 h; 11 species 50%.

fraction and the dark fraction. The latter would then suggest a response curve to constant light similar in principle to the well studied response curves to light flashes.

It is obvious that further investigations of both ranges of entrainment and of ranges of free-running periods are necessary before any firm generalizations can be made. Very few experimental studies of ranges of entrainment have been published (BRUCE<sup>28</sup>) and in most of those cases, data are not available on the range of free-running periods for the same organisms<sup>29</sup>.

**Zusammenfassung.** Quantitative Untersuchungen zur Häufigkeitsverteilung circadianer Periodenlängen der Schabe *Leucophaea maderae* zeigen, dass diese Art ein sehr enges Periodenspektrum besitzt. Die lichtbedingten Abweichungen von 24 h betragen im Mittel nur  $\pm 0,35$  h oder 1,5%. Die Befunde werden mit Angaben der Literatur verglichen.

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<sup>28</sup> V. G. BRUCE, Cold Spring Harb. Symp. quant. Biol. 25, 29 (1960).

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## PRO EXPERIMENTIS

### Use of Mixture of Ethanol-*n* Butanol-Xylene in Paraffin Method

During the study of cytology and development of some Indian Rusts Fungi infecting the leaves of their host plants, it was noted that during the monsoon season with high humidity, the transfers of dehydrated tissues in 100% ethanol to xylene, caused turbidity, perhaps due to the absorption of atmospheric humidity by absolute ethanol. While attempting to overcome this difficulty, the author noticed that the ethanol, which normally causes turbidity with xylene in high humid atmospheres, is easily miscible with it only in presence of *n* butanol.

Based on these observations, the author incorporated the following changes using the mixture of ethanol-*n* butanol-xylene, in conventional paraffin method. This method proved good for transferring the tissues of rust-infected leaves, without any turbidity or any other damage to the tissue, neither was the staining quality of the tissue affected. Moreover, the tissue remained soft during the process and the brittleness which is usually associated with xylene was avoided. Furthermore, the author also noticed that xylene is easily miscible with even much lower grades of ethanol, as much as 70% ethanol, in the presence of *n* butanol, without any fogginess being imparted to the mixture.

The use of the following working schedule is suggested to overcome the fogginess usually associated with transfers from 100% ethanol to xylene in humid atmosphere, and

to avoid the brittleness of the tissue usually associated with xylene.

**Working schedule.** The material dehydrated through ethanol grades to 100% ethanol and ready to be transferred to xylene is to be processed as follows: (1) a mixture of ethanol-*n* butanol-xylene (Ebxol) (1:1:1) 6 h; (2) *n* butanol-xylene (1:1) 1-4 h; (3) xylene pure 1 h; (4) xylene pure.

The material is now ready for the further process of paraffin infiltration. The material may be left in the second step of mixture of *n* butanol and xylene but not in xylene pure<sup>1</sup>.

**Zusammenfassung.** Es wird eine für die histologische Technik nützliche Methode beschrieben, um bei der Überführung von entwässertem Gewebe aus absolutem Alkohol in Xylol bei hoher Luftfeuchtigkeit Trübungen zu vermeiden.

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