

#### Supplement 4. Thermal responses during F-0 in *L. dubia* and *E. cynosura*

Also with respect to temperature, responses within the final stadium of spring species can show a similar variation as that dispersed to several stadia in other species. The combined effects of photoperiod and temperature on duration of two early morphogenetic substages in regulatory development within F-0 in *L. dubia* are shown in Figure S4. These substages precede the preferred overwintering substages (Norling, 1976). The photoperiods (including Civil Twilights), although constant, were initially simulating an early entry into F-0 (mid-late July, LD 19.3:4.7), and a very late one (late September, LD 13:11), much as seen in the field (Figure 1f). This corresponds to the slow leading edge (strong diapause component) and the fast trailing-edge (weak diapause component) of regulatory development, respectively, leading to convergence on a preferred overwintering stage. This is analogous to the development seen in semivoltine and univoltine F-3 and F-2 before the autumn cohort merging in F-1 in *C. puella* (Figure 1c). There is a distinct correlation between the high diapause intensity in long days and an inverse temperature response (increase in substage duration) between 15 and 25°C. The duration of post-apolysis development, always non-diapause, instead decreased to one third in this temperature interval (from 21.5 to 6.5 days), perhaps generally valid for non-diapause development.

In *Epitheca cynosura* (figure 2, p. 373, in Lutz, 1974a, <https://doi.org/10.2307/1935224>), the relationship of thermal response and diapause intensity was remarkably similar to *L. dubia* in two experiments with larvae collected in F-0 on 19 October and 21 December and tested in LD 11:13 and 14:10 at 15, 20, 25 and 30°C. At the source latitude of 36°N these photoperiods equal late November and mid April, respectively (estimated from Beck, 1968, p. 4; periods of Civil Twilight included). So, the responses (time from collection to emergence) should rather represent a premature thermal spring at winter and spring photoperiods. These larvae already had some natural short-day exposure, and in the December sample also some exposure to diapause terminating low temperatures. This has probably contributed to an observed faster development in all treatments in the latter group, most likely assisted by a more advanced interecdysis stage (cf. *L. dubia* in box on p. 3). Evidently the larvae were essentially reset to pre-emergence responses to long days but still in diapause at the start of experiments and not yet in a spring condition. The remaining diapause was still maintained by the winter photoperiod. Development time at the shorter photoperiod was thus the longest (average minimum 175 days at 20°C in the October sample, and 65 days at 25°C in the December sample), and it increased with temperature in the warmest 1 – 2 intervals as in the higher diapause intensities in *L. dubia*.

The larvae seemed to require additional photoperiodic activation, delaying complete diapause termination even in the long days, and the rate of development was still rather low (see box). The long-day temperature response above 15°C was almost or totally flat. Average time to emergence in the October sample was here c. **50** days (**60** at 20°C, **115** at 15°C), and **25** days in the December sample (**67** at 15°C). This relatively slow and flat response may partly depend on the LD 14:10 photoperiod. This mid April photoperiod may have had a somewhat attenuated and delayed diapause terminating effect (see main text; Hodek, 2002; Tauber et al., 1986, p.127 – 130), in particular for the October sample. This may be regarded

as a more elaborate version of the explanation offered by Suhling, Suhling & Richter (2015, p. 24). Thus, entirely non-diapause responses may not have been present, but they may have been in the spring treatment in figure 1 in Killian & Lutz (1985), with development at 25 and 30°C distinctly faster than at 20°C. Also other thermal responses described by Lutz (1974a, b) generally suggest similar interactions of diapause and temperature, although he cautiously did not use the term diapause.

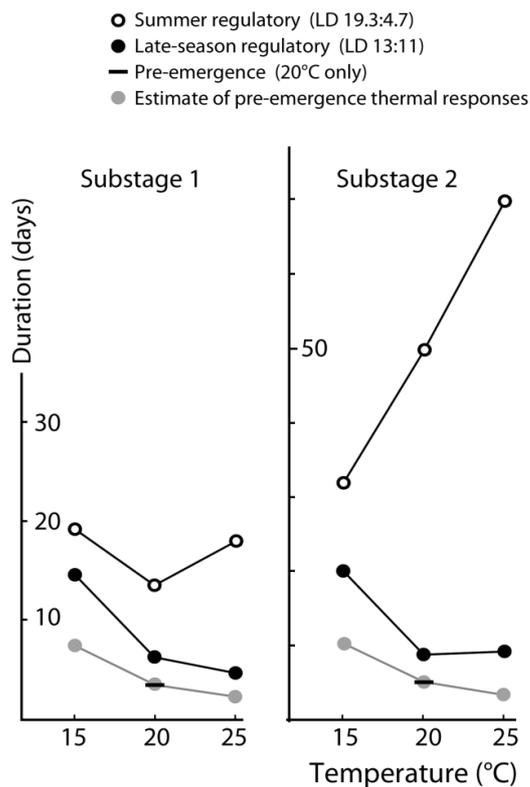


Figure S4. Thermal effects on development in the F-0 stadium in different photoperiods in a spring species, *L. dubia* from 58°N. Graph redrawn and simplified from Norling (1976, figure 4 on p. 253). Durations of two consecutive substages are shown, defined from the mesial expansion of the adult eye rim. Together they comprise the first 1/3 of F-0 if it is nondiapause (3.5 and 5 days, respectively, of totally 25 days, recorded at 20°C in one rare entirely pre-emergence individual; cf. also Corbet, 1999, figure 7.6 on p. 215). Larvae entered the experimental photoperiods before reaching F-0 from active development in earlier stadia. The likely non-diapause thermal responses (grey symbols) are estimated from thermal responses in the always non-diapause post-apolysis part of development, which at the used temperatures were the last 21.5, 10.5 and 6.5 days respectively. For full details, see Norling (1976). For comparison with *E. cynosura*, see <https://doi.org/10.2307/1935224>, figure 2.

### **F-0 responses at 20°C from early autumn to winter in *L. dubia* – possible relevance for *E. cynosura*?**

In true long days (LD 19.3:4.7 or LL) at 20°C, pre-emergence F-0 *L. dubia* was faster than F-0 *E. cynosura* in the described experiments. Total F-0 duration can be **25** days (cf. text to Figure S4). Time to emergence for September-collected F-0 was **19–32** days (Norling, 1976, figure 5c, cf. also August in 5b, l), and for cold-treated “winter” larvae **11 – 26** days (also including larvae from 68°N; Norling, 1976, figure 5d; 1984b, figure 8c). The variation was clearly correlated with interecdysis stage, but probably also with cold treatment, exact photoperiod and source population. Most overwintering F-0 from 58°N emerged after **15 – 20** days at 20°C. At 25°C times to emergence should be c. 60% of those at 20°C if post-apolysis thermal responses apply, in the latter case 9 – 12 days, and never longer than 20 days.

In short days (mostly LD 13:11, but also 19.3:4.7 for larvae from 68°N), larvae overwintering in early interecdysis stages remained in diapause, but the most advanced ones had largely completed diapause and emerged as in long days or showed a more moderate intermediate delay (Norling, 1976, figure 5g; 1984b, figure 8a, b). At 58°N, LD 16:8 appeared as a short-day photoperiod in September experiments, but elicited a long-day or intermediate response in winter larvae (Norling, 1976, figure 5h, i).

Interecdysis stage was unknown in the *E. cynosura* experiments. Since the final moult was supposed to be triggered by equinoctial photoperiods, and taking place in late September to middle October (Lutz, 1974a, b; cf. Figure 1e), many larvae were probably rather freshly moulted in the October sample and so comparable to the slowest *L. dubia*, which nevertheless were twice as fast in long days as average LD 14:10 *E. cynosura* at 20°C.

In the December sample, the larvae could be expected to have performed some amount of morphogenesis as also seen in winter specimens of other spring species in Sweden (e. g. own observations of *Libellula* spp., *Cordulia aenea* and *Coenagrion armatum*). Together with additional diapause development at low temperatures, a more advanced interecdysis stage may have contributed to a shorter time to emergence in the December larvae, as shown for *L. dubia*. Also an effect of changes in photoperiod at the start of experiments is possible. Taken together, an average time to emergence of 25 days at both 20, 25 and 30°C in LD 14:10 for the December sample appears long and suggests some remaining diapause.

### **References not present in the main text**

Beck, S. D. (1968). *Insect photoperiodism*. New York: Academic press.

Killian, J. C., & Lutz, P. E. (1985). Photoperiodic and thermal effects on nymphal development in *Epithea* (= *Tetragoneuria*) *cynosura* (Odonata: Libellulidae). *Environmental Entomology*, 14, 142 – 145. <https://doi.org/doi:10.1093/ee/14.2.142>