or supernumeraries in *B. maxima*. Chromosome No. 14 of *B. maxima* frequently had a secondary constriction (fig. 1), a marker for chromsome No. 7 in the other fire-bellied toad species[6–8, 10].

Heteromorphic chromosomes were not observed. We studied 8 embryos so it is unlikely that all of them were of the same sex. Male meiotic chromosomes (fig. 2) revealed interstitial chiasmata: one per chromosome arm. This is typical of the discoglossids.

Discussion. Apart from the chromosome number and the localization of the secondary constrictions, the chromosome set of *B. maxima* does not significantly differ in overall chromosome morphology from other species of *Bombina*. According to Morescalchi[5], karyological evolution in the discoglossids has led from a primitive, 'ascaphid' karyotype (i.e. *Alytes*) rich in acrocentrics with few microchromosomes, to a 'higher' type with few chromosomes all of which are bi-armed (i.e., *Bombina*). Karyotype evolution was accompanied by a loss of microchromosomes and acrocentrics resulting in a reduction in the total number of chromosomes. *B. maxima* ($2n = 28$) has a higher chromosome number than *B. bombina, B. variegata* or *B. orientalis*. These three species have $2n = 24$. However, as there are no acrocentrics in *B. maxima,* karyotypes of the latter species cannot be derived from the former by simple chromosome fusions. Therefore it is more reasonable to assume that *B. maxima* on one side and *B. bombina, B. variegata* and *B. orientalis* on the other represent two separate lineages within the genus, in which chromosome evolution proceeded independently. A common ancestor of all the *Bombina* species probably had a primitive karyotype with many acrocentrics.

To Mertens[14] and Bolkay[15] *B. maxima* was a close relative of *B. variegata,* whereas *B. bombina* was akin to *B. orientalis*. Present karyological data do not support such a relationship. Results of immunological[12] and electrophoretic[11] studies as well as artificial hybridization experiments[16] demonstrate that the European species, *B. bombina* and *B. variegata,* are the closest pair within the group. The East-Asian species are distantly related to them yet *B. orientalis* is closer to the European pair than it is to *B. maxima*. This relationship is also supported by the present chromosome analysis. *B. bombina, B. variegata* and *B. orientalis* have karyotypes practically identical to one another[7]. The split between *B. orientalis* and the European *Bombina* is estimated to have taken place in the Miocene, about 8–12 Myr ago[12]. Chromosome evolution in this lineage of small-bodied fire-bellied toads has been extremely slow and proceeded at a slow pace typical of the anurans in general[17]. If the same is true for the other lineage of large-bodied species from the *Bombina maxima* group, we predict that the other two Chinese species: *B. microdeladigitora* and *B. fortinuptialis* should have karyotypes very similar to, or indistinguishable from that of *B. maxima.*

Acknowledgments. We thank Dr J. W. Arntzen for help in obtaining the animals. This work was supported by a grant from the Polish Academy of Sciences (project CPBP 04.03) to J. M. S.

1 Estes, R., and Reig, A. O., in: Evolutionary Biology of the Anurans, p. 11. Ed. J. L. Vial. University of Missouri Press, Columbia 1973.
2 Lynch, J. D., in: Evolutionary Biology of the Anurans, p. 133. Ed. J. L. Vial. University of Missouri Press, Columbia 1973.
3 Duellman, W. E., and Trueb, L., Biology of Amphibia. McGraw-Hill, New York 1986.
4 Olmo, E., Morescalchi, A., Stingo, V., and Odierna, G., Mon. zool. ital. (N.S.) *16* (1982) 283.
5 Morescalchi, A., in: Cytotaxonomy and Vertebrate Evolution, p. 233. Eds A. B. Chiarelli and E. Capana. Academic Press, New York and London 1973.
6 Wickbom, T., Hereditas *35* (1949) 33.
7 Morescalchi, A., Boll. Zool. *32* (1965) 207.
8 Krzywda-Gałecka, U., Genet. Pol. *23* (1982) 151.
9 Sato, I., J. Sci. Hiroshima Univ. *6* (1938) 53.
10 Ueda, H., Sci. Rep. Lab. Amphibian Biol. (Hiroshima Univ.) *4* (1980) 185.
11 Szymura, J. M., in preparation.
12 Maxson, L. R., and Szymura, J. M., Amphibia-Reptilia *5* (1984) 245.
13 Gosner, K. L., Herpetologica *16* (1960) 183.
14 Mertens, R., Z. Morphol. Ökol. Tiere *11* (1928) 613.
15 Bolkay, S. T., Glasnik Zem. Muz. Bosn. Herceg. *41* (1929) 1.
16 Uteshev, V. K., and Borkin, L. J., Zool. Anz. *215* (1985) 355.
17 Wilson, A. C., Sarich, V. M., and Maxson, L. R., Proc. natl Acad. Sci. USA *71* (1974) 3028.
18 Levan, A., Fredga, K., and Sandberg, A. A., Hereditas *52* (1964) 201.

0014-4754/88/060521-03$1.50 + 0.20/0

Insect photoperiodism: various ways of regulating univoltinism in lacewings (Planipennia: Chrysopidae)

M. Canard and A. Grimal

Université Paul-Sabatier, Laboratoire d'Entomologie, 118 route de Narbonne, F-31062 Toulouse Cedex (France)
Received 2 November 1987; accepted 23 February 1988

Summary. Reproduction in *Tjederina gracilis* adults depends on short-day followed by long-day conditions. In *Nineta pallida,* development of overwintering first-instar larvae is retarded by short days; later on, that of second- and third-instar by long days. *Nineta flava* and *Chrysopa perla* prepupae automatically enter diapause, but in *N. flava,* photoperiod regulates diapause termination, and long days may retard egg laying.

Key words. Photoperiodism; dormancy; diapause; voltinism; overwintering; seasonal adaptation; Neuroptera; Chrysopidae.

The developmental strategies of insects in the temperate zone are variable, ranging from one generation every two years[1] up to 7 or 8 generations per year along the Mediterranean border of the tropical zone[2]. Whichever strategy of voltinism is chosen, the cycle has to be well synchronized with seasonal changes. This synchronization is most often established by perceiving variations in daylength, a perfectly repetitive geophysical signal. This cue, traditionally called 'photoperiod', is shown here to influence development in some Palearctic lacewings[3]. Four univoltine European species exhibiting different strategies were compared: *Chrysopa perla* (L.), *Nineta flava* (Scopoli), *Nineta pallida* (Schneider) and *Tjederina gracilis* (Schneider). All populations studied originated from the South of France, the southern extreme of their distribution.

Methods of investigation and terminology. All the comparative trials resulting in measures of duration were made in the laboratory at 20 or 21 ± 1 °C depending on the experiment. The insects were exposed to low intensity artificial light (ca 500 lux). The alternation of light and dark (L:D) was of the all-or-none type; the accuracy of illumination and extinction was within ±5 min per day. The various photoperiods used are globally referred to here as: a) short days (SD) when the photophase was 8 h or less, – b) medium days (MD) when the photophase was 12–14 h, – and c) long days (LD) when it was 16 h or more.

We call here primary dormancy that which coincides with overwintering, and secondary dormancies those which result in additional developmental pauses or arrests. An environmental factor is termed tachytelic[4] when it produces development at the fastest rate.

Results. In four species tested, overwintering is assured by three different methods. *Ch. perla* and *N. flava* overwinter as motionless full-grown larvae (prepupae) within the cocoon, in a state commonly considered an 'arrest of development'. In contrast *N. pallida* hibernates as first-instar larvae whose growth is slow but nonetheless continuous[5], whereas *T. gracilis* overwinters as adults whose reproduction is inhibited. Each species exhibits some alterations of development which qualify as dormancy in the broad sense[6].

Triggering of primary dormancy. In *N. flava*, the development arrest which occurs in larvae just after spinning the cocoon appears in the course of each generation, no matter what the daylength. All artificial photoperiods tested in the laboratory, as well as naturally increasing daylength (end of spring) or decreasing daylength (end of summer), produced the same result: the prepupae enter a diapause (parapause[6]) of strong intensity[7].

The phenomenon is qualitatively the same in *Ch. perla*; diapause invariably occurs in 75% of the population[8]. The other individuals, which are potentially multivoltine, exhibit the eudiapause[6] syndrome: they enter diapause in response to photoperiod during the embryonic and larval stages at a threshold close to L15:D9[9]; their sensitivity (minimal time necessary to enter diapause) increases until the third (last) larval instar. That a strictly univoltine strain has been selected in the laboratory after only 3 or 4 generations shows that this variability in primary dormancy involves genetic factors.

In *T. gracilis*, all experimental rearing of preimaginal stages under either constant or decreasing daylength has resulted in adults: a) having a very long lifespan, and b) whose reproduction is blocked, i.e., in reproductive diapause[10].

Finally, in *N. pallida*, the developmental delay of young overwintering larvae is conditioned by daylength which acts from the beginning of embryogenesis. This delaying effect varies with photoperiod; it is strong (longest) in SD, with a maximum at L12:D12, while LD result in shortest development of the first instar. The threshold for tachytely is around L15:D9[11].

Maintenance and termination of dormancy. In the absence of a specific reactivator, the prepupae of *Ch. perla* and *N. flava* remain in diapause until death, which can occur after a very long survival time of up to 18 months. We have not achieved activation by the light regime alone. Only prolonged periods at low temperatures are effective; for example, the reactivation of half the population of *Ch. perla* requires a 4-month stay at 4 °C[9]. A spontaneous resumption of development can nevertheless take place very rarely among individuals of this species (less than 1%), but only after 12 months or longer.

In *N. flava*, the date of pupal ecdysis (first visible manifestation of new morphogenetic activity) may vary. It appears to be modulated by the photoperiod to which the growing larvae were exposed during the preceding year. In experiments intended to demonstrate this, larvae were reared in the laboratory at 20 °C under either LD or SD; all the resulting cocoons spent the entire winter outdoors. The prepupae from SD-reared larvae ended their diapause about one month earlier than their sibling prepupae bred previously as larvae under LD[7].

The conditions of photoperiod which induce ovarian diapause in *T. gracilis* are not always sufficient to maintain it in the laboratory. Spontaneous oogenesis has sometimes been observed after 1.5–3 months if the photophase was constant, but only after 4 months when diapause was induced by transferring individuals to conditions of decreasing photoperiod. One can even avoid ovarian diapause by transfers from SD or MD to LD, after the end of active larval life. Similarly, an exposure to SD, during either the pupal stage or the first month of adult life, triggers ovarian activity[10].

Because dormancy in *N. pallida* is merely a slowing down of growth, one cannot really speak of its maintenance or termination. However, mortality after 3 months is higher under L12:D12 than under LD (about 30% vs none). Such a difference may indicate that a part of the population is unable to break dormancy without subsequent exposure to LD. Surviving larvae always continue development without hindrance: no specific reactivation factor is needed to break dormancy.

Possibility of a photoperiod-mediated secondary dormancy. A secondary dormancy induced by photoperiod could be evidenced in the studied population of *N. flava*. It concerns egg production, which can be more or less delayed in certain females. In contrast to many insects, it is the MD and particularly LD that inhibit oogenesis in this species. The pupae and young imagos are the sensitive stages. The response, which is very variable, does not permit a clear determination of a critical value for the duration of the photoperiod[12].

N. pallida is also capable of entering a second dormancy under the effect of long photoperiod, contrary to the situation which exists in the first instar. The larvae of second and third instars, collected in nature during and after spring, take longer to develop in the laboratory when they are subjected to LD[11].

In both *Ch. perla* and *T. gracilis*, no secondary dormancy appears in the natural cycle. However, one can artificially induce in *T. gracilis* a second – indeed a third – diapause of the same nature as that already cited; that is, one can re-inhibit ovarian activity by changing the photoperiod from LD to MD or SD[10].

Discussion. Univoltinism based on a short period of predatory activity in spring or summer following a long 'arrest of development', is a cautious strategy, necessitating an exploitation, brief but intense, of the feeding resources. It involves a demographic structure having a small and relatively stable population size. This strategy is rarely found. Instead, numerous species have evolved alternative solutions, giving their cycle – even if it is annual – more flexibility and adaptability in facing unfavorable external factors.

Among the four lacewings of which we have tried to ascertain the photoperiodic determinants regulating diapause, *Ch. perla* – especially the univoltine strain – is the most independent of photoperiod. Its life-history cycle is not at all (or is only slightly?) bound to photoperiod, either at the induction or at the completion of diapause. Developmental synchronization in this species seems to be governed principally by temperature. However, the genetically based voltinic polymorphism of the population studied allows some members an additional estival multiplication. These further generations would appear to give the multivoltines a distinct reproductive advantage over the univoltines, rapidly leading towards a purely multivoltine phenotype. But com-

Summary of dormancy and voltinism traits in four lacewings whose studied populations are in large part or totally univoltine

Species	Voltinism in population	Dormancy (and relevant stages)	Developmental response to short days	Developmental response to long days	End of dormancy induced spontaneously	by short days	by long days	by low temperature
Ch. perla	Multivoltine	Primary (PP)	D	T	0	0	0	T
	Univoltine	Primary (PP)	0 (diapause)	0	0	0		T
N. flava	Univoltine	Primary (PP)	0 (diapause)	0	T	D	T	
		Secondary (Ad)	T	P	T			
N. pallida	Univoltine	Primary (l_1)	D	T	T			
		Secondary ($l_2 + l_3$)	T	D	T			
T. gracilis	Univoltine	Primary (Ad)	D	D	P	succession of short days + long days		
		Occasional secondary (Ad)	D	T	T		T	

PP, prepupa within the cocoon; Ad, imago; l_1, l_2, l_3, first-, second-, third-instar larva. T, tachytelic (accelerating) effect; D, diapausing (delaying) effecta; P, polymorphism inducing various responses; 0, none.

pensatory – and not yet clear – mechanisms, like high summer parasitization or differential winter mortality, appear to readjust the proportion of the two phenotypes so that they remain constant. Such a scheme is not unique. For example, it has been noted in *Chrysopa formosa* Brauer[13], and one can reasonably assume that it is the same in a number of species called by various authors facultative multivoltines.

At the individual level, the preimaginal development of *N. flava* looks like that of *Ch. perla*: a short period as a free larva followed by diapause as a prepupa. But peculiarity appears at the population level. At first, the photoperiod experienced during larval growth determines the time of the pupal ecdysis in spring, and thus the time of emergence. Later on, LD more or less delay oviposition in certain females, and therefore increase their longevity. At the population level a) adult flight occurs from May to October, b) egg laying is also broadly spread over time, from July to October, c) exploitation by the larvae of the feeding resources is slow. So *N. flava* has the appearance of a multivoltine species, with a nonetheless reduced population[14].

In *N. pallida*, the SD and the LD act successively as quasi-permanent retarders of larval development. The predatory larva persists from the end of one summer (hatching) until the beginning of the next summer (spinning of the cocoon). Its winter activity and high abundance, together with the total larval duration of more than 10 months, make it unusual among the Chrysopidae. Due to these facts, its feeding requirements, qualitative and quantitative, can be considered unique among tree-dwelling Neuroptera. Only after the spring, do *N. pallida* larvae enter into competition with other predators exhibiting a more typical developmental pattern. Adult flight takes place in summer, sometimes extending to the beginning of autumn if the temperature is mild.

Of the four lacewings considered here, *T. gracilis* is the species most dependent on photoperiod. A sequence of LD after SD is a prerequisite for oogenesis; this excludes summer reproduction by the newly emerged imagos. The long critical photoperiod postpones oviposition to the solstice of the following summer. It is thus not surprising that this lacewing is scarce as an adult, because it is exposed for long periods to unfavorable factors.

According to this analysis, a second generation of *T. gracilis* is apparently impossible. Nevertheless, production of an earlier generation may be possible if the development of some of the parental generation is accelerated (early spontaneous termination of diapause), and the F_1 adults emerge before the summer solstice.

Regulation of the annual life cycle, similar in some aspects, was found in the American *Chrysoperla downesi* (Banks)[15]. In effect, a similar succession of relatively short days followed by days at least 4 h longer is prerequisite for the initiation of reproduction. However, the relatively short critical photoperiod permits *Ch. downesi* to reproduce in spring; but its photoinsensitivity until transition to SD prevents it from attaining bivoltinism, even occasionally.

In conclusion, analysis of the photoperiod constraints regulating univoltinism in some lacewings features a diversity of intervening possibilities and the common occurrence of multiple actions, sometimes opposite in their effects. It is probable that within a single species, geographically distant populations use analogous signals differently. In each habitat, the resulting response combined with the action of other cues must lead to an adequate synchronization or entail an inevitable elimination.

1 Canard, M., Ecol. Ent. *11* (1986) 27.
2 Neumark, S., Ilanoth *1* (1952) 54.
3 This paper is a synthesis of previous work, published or in print (see other refs), containing further information on methods and detailed data.
4 *sensu* Hodek, I., in: Diapause and Life-cycle Strategies in Insects, p. 9. Eds V. K. Brown and I. Hodek. Dr Junk Publ., The Hague 1983.
5 Canard, M., Neuroptera int. *3* (1985) 175.
6 Danks, H. V., Insect Dormancy: an Ecological Perspective, pp. 8 + 12. Biological Survey of Canada, Ottawa 1987.
7 Canard, M., Ent. exp. appl. *34* (1983) 111.
8 Canard, M., Ann. Zool. Ecol. anim. *5* (1973) 29.
9 Canard, M., Ann. Zool. Ecol. anim. *8* (1976) 393.
10 Grimal, A., Ent. exp. appl. *47* (1988).
11 Canard, M., in: Biologie des Populations, p. 487. Coll. CNRS, Villeurbanne 1987.
12 Canard, M., Neuroptera int. *2* (1982) 59.
13 Principi, M. M., and Castellari, P. L., Atti Acad. Sci. Ist. Bologna *7* (1970) 75.
14 Canard, M., in: Recent Research in Neuropterology, p. 145. Eds J. Gepp, H. Aspöck and H. Hölzel. Thalerhof Publ. Graz 1986.
15 Tauber, M. J., and Tauber, C. A., J. Insect Physiol. *22* (1976) 331.

0014-4754/88/060523-03$1.50 + 0.20/0