Chapter 6

DIAPAUSE/ DORMANCY

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6.1 INTRODUCTION: MECHANISMS AND DEFINITIONS

Some generalities need to be explained to make the reading of this chapter easier. The adaptive functions of diapause are: (i) to **synchronize** the development of active stages with favourable conditions and (ii) to enhance the **survival** potential during unfavourable periods. The modern definitions were coined by Lees (1955). He defined **quiescence** as direct inhibition of development, caused by the direct effect of ambient conditions (low temperature, lack of humidity), which can be terminated immediately by favourable conditions. **Diapause** is caused by conditions which do not directly prevent development, but which are merely signals of seasonal changes (cues, seasonal tokens; a typical signal is daylength). Thus diapause can begin before the onset of an unfavourable environmental change. For insects Danks (1987) also used the term **dormancy**, previously employed only for vertebrates and plants, to cover all states of suppressed development (i.e. both diapause and quiescence). Additional terms were coined to emphasize specific types of diapause (for details and relevant references see Hodek 1996, Saunders 2002).

A system of terms can never encompass the striking complexity of diapause mechanisms. Moreover, the same individual has the potential for alternative ways of arrest or resumption of development; this is clearly seen in *Chilocorus bipustulatus* (6.2.13). Therefore most authors use the term 'diapause' in a broad sense for any **adaptive** arrest of development which is accompanied by behavioural, structural and biochemical changes in insects, i.e. other than quiescence. The **variation in the ecological mechanisms** which govern the induction, maintenance and termination of diapause have to be a focus of our studies. For discussion of the period sensitive to diapause induction see 6.2.3.

Similar to the antithetic definitions for quiescence and diapause, there is a contrasting definition of the two basic types of diapause. If the potential for diapause is not realized in each generation in multivoltine (i.e. producing several generations per year) species or populations, diapause is termed '**facultative**'. A good example is *Har. axyridis* (Roy & Wajnberg 2008; 6.2.9). Its onset is regulated by appropriate environmental cues, e.g. photoperiod – the **photoperiodic response**. The **critical photoperiod** is the daylength that induces 50% response, i.e. in half of the sample diapause is induced, in the other half it is not. It should be stressed that **potentially multivoltine species** may show a univoltine life cycle in regions where the period of suitable conditions is unfavourably short. By contrast, '**obligatory**' diapause is entered by virtually every individual in each generation of the so-called obligatory univoltines, **regardless of the environment** (Lees 1955).

The original definition of obligatory diapause has become outdated by experiments with changing photoperiods. Changing conditions (e.g. exposure to short days followed by long days) for insects entering 'obligatory' diapause under, for example, a constant photoperiod in the laboratory, may be a prerequisite for their development. Zaslavskii (1970) demonstrated such phenomenon in *Chil. bipustulatus* (6.2.13). Changing photoperiodic regimes have not yet become a regular experimental procedure; we can expect that many examples of diapause currently regarded as obligatory may in future be considered facultative. A study of populations from northern Europe indicates this possibility for *C. septempunctata* (Semyanov 1978a; 6.2.1.3). It is therefore advisable not to restrict oneself to the use of constant photoperiods when studying the regulation of diapause.

6.1.1 Hibernation and aestivation

For quite a long time it is mostly hibernation diapause (induced principally by short days and low temperature) that has been studied, probably because in earlier studies diapause was thought of only as an adaptation to surviving sub-zero temperatures. Later, aestivation diapause was recognized, where the inducing environmental cues are long daylength and high temperature. While hibernation diapause can be completed spontaneously without any change of conditions, aestivation diapause is often terminated by a decrease in daylength and temperature (Masaki 1980). *Coccinella septempunctata brucki* shows this type of diapause in central Honshu, Japan (6.2.2.1). Typically, aestivation diapause serves to bridge the periods of drought in subtropical regions. Until recently it was wrongly assumed that diapause is superfluous in the tropics, but many cases have been quoted by Denlinger (1986), such as that of *Stenotarsus rotundus*. Some ethological and ecological traits of diapausing

S. rotundus are similar to those of coccinellids, but also changes in moisture operate in diapause regulation (Hodek 2003 and references therein).

6.1.2 Termination/completion of diapause

Ideas concerning the end of diapause have developed substantially. The classical concept assumed that the prerequisite for the termination of diapause was an exposure to 'chilling', i.e. low temperature above zero. The earlier variation of this concept contained the assumption that the inhibition of development was 'broken' by exposure to cold, whereas in the second variation Andrewartha (1952) stressed that at low temperature, often in the range from 5 to 10°C, a gradual **diapause development** ('physiogenesis') must be completed before normal development (morphogenesis) can be resumed. Because Andrewartha's assumption that 'chilling' is a prerequisite for the completion of a 'healthy' physiogenesis was later overgeneralized, the possibility that the programmed course of events might be modified by environmental signals such as an increase in temperature, was often neglected. In fact, there are **multiple pathways to diapause completion**; at least two should be considered here. Apart from **horotelic** processes of diapause development, diapause can be completed by **tachytelic** processes where the insects are **activated** by some environmental stimuli (Hodek 1983). According to Henderson et al. (1953), horotelic means 'evolving at the standard rate', while tachytelic means 'evolving at a rate faster than the standard rate'. Such a distinction has also been made by Danks (1987, 2001) when he speaks (1987, p. 397) about internal (genetically programmed) and external (environmentally determined) mechanisms.

Under natural conditions, the prerequisites for both diapause development and activation are usually fulfilled by seasonal changes. For example, in autumn and winter the development of hibernation diapause can proceed during the period of short daylength and low temperature. Vernal activation coincides with an increase in photoperiod, light intensity and temperature, the appearance of essential food (Chapter 5.2.11) and sometimes the arrival of rains. Chilling is not a prerequisite for the completion of hibernation diapause in many species (reviewed by Hodek & Hodková 1988). **Low temperatures** are important; they (i) conserve metabolic reserves, (ii) prevent a premature resumption of post-diapause morphogenesis and thus synchronize the life cycle and (iii) can provide a contrast to later increases in temperature, so that they are a component of the activating stimulus.

6.1.3 Phases of dormancy

In temperate zones diapause usually covers only the first periods of hibernation or aestivo-hibernation. In mid-winter or even in autumn, the potential for the resumption of development is already recovered. This **post-diapause** phase is, in fact, **a mere quiescence** because development is inhibited only environmentally (mostly by low temperature) and may be resumed in the laboratory. As stressed above by Andrewartha's term 'physiogenesis', diapause is a **dynamic state**. Insects undergo a series of changes in the course of diapause and only some of these are well known. There are conspicuous **ethological** changes, often a migratory phase in pre-diapause and end of dormancy (undertaken by coccinellids), changes in photo- and geotaxis, etc. **Physiological changes** include: (i) diapause intensification soon after its onset; (ii) decrease in diapause intensity due to the progress of horotelic processes of diapause development; (iii) consumption of food reserves accumulated during pre-diapause; (iv) dynamics of cryoprotectants (trehalose, polyols, antifreeze proteins) and ice nucleators, regulated both endogenously and by environmental changes (6.4.4); (v) a change in temperature prerequisites. The final phase of diapause and an early phase of post-diapause morphogenesis overlap.

Photoperiodic activation occurs in many insects (see e.g. Tauber et al. 1986, Danks 1987, Saunders 2002). As well as a change in temperature and/or photophase, both disturbance and injury can **terminate diapause** or at least accelerate its termination (Hodek et al. 1977).

6.1.4 Endocrinological aspects of adult diapause

Presumably exogenous stimuli influence the **neurosecretory cells of the brain** which are the prime movers of the neuroendocrine system. In the ecophysiological analysis of adult diapause in coccinellids, it is helpful to have a general idea of the underlying endocrinological pathways: (i) Environmental signals are perceived by **receptors** (photoreceptors are located in the brain or in compound eyes). (ii) Evaluation and storage of the information involves a **photoperiodic clock**; the exact nature of these processes is not yet fully understood. A recent survey is given in Saunders (2002). (iii) The stored information is transmitted to neuroendocrine organs; in adults these are neurosecretory cells of the brain, the ***corpora cardiaca*** (CC) and ***corpora allata*** (CA). The neurosecretory material is released from the CC where the axons of the neurosecretory cells terminate. The CC also contain glandular cells which produce their own hormones. The CA produce juvenile hormones (JHs), whereas the ovaries and possibly other tissues (testes, oenocytes, epidermis) produce ecdysteroids in adults. (iv) The neuroendocrine system controls the expression of diapause at the level of **target tissues** (ovaries, fat body, etc.). The most conspicuous feature of adult diapause is the **suppression of reproductive functions** (maturation of ovaries and probably male accessory glands and mating activity; 6.4.1.3). Metabolic reserves (glycogen, lipids) and cryoprotectants (polyols, trehalose, hysteresis proteins) accumulate and the metabolic rate is reduced. Specific diapause proteins are synthesized instead of vitellogenins (6.4.1.2, 6.4.3). For a survey of hormonal control of diapause (including the associated changes in gene expression) see Denlinger et al. (2005).

6.2 ECOPHYSIOLOGICAL REGULATION OF DIAPAUSE IN COCCINELLIDS

In the early studies, hypotheses were based on observations. While Dobrzhanskii (1922a, b; 6.2.1) (better known under his later spelling Th. Dobzhansky) underrated environmental cues (at least in *C. septempunctata*), other authors in the years 1922–48 acknowledged the involvement in diapause regulation of temperature, humidity and shortage of prey (for details see Hodek 1996).

6.2.1 *Coccinella septempunctata*

Regulation of diapause in this species is complicated, at least in the European populations. In two early studies of populations from the region of Kiev, **Ukraine** Dobrzhanskii (1922a, b) described a **bivoltine** developmental cycle for *C. septempunctata* and assumed a genetically fixed alternation of a generation with unbroken development and a generation entering diapause (details on p. 287 in Hodek 1996). Since then experimental research has indicated a very wide plasticity in *C. septempunctata* (Obrycki & Tauber 1981, Phoofolo & Obrycki 2000, Hodek & Michaud 2008) enabling heterogeneous activity in different fractions of populations (6.2.1.6). The two short studies by Dobrzhanskii (1922a, b) are important, because for the first time imaginal diapause in Coccinellidae was recognized together with its adaptive significance for survival, i.e. enabling the species to withstand a long period of shortage of aphids.

6.2.1.1 Central Europe

Jöhnssen (1930) reported a univoltine cycle for *C. septempunctata* in **Germany**. However, he admitted the possibility of a second generation occurring under favourable conditions, though it would later die out in the egg or larval stage.

In **Bohemia** (50° N, western Czech Republic), the **population in the autumn** was found to consist of **two fractions.** Although in some years aggregations of both sexes of dormant *C. septempunctata* may be found in their hibernation quarters from early August onwards, one can also find actively feeding coccinellids on vegetation with aphids (often on different weeds, such as *Carduus* spp. and Daucaceae) for the whole of September and in early October (Hodek 1962). This agrees with observations by Telenga (1948), who also used to find *C. septempunctata* in the Ukraine both partly dormant and partly feeding at that same season.

The **physiological condition** of these two fractions from Bohemia was determined by dissection immediately after sampling, and after rearing (Hodek 1962; Table 6.1). Whereas the alimentary canal in the sampled **dormant beetles** was empty of food and there were no traces of vitellinization in the ovaries, the digestive tract was full of food in more than half of the sampled **active adults** and 13–20% of females possessed one or more vitellinized oocytes or even eggs. The difference between the dormant and active parts of the population became more striking when beetles from **both fractions** were **reared** for 3 weeks under long-days, at 19–22.5°C and with plentiful essential aphid food. The ovarioles of about 85–90% of dormant

Table 6.1 Difference between two population fractions of autumnal adults of *Coccinella septempunctata* (Hodek 1962).

Date sampled*,†	Condition	*n*	Dissection of sampled or reared adults	Digestive tract		Fat body			Ovarioles with	
				empty	full of food	+	++	+++	germaria only	at least one vitellinized oocyte
18.9.*	active	30	sampled	4	26	3	8	19	26	4
27.9.†	active	20	sampled	9	11	5	3	12	16	4
18.9.*	active	18	reared‡	—	—	—	—	—	2	16
8.8., 6.9.*	dormant	17	sampled	17	0	2	5	10	17	0
8.8.*	dormant	29	reared‡	—	—	—	—	—	26	3
	dormant	25	reared‡	—	—	—	—	—	21	4

*Collected in N. Bohemia, near Louny.

†Collected in S.E. Slovakia, near Kral. Chlumec.

‡3 weeks, long days, 19–22.5°C, surplus of essential aphids.

females remained without any vitellinization, while about 90% of the females collected on plants possessed vitellinized oocytes after this period of rearing (Hodek 1962; Table 6.1). Dissections in **summer** (mid-July) of females collected **outdoors** in central Bohemia (50° N) a fortnight after adult emergence indicated a strong **tendency to univoltinism**: 84–93% of the females entered diapause.

Preliminary experiments

The incidence of diapause was high (65–80%) when small groups of coccinellids were **reared indoors** under conditions approaching those in the field (except for extremes) and with the natural photoperiod of late June and early July. In addition to these experimental small groups dissected, two simultaneous massive cultures of 200 adults each were reared to check the reproduction. A very low oviposition was observed in spite of plenty of essential aphid food and heating with a lamp during cool days. Most adults aggregated in corners of the cages from mid-July, leaving only 5–10 beetles moving about, even though the aggregations were disturbed every day. Only one or two egg batches were obtained per day, and in late October about 80% of females were still alive. Adults from the second generation, reared from the first egg batches, emerged from pupae from mid-August onwards; they remained very active and feeding for about three weeks, but then aggregated after four weeks (Hodek 1962).

The above experiments demonstrated that *C. septempunctata* adults feed before they enter diapause (in contrast to Yakhontov's 1962 assumption for *Cer. undecimnotata*) and that the onset of diapause is not prevented by surplus essential food (in contrast to Hagen's 1962 report on *Hip. convergens*). The data showed the plasticity of the *C. septempunctata* life cycle and a weak effect of daylength on the prevention of diapause, since in some replicates the beetles entered diapause in spite of having emerged from pupae under the longest possible daylength. The slight difference between the outdoor and indoor results suggested the possibility that temperature extremes play some part in inducing diapause.

Attempts at diapause prevention in Slovakia (48° N)

A premature development of the first generation (the adults emerged from pupae as early as mid-May 1968) was employed to assess the **potential for bivoltinism** in the population from a warm region in southwestern Slovakia (Zohor near Bratislava). Almost natural conditions were used: extremes were excluded by the transfer of beetles on cold days to the greenhouse, or into the laboratory at a constant 25°C, into shadow during strong sunshine and indoors for the night. The proportion of diapausing females (i.e. ovarioles only consisting of germaria without any trace of vitellinization) ranged in the replicates between 66 and 90% (average = 79%, n = 184) (Hodek 1973). In spite of particularly favourable conditions (a warm spring, a warm region) the tendency to a univoltine cycle prevailed in a high percentage of *C. septempunctata* females. The last beetles were dissected on the summer solstice, 21 June: enterning diapause could therefore not be affected by the shortening of long days.

Laboratory diapause prevention and selection

A series of experiments were carried out under controlled photoperiod and temperature conditions (Hodek & Cerkasov 1960, 1961, Hodek 1962, for details see Hodek 1996). Cultures were started in five successive seasons (1956–60) with beetles collected from the field after hibernation. The eggs originated from non-diapausing females and thus there was **selection against the tendency to diapause** and a gradual decrease in diapause incidence was achieved. In spite of favourable conditions (i.e. long-day conditions of 16L:8D or 18L:6D, a constant 25°C and an excess of essential aphids), the **incidence of diapause** remained rather high in the first generations, and usually **fluctuated between 60 and 90%**. These fluctuations were apparently related to varying conditions: e.g. the number and origin of specimens starting the culture, and the age of the females founding the progeny. In the subsequent generations a progressive decrease was usually obtained within the first three generations, as in 1956 or in 1958. A steady excess of essential prey, cleanliness and a population density not exceeding 25 pairs in a cage of about 8 l is needed to achieve a very low incidence or even absence of diapause in later generations. Due to long laboratory breeding under constant conditions and inbreeding, the vitality of the beetles decreased, so that in the sixth generation the oviposition period and longevity had decreased to 8 and 45 days respectively (Hodek & Cerkasov 1960, 1961, Hodek 1962). A decrease in diapause incidence down the sequence of generations indicates selection against a tendency for obligatory diapause under long days. Such results are not exceptional in the literature on diapause (Hodek & Honěk 1970). It is assumed that multiple genes control the tendencies for diapause (Tauber et al. 1986; Danks 1987).

Induction of diapause in selected lines

When the tendency to the ‘obligatory’ entry into diapause was selected out, the way was open to study the effect of **environmental factors** on diapause induction (Hodek & Cerkasov 1960, 1961). This was done gradually, with coccinellids obtained from different generations and cultures (Table 6.2). Up to the time that the larvae were transferred to the experimental conditions, they had been reared under the usual

Table 6.2 The incidence of *Coccinella septempunctata* females entering diapause under laboratory conditions (Hodek & Cerkasov 1961).*

Culture/ generation	Age of larvae at experiment onset a (days)	Temperature (°C) (at dark/light phase)	Photophase (h/24 h)	Age at dissection (days)	*n*	Diapausing females (%)
I/7	3	17–18 (D) / 20–22 (L)	12	42–51	15	94
I/7	pupae	17–18 (D) / 20–22 (L)	12	56–62	15	87
I/9	5–8	17–18 (D) / 20–21 (L)	12	24–41	30	87
III/4,5	2–3	18 ± 0.5	12	26–43	20	85
III/7	6–7	18.5 ± 0.7	12	20–37	38	50
I/9	5–7	22 ± 0.5	8	33–35	10	70
II/4	4–5	22 ± 1.0	12	24–34	42	60
I/9	3–7	25 ± 0.5	12	30–33	40	33
I/6	eggs	24–25 (D) / 27–28 (L)	8	24–26	20	10
III/4, 5	2–3	18 ± 0.5	19	26–43	24	13
III/7	6–7	18.5 ± 0.7	19	20–37	46	4

*Before the transfer to experimental conditions the insects were reared at 25°C, 16 or 18 hours photophase; both before and during the experiment an excess of essential food was supplied.

breeding conditions described above. Although the age of the larvae differed in individual experiments, the results are consistent, and separated out the effect of two environmental variables, photoperiod and temperature. The **importance of photoperiod** was **dominant**: diapause was prevented by long-day conditions (19L:5D) even at low temperatures of 18 or 18.5°C in, respectively, 87 and 96% of females. The response of diapause to short-day conditions was considerably **modified by temperature** (Table 6.2). At lower temperatures around 18°C the incidence of diapause reached 85 to 94% (with one unexplained exception of only 50%). An increase in temperature to 22 and 25°C led to a marked decrease in the incidence of diapause, and at temperatures fluctuating between 24–25°C (night) and 27–28°C (day) it amounted to just 10%. No significant differences were found in the incidence of diapause between the lengths of short photophase of 12L or 8L, or between the exposure of younger or older larvae or of pupae to the experimental conditions. The critical photoperiod was not ascertained; >16L:8D prevents diapause.

The central European population of *C. septempunctata* is evidently heterogeneous, but with a prevalence of univoltines (Hodek 1962). As this species is reported as univoltine in northern Europe (6.2.1.3) and obligatory (Dobrzhanskii 1922a, b) or facultatively bivoltine in the Ukraine (Dyadechko 1954), it seems probable that central Europe lies in a **transition zone** (in the sense of Bodenheimer & Vermes 1957) **between** the distribution areas of **uni- and multivoltine populations** (Table 6.3; see also 6.2.1.6 and 6.2.16).

The above studies of diapause regulation in *C. septempunctata* from central Europe did not include experiments with variable photoperiods. However, it has been assumed (Hodek 1973) that obligatory entry into diapause may result when the sequence of long day after short day conditions is not experienced under effective temperatures. Such a sequence may be a prerequisite for maturation and oviposition. In the studies described above, a gradual negative selection for this requirement appears to have been executed. Such an experimental response to the increase in daylength after a period of short days was then reported in populations from northern Europe (Semyanov 1978b; Zaslavsky & Semyanov 1983; 6.2.1.3) and could also operate in central Europe.

Diapause development/photoperiodic activation

The course of diapause development was followed first by **preliminary checks.** While in the August samples only 10–20% (n = 72) of females were activated by transfer to long days and a surplus of suitable aphid prey, in November it was 78–100% (n = 40) (Hodek 1962). To analyze approximately the role of temperature in diapause development, the August samples were also exposed for 3 or 6 weeks to 0, 5 or 12°C before transfer to the above re-activating conditions. The 6 week exposure resulted in 27–56% more reproducing females than the 3 week exposure, and there was no significant difference between the effect of 5 versus 12°C. The temperature of 0°C was the least effective (Hodek 1970; also p. 298 in Hodek 1996).

Table 6.3 Hypothetical condition of *Coccinella septempunctata* populations in early autumn (Hodek 1962).

Activity	Ovaria	Voltinism	Type of diapause	Generation	Origin
Dormant at hibernation quarters	Without vitellinization	univoltine	'obligatory'	1st	(early batches)
Active (feeding) on plants	Without vitellinization	univoltine	'obligatory'	1st	(later batches or slower development)
	(primarily or retrogressed	multivoltine	facultative	1st or 2nd	(later batches or slower development)
	reproductive	multivoltine	facultative	0	(still surviving overwintered adults)
				1st	(early batches)

Table 6.4 Response to photoperiod in *Coccinella septempunctata* females transferred from a dormancy site to laboratory in October (Hodek & Ruzicka 1979).*

		Normal oviposition				Transient oviposition			No oviposition
		after short pre-OP		after long pre-OP					
Photoperiod	***n***	**%**	**duration of pre-OP (days)**	**%**	**duration of pre-OP (days)**	**%**	**duration of pre-OP (days)**	**duration of OP (days)**	**%**
18L:6D	31	74.1	13.6 (9–26)	6.5	38, 52	0†			19.4
12L:12D	32	3	13	25	67.5 (56–72)	9.5	13, 15, 18		62.5

*The experiment was discontinued the 73rd day; the total duration of oviposition (OP) and post-oviposition was not followed.

†Till the 26th day of oviposition, when the females were transferred to short day.

These preliminary experiments established (i) that in **August**, soon after arrival at dormancy sites, the females are refractory to photoperiodic activation; (ii) the decrease in diapause intensity with time, both in the open between August and **November** and in the laboratory; (iii) the temperature of 12°C as suitable for diapause development.

The progress of diapause development was later investigated in more detail by **comparative transfers** to laboratory short- and long-day regimes of October and May samples from hibernation sites. In **October**, when diapause development was not yet completed, the photoperiodic response still controlled the reproductive activity of females from Bohemia, and many more females reproduced under long day (81%) than under short day conditions (36%). This experiment revealed the polyphenic composition of the population: at a short daylength only 3% of females oviposited normally, the other females either showed a much delayed oviposition (mean pre-oviposition period of 68 days) or transient oviposition lasting less than 3 weeks (Table 6.4). Diapause was completed in late winter/early spring; then the maturation of ovaries was prevented only by low ambient temperature. By **May** some maturation occurred due to the increase in temperature in the field and the pre-oviposition period after transfer was therefore very short at both photoperiods (Hodek & Ruzicka 1979).

In general, the above two experimental results from Bohemia are consistent with those from the Paris region (Bonnemaison 1964) and from southeastern France (Hodek et al. 1977; 6.2.1.2) and with findings on other insects (Hodek 1983): (i) diapause development proceeds with time, i.e. the **intensity of diapause** gradually diminishes in the course of late summer, autumn and early winter, and is **completed** in almost the entire population **by December**; (ii) processes of diapause development that end diapause can proceed at 12, 15 and even 25°C, and thus low temperatures near 0°C are not a prerequisite for diapause completion; (iii) the photoperiodic response disappears with the termination of diapause.

6.2.1.2 Western Europe: France

Diapause induction

Although the climate around **Paris** (Ile-de-France, c. 49°N) is different from that of central Europe, the results from a study of diapause in *C. septempunctata* in this region (Bonnemaison 1964) are **similar to** those from **Bohemia** (6.2.1.1). A large proportion (85–95%) of the first generation entered diapause despite favourable conditions (long day of 16L:8D or 18L:6D, 22°C, excess of aphids), under which selection proceeded from 85% diapausing individuals by steps to 40, 20, 5 and 5% in the first five generations to a culture consisting solely of non-diapausing coccinellids by the sixth generation. In the second generation the incidence of diapause was increased by short days or shortage of food. Bonnemaison accepted the hypothesis of a mixture of uni- and multivoltine phenotypes and assumed that immigrants are brought by air movements and human transport. He did not think the development of a second generation in the Paris area possible.

Diapause in males

As explained in 6.4.1.3, diapause in coccinellids is not connected with inactivity of the tissues of the testicular follicles. Because of difficulties of method, diapause regulation has only rarely been studied in coccinellid males (Hodek & Landa 1971, Ceryngier et al. 1992, 2004) and accessory glands were not included in the analysis. From knowledge of other insects, one might expect diapause to result in inhibition of the accessory glands and the copulatory aptitude of males. When Bonnemaison (1964) reared emerged males of the fourth selected generation of *C. septempunctata* under 12L:12D and 14 or 18°C for 15 or 25 days, he could not find these symptoms of diapause. After transfer to favourable conditions where females were being reared (16L:8D, 20°C), the males copulated after 2–7 days, and the females laid apparently (not specified) viable eggs after 3–23 days.

Diapause development

Near Paris, Bonnemaison (1964) found that diapause lasted 3–6 months in *C. septempunctata* females which had emerged from pupae in early August and were kept at 20–22°C and under a natural photoperiod. In contrast to the Czech results, he failed to activate the diapausing females (probably collected too early between late July and early September) by an exposure to 5 or 8°C for 5, 9 or 13 weeks and by subsequent rearing at 20°C and 16L:8D for 15, 11 or 7 weeks. His findings on the onset of previtellogenesis in females in the field from late September onwards indicate, very similarly to the results from Bohemia (6.2.1.1) some kind of lifting of the diapause inhibition in autumn. In contrast to central Europe, however, the apparently **higher temperature** of the Paris region enabled the **first stage of maturation** to proceed **in the field** and to be recorded by the dissection of field samples, whereas in central Europe the potential for maturation could only be revealed by laboratory rearing (Table 6.1).

As pointed out above, the French population does not differ from the general picture. Diapause was completed **in December** when the **photoperiodic response** was **lost** in almost all adults. This was indicated by almost identical incidence of reproduction and duration of the pre-oviposition period at 18L:6D and 12L:12D photoperiod regimes.

In **southeastern France** (Basses Alpes, c. 44° N), *C. septempunctata* has been reported as univoltine or partly bivoltine (Iperti 1966a). Later, two observations on diapause development were made on this population: (i) a comparison of the coccinellids from a hill (about 600 m) in the plain with those from a high mountain (Cousson, 1512 m), both close to the town of Digne, and (ii) the activating effect of injury. There is a strong indication that the intensity of diapause in early December was greater at the **high altitude** (medians of oviposition delay were 27 days at 12L:12D and 17 days at 18L:6D) than at the **lower altitude** (13 and 10 days, respectively; Hodek et al. 1977). The reason for this difference may be the high incidence of 'obligatory' univoltines in the high altitude dormancy sites, which are usually occupied earlier and by larger individuals (Honěk 1989; 6.3.2.3).

Half of the sample from Digne were **injured** by cutting off the second pair of wings. This treatment shortened the mean pre-oviposition period in the beetles from both altitudes (Hodek et al. 1977). Wounding has been reported in several insect species as a stimulus that decreases diapause intensity (Hodek 1983). It may act directly, through metabolic changes, or indirectly via sensory pathways affecting the neuroendocrine system. A similarly activating effect of parasitization on diapause was observed in another study (Ceryngier et al. 2004).

6.2.1.3 Northern Europe

From **northern Europe,** most authors have reported a univoltine cycle for *C. septempunctata* (Banks 1954 from **England**; Sundby 1968 from **Norway**; Semyanov 1978a from **northern Russia**).

However, in a sample from Helsinki (**Finland**), surprisingly all beetles reproduced in the first generation when reared under constant long day conditions, i.e. they responded like potential multivoltines (Hämäläinen & Markkula 1972). Such a response, never found in central or western Europe, is unexpected so far north and is different from the populations from **northern Russia** (Semyanov 1978a).

Semyanov activated beetles, sampled at the beginning of dormancy, by exposing them to 18L:6D at 25°C for at least 30 days and he considered the reproducing individuals as multivoltines. In fact, activation by photoperiod was involved (similar to that of the Czech *C. septempunctata*; 6.2.1.1). Activation was achieved in 33% in the population from the Khibiny Mountains and 55% in the St. Petersburg population. This may be compared with 81% activated females in the October sample in the population from Bohemia.

Zaslavsky and Semyanov (1983) reported that the 'obligatory' univoltines need a short-day sensitization before exposure to long days in order to reproduce. Semyanov (1978a) executed such experiments with a population (from the Khibiny Mountains on the Kola peninsula) having the **critical photoperiod** (term explained in 6.1) of 17L:7D at 25°C. He used 10L:14D at 20°C or 14L:10D at 25°C for the 'short-day sensitization' and then 20L:4D at 20°C or 18L:6D at 25°C as the long day regime, and achieved reproduction in all individuals. The principal treatises on diapause (Tauber et al. 1986; Danks 1987; Saunders 2002) refer to a short day/long day requirement for diapause prevention. The surprising results mentioned earlier from Finland with the *C. septempunctata* population from the Helsinki region (Hämäläinen & Markkula 1972) could have been produced by a similar sequence of conditions, and the authors may have omitted the information that young adults (or larvae) were kept under short days before exposure to long days.

In experiments on populations from the Pskov and Novgorod regions of Russia, diapause of *C. septempunctata* was intensified by the combined action of short days and the absence of aphid prey (Zaslavsky & Vaghina 1996).

6.2.1.4 Mediterranean region

On the coastal plain of **Israel,** Bodenheimer (1943) recorded reproduction in late September after aestivation diapause that was not induced by a lack of aphids. There *C. septempunctata* develops one complete and one partial generation in spring which is repeated in autumn. Hibernation of *C. septempunctata* is apparently a quiescence rather than diapause in this region.

In **northern Greece** near **Thessaloniki** (about 41°N), the coccinellids aggregate in autumn on mountain tops, although the absence of a photoperiodic response recorded in the sample collected in mid-November (Hodek et al. 1989) would enable them to reproduce also in short days. It cannot be excluded that the populations from Thessaloniki belong to a Mediterranean biotype of *C. septempunctata*, less dependent on photoperiod in the regulation of its life cycle. Preliminary results obtained with the population from southern Spain also indicate this tendency (Hodek & Okuda 1993).

Although no similar aggregation of *C. septempunctata* adults was found in autumn in the **Athens** region, coccinellids resting individually on dry plants or small conifers were sampled on the slopes of hills. The beetles were apparently on their way to hibernation sites. This autumnal behaviour was taken as an indication that winter dormancy also occurs in central Greece, at least in a proportion of the population (Hodek et al. 1989).

This assumption was confirmed by two detailed field and laboratory studies on populations from **central Greece** (Katsoyannos et al. 1997a, b), although some aspects, e.g. natural voltinism and the probability of a second reproduction period in autumn, were not completely resolved. Katsoyannos's (1997b) statement that *C. septempunctata* is a multivoltine species was based on laboratory rearings; in shaded outdoor cages in the Institute's yard, four generations were achieved (Fig. 6.1a, b). While there was evidently a natural photoperiod, the other conditions (continuous feeding on essential prey and protection from direct insolation) were far from what happens in the field. Eggs, larvae and pupae were abundant in the plain only between April and June, and absent in winter. In June, most adults migrated to **mountains**, where they were recorded the whole year except in May, but with immature ovaries and empty guts (Katsoyannos et al. 1997b). That aestivation diapause continues in the open as hibernation quiescence was shown by reproduction after a short pre-oviposition period in late autumn and winter samples transferred from dormancy sites to the laboratory (Fig. 6.2). Field observations therefore indicate a univoltine life cycle in central Greece, but the tendency to obligate univoltinism is probably present in only a low proportion of the population. This is shown by the high incidence of oviposition in the first generation in the vial cultures (Fig. 6.1b). Beetles in outdoor cages spent the winter in dormancy and resumed egg laying in spring (Katsoyannos et al. 1997a, Fig. 6.1a, b).

Iperti (1966a) recognizes *C. septempunctata* as univoltine or partly bivoltine in the **French Riviera**. The first generation only oviposits exceptionally before diapause.

In the mountainous region of Sweida, in **southern Syria** (1400–1500 m) *C. septempunctata* are actively

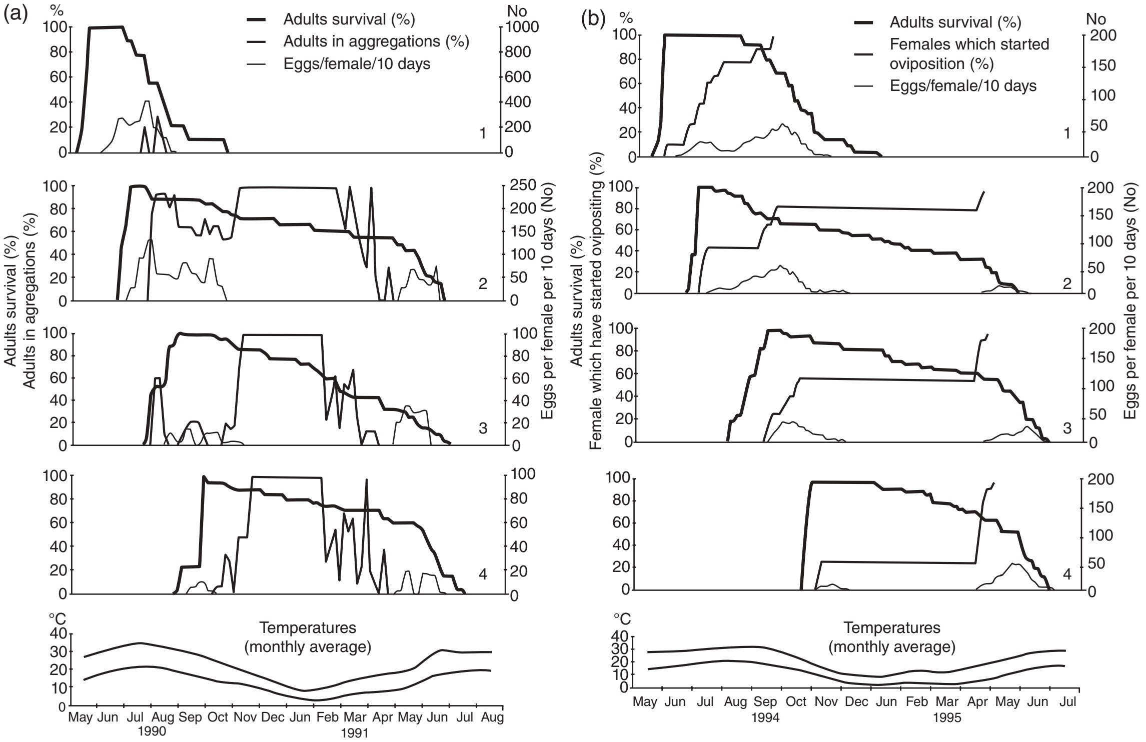

Figure 6.1 Subsequent cultures (1–4) of *Coccinella septempunctata* adults reared outdoors under temperatures (monthly averages of minimum and maximum) indicated below; Kifissia, Athens (from Katsoyannos et al. 1997a). (a) Ladybirds were reared in outdoor cages in 1990–91; (b) Outdoor rearings in vials 1994–95.

preying on *Eriosoma lanigerum* in April and May. The authors recorded aestivation quiescence from late June to early September and no reproduction before the winter diapause (Almatni et al. 2002). They agree with Bodenheimer's (1943, 1957) interpretation, although it is in fact different (see above).

In the region of **Ankara** (**Turkey**), Bodenheimer (1943) did not record much difference from the central-European pattern of aestivo-hibernation terminating in late May and June. Although Bodenheimer (1943) found active adults of *C. septempunctata* in late March and in September, he maintained that there is only one annual generation in the Ankara region. From similar observations in central Europe, the possibility of a partial second generation has been based on dissections and experiments (6.2.1.1; Table 6.1).

Long-term phenological observations also in Italy and Spain would clarify the cycle of *C. septempunctata* in this region. Oportunistic traits may be involved such as those described e.g. in coccinellids from eastern Australia (6.2.10, 6.2.11).

6.2.1.5 Nearctic region

The establishment of *C. septempunctata* in the Nearctic region was first recorded in New Jersey during the years 1973–74, and the species was reported as almost entirely univoltine, with only occasional females producing a second generation (Angalet et al. 1979). Obrycki and Tauber (1981) also found a univoltine cycle in New York State. In a comparison between four populations, Phoofolo and Obrycki (2000) recorded, however, that 47 and 70% of ladybirds from Iowa and Delaware (USA), respectively, laid their first eggs within the first two weeks. These figures agree with those obtained over 5 years in the first generations of Czech populations (6.2.1.1).

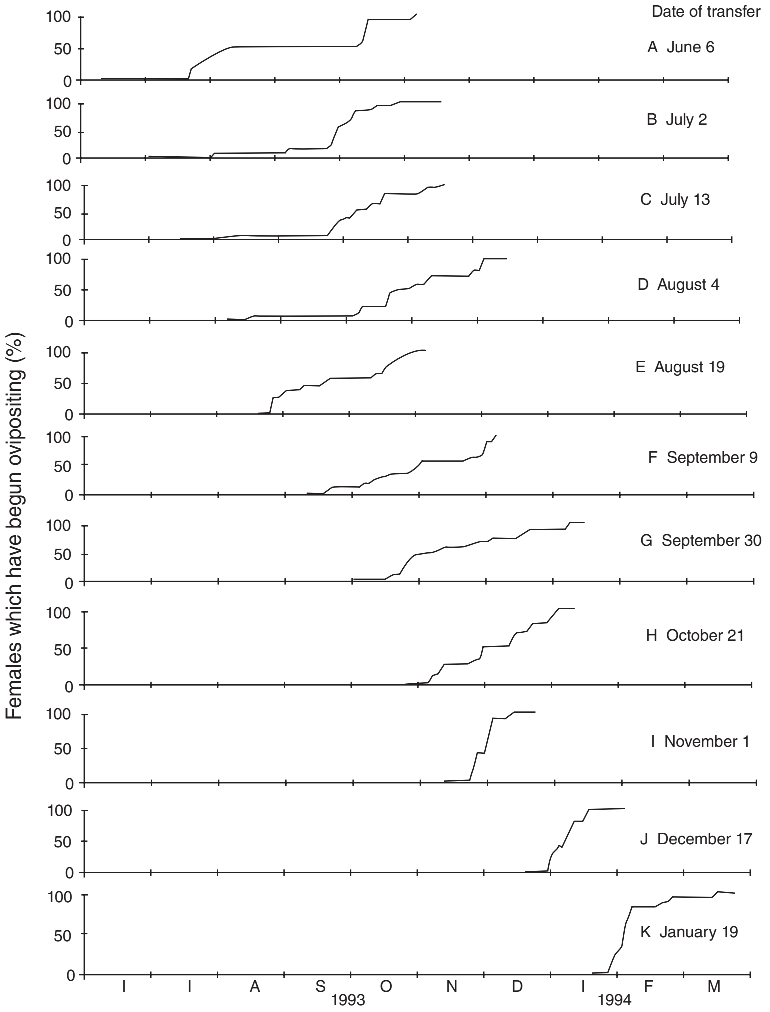

Figure 6.2 Pre-oviposition periods of *Coccinella septempunctata* females collected in aestivo-hibernation from Mt. Kitheron (1993–94) and transferred at different dates (a–k) to 25°C, 16L:8D and presence of aphids in the laboratory (from Katsoyannos et al. 1997b).

6.2.1.6 Potential multivoltines in univoltine populations

It seems remarkable that more than 85 years after the classic Dobrzhanskii (1922) papers, we cannot yet fully answer the question: how is the voltinism of *C. septempunctata* regulated? (See later that this is not different in other species, such as *Hip. convergens*, 6.2.7.) While in temperate climates of Europe and USA we see the scenario of a rare and partial development of a second generation, a similar situation often obtains in the Mediterranean climate, with the exception of the reports from the coastal plain of Israel (Bodenheimer 1943) and an isolated observation from southern Spain (Hodek & Okuda 1993). A bivoltine life cycle with two reproduction periods in spring and autumn, isolated by two dormancies, has also been found in central Japan in *C. s. brucki*, but there it is based on reverse photoperiodic responses (6.2.2.1). The low level of understanding of diapause regulation in *C. septempunctata* is caused by the great plasticity of this species, evolved due to a constant instability of the occurrence of aphid prey (Chapter 5) and evidently maintained by the vagility that prevents the formation of isolated gene pools in individual regions.

To simplify, we may say that researchers bring to the laboratory samples of a bimodal population, composed of 'obligatory' univoltines and potential multivoltines. The proportion of these two fractions of the population varies between localities and most probably also between years. We cannot be sure that our 'favourable' experimental conditions enable the maximum expression of the potential multivoltinism. Some of the conditions used (temperature, prey, population density, relative humidity) may decrease the incidence of reproducing females. Thus, we can only state what proportion of our sample produces a second generation under our experimental conditions. We do not, however, know the maximum proportion of potential multivoltines in a sample of a given population. Anyway, the potential for multivoltinism is only rarely realized in the field.

Phoofolo and Obrycki (2000) carried out a detailed comparison of multivoltine tendencies in four geographically distant populations. Perhaps a two-stage response (Danks 1987) underlies the adult development of *C. septempunctata*. This phenomenon may be similar to the northern population of *Chil. bipustulatus* (Zaslavskii 1970). Therefore the possibility of an 'artificial' multivoltine cycle may be revealed in the laboratory under a short day/long day photoperiodic programme.

6.2.2 *Coccinella septempunctata brucki*

6.2.2.1 Central Japan (central Honshu)

The life cycle of *C. s. brucki* in central Japan is quite the reverse of that of *C. s. septempunctata* as described from Europe and North America. Regulation of diapause was first studied in the regions of Nagoya and Tokyo in central Honshu. The conditions of the Nagoya plain are suitable for reproduction and development of larvae in spring (from mid-April to late June) and again in autumn (in September and October). These periods of active life alternate with periods of developmental arrest, a deep aestivation (summer diapause) and a weak hibernation which is a mere quiescence ((Sakurai et al. 1981a; 6.1). In spite of the natural photoperiod in winter, hibernating adults were quickly activated by a simple increase in temperature to 25°C (Sakurai et al. 1981a, 1982, 1983, 1986, 1987a, b, Okuda & Chinzei 1988).

Diapause induction/prevention

Preliminary experiments indicated that **short days** and low temperature **prevent diapause** in beetles from the Nagoya region (Hirano et al. 1982). A similar but much weaker response was reported in the Tokyo population (Niijima & Kawashita 1982). When the Nagoya coccinellids were reared under short days (11L:13D or 10L:14D) and a low temperature (18°C), the beetles showed a high respiration rate and females oviposited. High temperature (25°C) weakened the diapause-preventing effect of short days: the females oviposited for only 10 days and their respiration rate was low (Sakurai et al. 1987a).

Short days prevented diapause, but not in the whole sample of the Nagoya population. The experimental photoperiod and temperature, which roughly simulated autumn conditions in that region (13.5L:10.5D, 18°C), stimulated reproduction in 15 females (62%) within 36 days, while only one female (5%) oviposited within the 50 days of rearing under control conditions of long days (18L:6D) and high temperature (25°C). Another sample responded similarly to a still shorter photophase (12L:12D) combined with high temperature (25°C): 79% ($n = 29$) females oviposited within 30 days while in the control (18L:6D, 25°C) only 36% females oviposited (Okuda & Hodek 1983, Hodek et al. 1984).

Populations of *C. s. septempunctata* are very sensitive to shortage or lower quality of food and respond by increasing the incidence of diapause (6.2.1.1 and Chapter 5). This phenomenon was also observed in *C. s. brucki* (Niijima & Kawashita 1982, Kawauchi 1985) and might have partially affected the above-mentioned variation in incidence of ovipositing females. Anyway, the same tendency was recorded in all quoted experiments: In central Japan, *C. s. brucki* is a short-day insect and the induction of diapause of the populations from central Honshu is regulated by the reverse photoperiodic response of that found in *C. s. septempunctata* (which is a long-day insect; 6.2.1).

The bivoltine cycle of *C. s. brucki* in central Honshu may be modified if the pupae (and/or eggs) develop in directly insolated artificial microhabitats, such as metal cans or paper and wooden material on slopes exposed to solar radiation. While most beetles of the population hibernate in quiescence, reproductive adults can therefore occur at the same time (Ohashi et al. 2005).

Diapause development and termination

A short-day photoperiodic response also operates in the development and termination of diapause. Adults collected in September in aestivation sites in the Nagoya region were **activated** remarkably well **by autumn-like conditions** (13.5L:10.5D, 18°C) with 36% of the females ovipositing within just a fortnight and 77% within 80 days ($n = 13$). Under long days and high temperature (18L:6D, 25°C), only 18 and 29% of control females oviposited within 14 and 80 days, respectively ($n = 17$) (Hodek et al. 1984).

In contrast to hibernation, aestivation diapause is often terminated by environmental factors (Masaki 1980), i.e. by tachytelic processes (*sensu* Hodek 1981, 1983; 6.1). The aestivation of *C. s. brucki* is thus terminated in the usual way. This laboratory finding needs to be verified by observation in the field; the laboratory results may reflect only one of the possible multiple ways of diapause termination and another pathway may be operating in the natural situation. In general, hibernation diapause can be terminated by photoperiodic activation in the laboratory, but usually this is not the case in nature where diapause ends spontaneously around the winter solstice (Hodek 1971b; Tauber & Tauber 1976).

Life cycle in central Honshu

In the Nagoya plain, the progeny of hibernated females can develop from mid-April to late June and is thus exposed to an increase in daylength from 14 to 16 hours and in average temperature from 15 to 25°C. These conditions ought to induce aestivation diapause in a large proportion of adults. We recorded a high incidence (64 and 95%) of diapause under a constant, but longer daylength of 18 hours. The resumption of reproductive activity in September coincides with shortening daylengths from 14 to 13 hours and with a decrease in mean temperature from 25 to 20°C. Our laboratory conditions, which simulated the autumn in Nagoya, stimulated the resumption of oviposition. Thus *C. s. brucki* from central Japan shows environmental regulation of the life cycle which is the reverse of that of *C. s. septempunctata*. All results indicate that the photoperiodic and temperature responses are directed towards the induction of aestivation diapause.

The reversal of the photoperiodic response might be the consequence of **divergence in allopatric populations** during the process of geographic differentiation which has reached the subspecies stage (Mayr 1970). If the distribution of *C. s. brucki* is limited to Japan, the subspecies probably represents a peripheral isolate (Mayr 1970) whose **first arrivals in the south** of the Japanese archipelago may have been multivoltine individuals predominantly lacking the photoperiodic response. The short-day type of photoperiodic response was then acquired under selective pressure of the climate with hot summers and mild winters. Of course, this speculation relates to just one of several possible evolutionary pathways. An analysis of the problem should begin with the study of photoperiodic responses of *C. s. brucki* (or eventually other subspecies) populations not only from Japan but also from adjacent regions, particularly from southern Korea and Ryukyu.

6.2.2.2 Sapporo, Hokkaido (Japan)

In a population from Sapporo, long-day photoperiod combined with high temperature averted diapause (Okuda & Hodek 1994). This was evidenced both by the relatively fast activation of the entire autumnal sample and by the incidence of reproductive females in the F1 progeny. In long days of 16L:8D and 25°C, all females collected in early September started oviposition within 32 days. Short days of 12:12D combined with 20°C, however, inhibited the reproductive activity of most females from the sample ($n = 34$), so that only three started ovipositing after 10 weeks. In the F1 generation, diapause was averted in 37 and 63% of the females when a long-day photoperiod of 16L:8D was combined with 25 or 30°C respectively. In short days of 12L:12D and 20°C, the beetles of the F1 generation aggregated after a short period of feeding and did not begin to oviposit, at least until they were 68 days old, when the experiment was discontinued (Okuda & Hodek 1994).

In contrast to the populations from central Honshu, these results strongly indicate that the **Sapporo population** of *C. s. brucki* has the **long-day photoperiodic response**. In this respect *C. s. brucki* from Hokkaido is

similar to *C. s. septempunctata* from **central Europe** (Hodek & Cerkasov 1961, Hodek & Ruzicka 1979) and some other European populations (Bonnemaison 1964, Hämäläinen & Markkula 1972, Hodek et al. 1977, Semyanov 1978b). This similarity in photoperiodic response is appropriate to ensure a similar life cycle, i.e. to induce winter diapause in two climatically similar regions with long harsh winters**.**

6.2.2.3 Northern Honshu (Japan)

An intriguing question still remains to be solved: what happens in northern Honshu in the transition zone between the two above populations. A group of Japanese researchers has recently tackled this problem by sampling and dissecting *C. s. brucki* samples in Hokkaido and several regions of Honshu (Ohashi et al. 2003). The absence of summer diapause in Hokkaido and its presence in the plains of central Honshu was corroborated.

At **higher altitudes of central Honshu**, and in **northern Honshu** that is an intermediate area between regions of short-day and long-day populations, the frequency of diapause expression varied greatly among samples from different localities and years. Moreover, diapausing and non-diapausing adults co-existed in 31% of summer samples. The authors rightly concluded that there is genetic variation in diapause tendency within the local populations. They suggest that diapause is prevented there if the average daily mean air temperature in July is lower than 21.5°C (Ohashi et al. 2003).

6.2.3 *Coccinella novemnotata*

The **bivoltine** *C. novemnotata* (McMullen 1967a, b) undergoes diapause twice during the annual cycle. The adults of the **spring generation** pass the hot, dry summer months in diapause and lay eggs in the early autumn. The adults of the **autumn generation** pass the winter in diapause and reproduce in the early spring. In the spring, the teneral adults are subjected to a photophase which increases from 17.5 to 18 hours and in the autumn decreases from about 14 to 13 hours (Fig. 6.3). Temperature and the amount of prey are also involved: Particularly low temperature contributes to the induction of diapause in the autumn generation. The intermediate photophase of 16 hours consistently determines non-diapause development in 90–100% of coccinellids, even when feeding rates are reduced to one-quarter and the temperature ranges between 15.5 and 32°C.

One of the studies (McMullen 1967b) analyses the **stage sensitive to diapause induction** (Fig. 6.4). The conclusion that it is the young adult aged 1–7 days that is sensitive is, however, rather questionable because the percentage of diapause was estimated by the dissection of females when only 14 days old, i.e. only 4–7 days after transfer. In such a short time the processes controlling the maturation or regression of ovaries could not have been completed at 21°C. In order to exclude what seems indeed very probable, i.e. that adults aged more than 7 days are also responsive, it would be necessary to dissect females much later after the transfer. These experiments did not exclude the possibility that, as in other insects diapausing as adults (Hodek 1971a), the pre-imaginal stages are also sensitive to the cues controlling diapause induction. This was recorded in *Chil. bipustulatus* (Tadmor & Applebaum 1971; Table 6.12; 6.2.13). In *C. novemnotata* the sensitivity of pre-imaginal stages is indicated

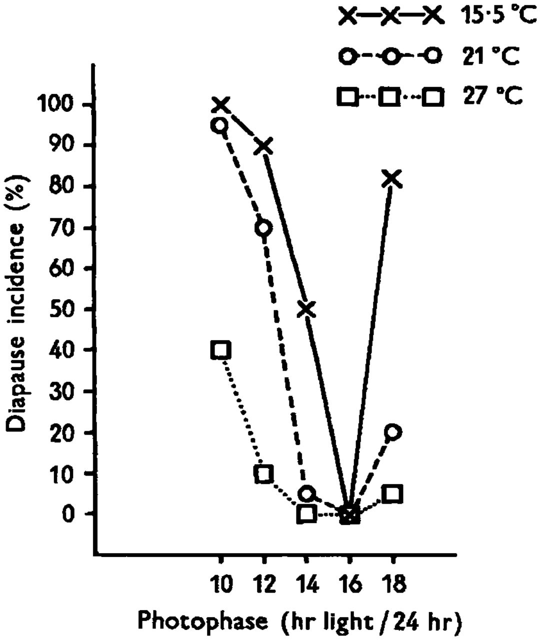

Figure 6.3 Modification of photoperiodic response by temperature in *Coccinella novemnotata* (from McMullen 1967b).

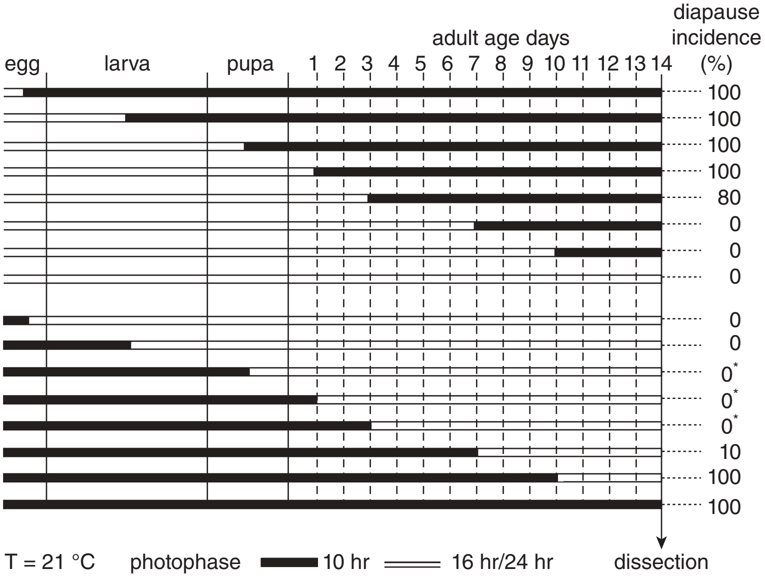

Figure 6.4 Effect of change in photoperiod on diapause incidence in *Coccinella novemnotata*. Females were dissected at the age of 14 days. Under the treatments marked by * ovogenesis in non-diapause individuals was retarded, compared with controls (from McMullen 1967b).

by retarded ovogenesis in females (marked with 'a' on Fig 6.4) which were transferred to a non-diapause photoperiod as teneral or young adults.

The **sensitivity** of earlier stages may be **masked** when later stages are influenced by the **reverse stimuli**; the effect may thus be reversed. The sensitivity of the pre-imaginal stages may be distinguished when the adults are kept under neutral conditions. In fact, we do not search for a specific sensitive stage, but rather for the stage of development in which we can still succeed in preventing oviposition completely (i.e. to reverse the former opposite effect) by a transfer from non-diapause to diapause-inducing conditions. A suitable criterion for the sensitivity of larvae may be the length of the pre-oviposition period in females kept as larvae for different periods of time under diapause conditions (Hodek 1971a).

6.2.4 *Adalia bipunctata*

Due to the low developmental threshold (Chapter 3.6) for post-diapause and non-diapause development ***A. bipunctata*** can start mating and ovipositing early in spring (Hodek 1960, Obrycki et al. 1983, Hemptinne & Naisse 1987) and have a **multivoltine** life cycle. Four generations are reported from the region of Bologna, northern Italy (Bazzocchi et al. 2004). Short daylengths induce diapause, while long daylengths prevent diapause in all individuals. The **critical photoperiod** for diapause induction in the Ithaca, New York, USA (about 42°N) population lies between 13L:11D and 14L:10D at 23°C (Obrycki et al. 1983; Table 6.5). In two samples from southeastern France, Iperti and Prudent (1986) found oviposition in 54 and 85% of females at 13L:11D and 25°C, while at 12L:12D no female reproduced during a period of 4 weeks. Thus, the critical photoperiod of both populations, collected in different continents, appears to be very similar.

Diapause completion does not require exposure to low temperatures. At 23°C, duration of diapause is quantitatively related to photoperiod. The progress of **diapause development** is indicated by the photoperiodic response diminishing substantially in late December and being almost absent in March (Obrycki et al. 1983; Table 6.6).

Table 6.5 Induction and duration of diapause in *Adalia bipunctata* under a series of constant photoperiods (23 ± 1°C) (Obrycki et al. 1983).

Photoperiod	Preoviposition period (x ± SD) (days)	Diapause (%)	Ovipositing females (*n*)
16L:8D	8.4 ± 2.2	0	18
15L:9D	8.9 ± 2.3	0	11
14L:14D*	10.4 ± 2.4 (A)	—	7
	26.7 ± 6.4 (B)	46	6
13L:11D	61.8 ± 16.2	100	13
12L:12D	98.5 ± 8.2	100	11
10L:14D	113.3 ± 18.1	100	11

*Group A females oviposited in about the same time as observed at 16L:8D and 15L:9D, no diapause; group B females took substantially longer to oviposit, weak diapause.

Table 6.6 Pre-oviposition period in *Adalia bipunctata* after transfer from outdoors to various photoperiods (Obrycki et al. 1983).

	Photoperiod				
Sample date	16L:8D	14L:10D	12L:12D	10L:14D	Natural
22 Oct.	14.6 ± 1.6 (9/9)	12.3 ± 3.3 (8/10)	75.0 ± 8.4 (3/9)	144.5 ± 34.6 (2/10)	116.0 ± 37 (4/10)
22 Dec.	A -	10.8 ± 1.8 (10/11)	20.8 ± 7.7 (7/13)	18.0 ± 5.3 (3/10)	21.5 ± 4.9 (2/10)
22 Dec.	B -		117 (1/6)	97.0 ± 10.8 (3/7)	61.3 ± 30.3 (3/8)
7 Mar.	—	7.0 ± 1.7 (6/8)	10.8 ± 3.1 (8/12)	12.0 ± 4.3 (8/11)	12.6 ± 2.7 (7/8)
21 May	—	2.8 ± 1.3 (7/8)	2.5 ± 1.0 (6/6)	2.4 ± 1.0 (7/9)	3.0 ± 1.2 (4/4)

Numbers in parentheses indicate number of ovipositing females per total number of females in each condition. Temperature under constant photoperiods, 23 ± 1°C; temperature under natural daylength, 23 ± 2°C. For the definition of groups A and B see Table 6.5.

Adalia bipunctata has recently **invaded Japan**. Larvae and pupae are found in April and May, while during summer (July, August) the inactive adults are dormant in groups of up to 10 in rolled dry leaves. In autumn some actively foraging and preying adults were observed, but no larvae. In addition to the sites recorded during aestivation, the bark of maple is used for overwintering (Sakuratani et al. 2000).

6.2.5 *Propylea quatuordecimpunctata* and *P. dissecta*

Obrycki et al. (1993) studied the photoperiodic induction of diapause in three populations of ***P. quatuordecimpunctata*** from widely separated geographical regions (Quebec 45° N, Turkey 40° N, southern France 44° N). Little difference was found in their **critical photoperiod** for diapause induction. The authors explained this similarity by the previous 2–4 generations reared under long days. However, the critical photoperiod depends mostly on the latitude, and this was similar in all three regions. At long days and 26°C Phoofolo and Obrycki (2000) recorded in the above three populations, respectively, 83, 56 and 80% of females ovipositing within 2 weeks of emergence, which is a higher proportion of 'multivoltines' than in *C. septempunctata* (6.2.1.5).

In **northern India**, active adults of ***P. dissecta*** are found from February to April and again from August to October (Omkar & Pervez 2000). These activity periods are a little different from those reported for *C. septempunctata* for the same region: December to March and July to September (Omkar & Pervez 2000).

6.2.6 *Hippodamia tredecimpunctata*

Populations of *Hip. tredecimpunctata* from Maine (USA) are bivoltine. At a constant temperature of 21°C, diapause can be prevented in all females by long days of 16L:8D. The photoperiod of 14L:10D (near the critical threshold) enabled reduced (i.e.halved) oviposition in 70% of females. At 12L:12D still one third of females laid eggs for 14 to 69 days. The results were slightly modified by the photoperiod during larval rearing, indicating that also the larvae are sensitive to factors inducing adult diapause (Storch & Vaundell 1972).

Below, the data for **three long distance migrants** are discussed. Fifteen years ago, it was surprising (Hodek 1996) how meagre was the experimental evidence on the regulation of diapause in the three best-known long-distance migrants, the Nearctic *Hip. convergens*, the Palaearctic *Cer. undecimnotata* and *Har. axyridis* from Far East Asia. There were only studies in southeastern France and central Europe on *Cer. undecimnotata* (6.2.8). This situation has much improved in the last decade.

6.2.7 *Hippodamia convergens*

In **northern California,** Hagen (1962) assumed that there were three types of dormancy in *Hip. convergens*. The majority of the population has a univoltine cycle. This is the original pattern, as before irrigation was introduced, the species was dependent on aphids on prairie grasses in the spring. The irrigated crops enable introduced aphids to maintain themselves during the summer and autumn. Some *Hippodamia* spp. (including *Hip. convergens*) have reacted to this later abundance of aphids by reproduction in the summer and multivoltinism. The **multivoltine** adults of *Hip. convergens* enter hibernation in the autumn. Their diapause is induced mainly by **photoperiod and temperature** (Hagen 1962). For the Californian populations, the photoperiod of 14L:10D and 25°C was reported as preventing diapause (Davis & Kirkland 1982).

Hippodamia convergens often joins the other *Hippodamia* spp. in the valley aggregations. Most **univoltine** *Hip. convergens* adults show a **facultative diapause** that appears to be largely **nutritionally induced**. In the laboratory, however, there were occasions when 10–20% of the beetles entered diapause, although conditions were optimal and the beetles were supplied with an excess of essential aphid food (Chapter 5.2.11, for the term). Hagen (1962, p. 305 therein) supposed that strains of *Hip. convergens* exist which possess an obligatory diapause (see the discussion on *C. septempunctata*, 6.2.1.6).

In the **Great Plains region** of central USA, various cases of nutritional regulation of reproductive diapause were analyzed in females of *Hip. convergens* (Michaud & Qureshi 2005, 2006). The **importance of drinking sap** on sunflowers in the summer months in West Kansas was examined in this arid region. Sunflower petioles and pollen as well as lepidopteran eggs were provided to the beetles collected in early June. While these females did not oviposit in the absence of protein food, feeding on eggs of *Ephestia kuehniella* followed by pollen enabled 66% ($n = 171$) of the females to lay viable eggs at a low rate of 6.6 eggs/day. The females, transferred on 14 August to essential aphid food (*Schizaphis graminum*), laid six times more eggs.

These experiments stressed the **adaptive role** of the life cycle in *Hip. convergens* in that it enables survival during arid summer conditions when there is a shortage of the essential food, aphids. In the absence of protein-rich food, the first generation can enter diapause. Another tactic could be to wait in a **state of lowered metabolism** (but less lowered than in diapause) for the re-appearance of essential aphid food, relying meanwhile on alternate foods. Then a switch to intensive egg laying can be quick, as was shown by a short oviposition delay of only 4 or 6–9 days on essential prey (Michaud & Qureshi 2005, 2006). For *Hip. convergens*, we still need to know more about the combined action of food and photoperiod.

In the upper coastal plain of **South Carolina**, diapausing adults of *Hip. convergens* were recorded feeding on eggs and larvae of the moth *Heliothis zea* in spite of a short day photoperiod of 12L:12D, when they were transferred during December/January to temperatures >15.5°C (Roach & Thomas 1991). This may indicate that **diapause development** was already **completed**.

6.2.8 *Ceratomegilla (=Semiadalia) undecimnotata*

Detailed studies in central Greece (Katsoyannos et al. 1997a, b, 2005) have widened our knowledge on diapause of *Cer. undecimnotata*. In one season, five generations were reared in outdoor cages with a surplus of

Table 6.7 Duration of preoviposition period of females of *Ceratomegilla undecimnotata* collected during their aestivo-hibernation from the summit of Mount Kitheron (1409 m) and transferred to the laboratory (25°C, 16L:8D, and presence of aphids) (Katsoyannos et al. 2005).

Collection date	Females collected	Duration of preoviposition (days)				
		Median	Mean	sd	Minimum	Maximum
04-Jul-93	16	92	94	4	89	101
21-Jul-93	15	68	72	9	68	103
04-Aug-93	22	64	64	4	58	68
19-Aug-93	20	42	44	6	40	60
09-Sep-93	17	21	22	7	11	34
28-Sep-93	18	24	30	16	15	66
21-Oct-93	19	29	30	6	21	48
01-Nov-93	18	30	31	3	28	42
18-Nov-93	19	28	29	10	13	46
17-Dec-93	20	17	22	10	17	49
19-Jan-94	16	16	20	7	12	37
30-Mar-94	24	14	15	5	9	29

aphids. It was demonstrated that **diapause** is **facultative** in a part of population: about 30% of females remained immature in the first three generations. Thus the population appears to be **heterogeneous** as regards the **induction** of diapause (similar to *C. septempunctata*, 6.2.1.4 and 6.2.1.6, and *Hip. convergens*, 6.2.7). The results from these cultures corresponds to that from the dissection of samples **from the plain**, where about 40–50% non-reproductive females were recorded from mid-June. At that time most females (70–100% in different years) collected **on mountain summits** were immature (Katsoyannos et al. 2005). Regularly transferring samples from the mountain tops to long days of 16L:8D and prey surplus at 25°C led to activation. Females laid eggs after a gradually shortened pre-oviposition period, that was long in summer (92 and 64 days in July and August, respectively) and decreased to about 20 days in September. This clearly showed the **progress of diapause development**, i.e. a decrease in diapause intensity (Table 6.7). Katsoyannos et al. (2005) plausibly assumed that since late autumn the ladybirds are quiescent rather than diapausing. The transfer experiment indicates that *Cer. undecimnotata* is a long-day insect. The authors believed (apparently wrongly) that diapause of *Cer. undecimnotata* in Greece is induced by long days and high temperatures, because of the above-mentioned 30% of immature females in cages in summer in spite of a surplus of aphids.

The evidence obtained from *Cer. undecimnotata* in France (Iperti & Hodek 1974, Hodek & Iperti 1983) also shows it to be a long-day insect while supporting the possibility that **lack of food** also plays some role in diapause induction. In spite of the important studies made in Greece, the regulation of diapause induction in the field is not yet clear. While under experimentally improved conditions several generations can be reared within a year, the natural life cycle seems univoltine and the relative role of the factors (photoperiod and food) inducing diapause outdoors is not clear (see later for Czech populations).

Similarly to *Hip. convergens* (Hagen 1962; Michaud & Qureshi 2005, 2006) *Cer. undecimnotata* shows indications of a **nutritional induction** of diapause. When the young adults of *Cer. undecimnotata* were reared at 20°C for only 2 days on *Myzus persicae* and then for 5 days on a 1:1 mixture of honey and agar, they completed the regression of ovaries and accumulated large reserves in the fat body (Iperti & Hodek 1974). The survival of these adults, induced to diapause by alternative food, was quite long at 5–8°C (the average, the median and the maximum longevity were respectively 124, 136 and 198 days). The other 11 combinations of aphid and carbohydrate food yielded similar results. Diapausing adults collected in the hibernation sites in December lived about twice as long at 5–8°C, with median values between 230 and 260 days (Iperti & Hodek 1974). Although these results

Table 6.8 Effect of the physiological condition of the host plant of aphids (*Aphis fabae*), used as food for larvae of *Ceratomegilla undecimnotata*, on the incidence of diapause in the adult coccinellids (Rolley et al. 1974).

Host-plants of prey-aphids fed to larvae	Incidence of reproducing females*		Frequency of quantity of fat reserves (%)				Sex
	n	%	n	no or low	medium	high	
Young, reared in the lab	—	—	21	75.5	10	14.5	females
	21	95	24	33.5	37.5	29	males
Senescent, collected in the field	—	—	27	11	0	89	females
	27	44	17	6	6	88	males

The coccinellid adults (emerged from pupae in mid-July) were fed with aphids reared on young plants from the laboratory. Both larvae and adults were reared in screened outdoor cages. The mean of daily minimum temperatures was 14°C and of maximum temperatures 28°C, whilst the absolute extremes were 10 and 31°C.
*Females with vitellinized oocytes or eggs.

might indicate that there are nutritional components in diapause induction in *Cer. undecimnotata*, the adults have been observed to migrate to dormancy sites when aphids are still abundant in the valleys where they breed (G. Iperti, unpublished). Thus the situation appears different from that reported by Hagen (1962) for *Hip. convergens*.

Also the physiological **age of the host plant** of aphids plays an important role. This was tested by rearing *Cer. undecimnotata* larvae on two food regimes: aphids from young versus old bean plants. The incidence of diapause was increased by feeding **larvae on aphids from old plants** (Rolley et al. 1974; Table 6.8). All adults reared as larvae in the two food regimes were fed after emergence from pupae with aphids reared on young plants. Both larvae and adults were reared in screened outdoor cages. The mean daily minimum and maximum temperature was 14 and 28°C, respectively.

An **inhibition** of reproduction **by short daylength** (12L:12D) in *Cer. undecimnotata* at 25°C is weaker than in some other coccinellids such as *C. septempunctata*. Although the difference in the duration of the pre-oviposition period between the two photoperiods (12L:12D versus 18L:6D) was great (60 versus 8 days, respectively), the short days merely delayed, and did not prevent reproduction, except 10% of females, and the resulting total fecundity was almost twice as high (824 eggs) than at long days (455 eggs) (Table 6.9; Fig. 6.5). Also the oviposition rate was substantially higher

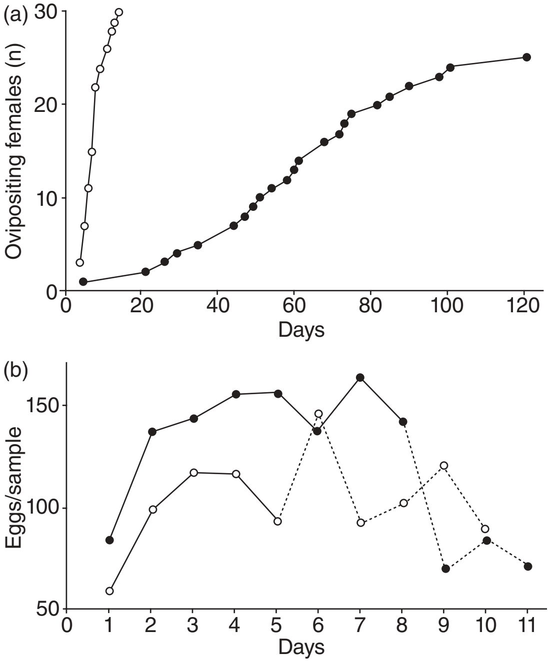

Figure 6.5 Reproduction in *Ceratomegilla undecimnotata* at 25°C, 12L:12D (solid circles) and 18L:6D (open circles) (from Hodek & Iperti 1983). (a) Duration of pre-oviposition period; (b) Oviposition rate, oviposition by individuals aligned for a uniform start in both photoperiods.

Table 6.9 Comparison of reproductive activity of *Ceratomegilla undecimnotata* reared at 25 ± 1°C and 12L:12D or 18L:6D (Hodek & Iperti 1983).

			Duration (days)			
Photophase	***n***		**pre-oviposition**	**oviposition**	**post-oviposition**	**Fecundity (eggs/female)**
12L	25	aver.	60.3	38.5	2.7	823.6
		med.	61	39	3	789
		range	5–121	7–78	1–9	35–2277
18L	30	aver.	7.7	25.5	2.9	454.4
		med.	7.5	23	3	414
		range	4–14	4–71	1–7	65–1284

at a short daylength (Hodek & Iperti 1983). It can be speculated that, in this mostly univoltine population, the **precocious photoperiodic activation** by long daylengths (i.e. by tachytelic processes) was not adequate for the ‘normal course of events’, which involve the horotelic termination of diapause under short daylength (for definitions see 6.1.2). It is still too early to distinguish definitively the relative importance of individual factors (food, photoperiod, temperature and others) in diapause induction in *Cer. undecimnotata*, but it seems that photoperiod is a major cue also in this species.

In *Cer. undecimnotata* the photoperiodic response is lost during **diapause development**, so that after diapause, in spring, adults reproduce without respect to daylength (Hodek & Ruzicka 1979). The development of diapause (monitored by the decrease in the duration of pre-oviposition) follows the same course in south-eastern France and in central Europe (Iperti & Hodek 1974). In early diapause (before mid-August) the French samples had two patterns of pre-oviposition duration. The long durations were around 25 days. The other pattern in summer was short pre-oviposition periods (around 10 days – the duration normally found in December–January), apparently occurring in the females which had just arrived in the hibernation sites; their diapause was not yet fixed. This phenomenon of **diapause fixation** (6.1.3) has already been reported from several other insect species (Hodek 1983; Danks 1987). Adult diapause of *Cer. undecimnotata* females could be prematurely but efficiently terminated (and oviposition started) in September by application of 6 of 18 juvenoids (artificial analogues of juvenile hormone) tested (Hodek 1973; 6.1.4).

6.2.9 *Harmonia axyridis*

Telenga and Bogunova (1936) reported from **East Siberia** that only those adults of *Har. axyridis* which emerge from the pupa before mid-August can oviposit in the same season. Ulyanova (1956) confirmed the **multivoltine** character of this coccinellid after it was imported to the warm climate of **central Asia** (Tashkent, Uzbekistan), where the beetles terminated hibernation in mid-February. When the first generation then emerged from pupae in April and the second in June, the females were reproductive. In the next two generations that emerged in July and August, most females were diapausing. In neither of these two papers were the diapause inducing factors discussed.

In **central Japan** *Har. axyridis* has a **bivoltine cycle** interrupted twice, by aestivation and hibernation. **In contrast to *C. s. brucki***, which has a similar life cycle in that region, but is a short-day insect **aestivating in diapause** (6.2.2.1), a study suggests that ***Har. axyridis* hibernates in diapause** while its **aestivation** may be a **mere quiescence** (Sakurai et al.1988). This assumption of direct inhibition by high temperature is supported by observation of mating and oviposition in August. In adults of the second generation, appearing in mid-October, the ovaries remained undeveloped and the corpora allata small, and thus no vitellogenins were found by electrophoresis in the haemolymph of females. Mating and oviposition were not observed before April. Low temperature (18°C) and short daylength (10L:14D) was reported to stimulate the respiration rate, which was suppressed by contrasting conditions (25°C, 16L:8D). These data would not support the above assumption that the aestivation is a

Table 6.10 Results of dissections (% of females) of *Harmonia axyridis* collected at hibernation sites. F, full; E, empty; s, small; m, medium; l, large; Im, immature; M, mature; R, regressed; transfer, date of transfer to the hibernation site (Iperti & Bertand 2001, modified).

Locality	**Midgut**		**Fat body**			**Spermatheca**		**Ovaries**		
Month	**F**	**E**	**s**	**m**	**l**	**E**	**F**	**Im**	**M**	**R**
Alpilles 230 m a.s.l. n = 1690 transfer: 16 Dec.										
Dec.	50	50	20	40	40	50	50	90	0	10
Jan.	0	100	0	37	63	50	50	100	0	0
Febr.	0	100	50	10	40	100	0	100	0	0
March	0	100	40	0	60	50	50	100	0	0
April	30	60	90	10	0	25	75	100	0	0
Roquebrune 280 m a.s.l. n = 1500 transfer: 9 Dec.										
Dec.	30	70	10	10	80	90	10	90	0	10
Jan.	0	100	0	10	90	70	30	100	0	0
Febr.	0	100	0	70	30	100	0	100	0	0
March	0	100	10	30	60	90	10	100	0	0
April	0	100	90	10	0	10	90	70	0	30
Tourniol 1180 m a.s.l. n = 1788 transfer: 10 Jan.										
Jan.	25	75	50	50	0	25	75	100	0	0
Febr.	0	100	33	33	34	66	34	100	0	0
March	0	100	0	40	60	50	50	70	30	0
April	0	100	0	100	0	20	80	60	40	0
May	20	80	100	0	0	10	90	40	60	0
Courbons 1200 m a.s.l. n = 1818 transfer: 23 Dec.										
Dec.	20	80	10	20	70	78	22	100	0	0
Febr.	0	100	0	30	70	100	0	100	0	0
March	0	100	0	60	40	70	30	100	0	0
April	0	100	100	0	0	40	60	80	20	0
May	0	100	90	10	0	23	77	56	44	0

mere quiescence. Both the early studies by Telenga and Bogunova (1936) and Ulyanova (1956) and the more recent studies described below indicate that *Har. axyridis* is a long-day insect entering hibernation diapause in response to short days. Therefore it is strange that the autumnal conditions inducing diapause merely double the duration of the pre-oviposition period (Sakurai et al. 1988).

In **southeastern France**, the imported population of *Har. axyridis* (probably from China) hibernated succesfully with <10% mortality (at some sites it was only around 2%) on five sites varying in altitude from 40 to 1200 m. The beetles were **fed before overwintering on eggs** of the moth *E. kuehniella* at outdoor conditions of decreasing temperature of <22°C and a photophase (<14 hours 40 minutes) that induced diapause. Ovaries did not mature before April, but the spermathecae contained sperm in March in 30–50% of females. A much higher proportion of females (75–90%) contained sperm in their spermathecae later, before the flight from hibernation sites (Table 6.10). The intensity of diapause, indicated by the pre-oviposition period after transfer to 22°C and 16L:8D, gradually decreased during overwintering. The delay of oviposition in March was only 7–8 days (Iperti & Bertand 2001; Table 6.11). In **northeast Canada** no winter survival of *Har. axyridis* was observed outside buildings (Labrie et al. 2008) because they do not withstand temperatures around −20°C (Koch et al. 2004).

Bazzocchi et al. (2004) reported four generations per year in the region of Bologna (**northern Italy**) for *Har. axyridis* of commercial origin. They did not record aestivation diapause (reported by Sakurai et al. 1988, for central Japan) probably due to the continuous availability of aphid prey, the screening from sunlight and favourable air humidity caused by surrounding vegetation. In the commercial culture, *Har. axyridis* was fed on frozen eggs of the moth *E. kuehniella*.

Table 6.11 Preoviposition period (days) in *Harmonia axyridis* females collected at hibernation sites and reared at 22 ± 1°C and 16L:8D on abundance of eggs of *Ephestia kuehniella* (Iperti & Bertand 2001, modified).

Dates\Sites	Antibes	Roquebrune	Alpilles	Courbons	Tourniol
November	11 (7–19)	—	—	—	—
December	16 (12–20)	—	—	—	—
January	14 (10–24)	12 (7–17)	14 (11–18	—	—
February	9 (7–12)	11 (10–13)	12 (9–14)	11 (8–14)	11 (8–13)
early March	—	9 (6–10)	12 (11–17)	13 (12–14)	13 (10–18)
late March	7 (4–10)	7 (6–9)	8 (6–12)	—	—
April	—	—	—	7 (4–12)	9 (7–11)
May	—	—	—	—	7 (5–13)

In experiments executed in the region of Ghent, **Belgium** (Berkvens et al. 2008), diapause was induced by short days of 12L:12D at 23°C, similar to the experiments in France (Ongagna & Iperti 1994). The diapause lasted 1–3 months when the ladybirds were fed on *Acyrthosiphon pisum*, but was longer when frozen *E. kuehniella* eggs were the food. A laboratory population of commercial origin was reared for c. 50 generations at long days of 16L:8D; its response to diapause induction was weaker than in populations founded by beetles collected in the field (Berkvens et al. 2008).

6.2.10 *Coccinella leonina (=repanda)*

The facultative diapause of *C. leonina*, a common and widely distributed aphidophagous coccinellid of **eastern Australia**, is reported to be induced mainly by non-aphid diet, i.e. pollen and sugar. When fed with aphids, almost all females reproduce at temperatures of 20, 22, 28 and 32°C without respect to the photoperiods 10L:14D, 12L:12D or 14L:10D. There is some incidence of diapause at the highest temperature, but there is also a certain tendency to diapause at the lowest temperature (Anderson et al. 1986). The authors conclude that this result, together with field data, may indicate the capacity to enter both summer and winter diapause. They characterize the life cycle strategy of *C. leonina* as opportunistic, enabling the use of fluctuating food supplies.

6.2.11 *Apolinus (=Scymnodes) lividigaster* and *Illeis (=Leptothea) galbula*

Around Sydney, **Australia**, the aphidophagous *A. lividigaster* and mycophagous *I. galbula*, both multivoltine, show a very **plastic reproductive strategy** adapted to the unpredictable conditions there. Apart from the periods of dormancy (aestivation and hibernation in *A. lividigaster* and only hibernation in *I. galbula*), **diapausing** adults also **occurred concurrently with ovipositing** females during the periods when reproduction normally occurs (Anderson 1981). In another paper, Anderson et al. (1986) supposed that the diapause of *I. galbula* was controlled by photoperiod.

6.2.12 *Harmonia sedecimnotata*

An experimental culture of this Asian ladybird was founded from individuals sampled near the town of Guangzou, southeastern China. The species does not show a photoperiodically induced diapause. In reproducing 30 day old adults fed on aphids (*M. persicae*), **trophic dormancy** was induced by feeding them on only 10% sucrose solution. In the course of 4 weeks the ovaries were resorbed and only small germaria were found by dissection. The beetles aggregated similarly to diapausing individuals. When feeding with aphids was resumed, the females recommenced egg laying, even with a very low ration of prey. The authors assumed that the beetles were activated by a food signal and not metabolically, and thus considered this dormancy a diapause (Zaslavsky et al. 1998). More probably a quiescence was concerned, as both induction and termination of the dormancy were directly produced by absence or presence of suitable food (6.1).

In a culture with only **intermittent presence** of aphid prey, monthly oviposition was doubled (207 eggs, $n = 39$) compared to a culture with a continuous surplus of aphids (107 eggs, $n = 48$). Furthermore, the longevity of females was increased by this manipulation (Semyanov & Vaghina 2003).

Adults in trophic dormancy can be used for **storage** at 12°C with the low mortalities (n = 420) of 9.6, 25.5, 41.8 and 55.1% in the first, second, third and fourth month respectively. At 25°C, 25% of beetles (n = 1145) died in the first month (Semyanov 2000). Similar trophic dormancy was induced in *Cer. undecimnotata* (Iperti & Hodek 1974; 6.2.8).

6.2.13 *Chilocorus* spp.

Chilocorus rubidus, a species from **eastern Siberia** is strictly **univoltine**. The dormancy lasts from late August/mid-September to late April, while diapause (which is marked by very low oxygen consumption) ends as early as late December to early January. The rest of the dormancy is spent in mere quiescence during which oviposition can be started by transferring the beetles to a higher temperature. Pantyukhov (1968a) found that neither low temperature, nor any particular photoperiod, was necessary for the **termination of diapause** and that the passage of 3.5–4 months was sufficient under field conditions or at temperatures of 25, 20 or 5.8°C.

In contrast to *Chil. rubidus,* diapause can be prevented in the majority of ***Chil. renipustulatus*** individuals in both populations studied. Thus only 12% of beetles from Maikop (**southern Russia**, 45°N, 40°E) entered diapause under long days, whereas in the population from **St. Petersburg**, 60°N, the figure was 38%. The critical photoperiod (6.1) is insensitive to temperature (within the range 20–25°C) and is 2 hours longer in the beetles from St. Petersburg than in the Maikop strain (Pantyukhov 1968b).

Chilocorus bipustulatus from **St. Petersburg** (60°N), a northern population, reproduced when a three week exposure to **short days** (9L:15D) was **followed by long days**; they do not lay eggs if the larvae, pupae and adults are reared continuously in long days (20L:4D) (Zaslavskii 1970). The short day treatment is equally effective when given to the larvae. The alternative way to achieve oviposition is an exposure to +7°C for about a month followed by long days. If the beetles are constantly left in long days, diapause ends spontaneously after 2–4 months. However, after about 1.5–2 months of egg laying in continuous long days, the beetles gradually cease ovipositing and have to be activated from this secondary diapause by a fresh experience of about 25 days in short days.

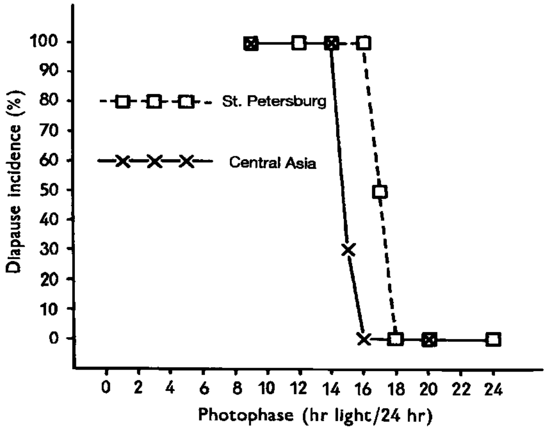

Figure 6.6 Effect of photoperiod on diapause incidence in strains of *Chilocorus bipustulatus* (from Zaslavskii 1970).

The **critical photoperiod** for the beetles from St. Petersburg is 2 hours longer (17L:7D) than for the central Asian population (Fig. 6.6). It does not change with temperature within the range of 20–27°C. The results of Zaslavskii (1970) are of extreme importance, as they were an early confirmation that a number of alternative pathways can lead to the triggering of the neuroendocrine system regulating oviposition.

In ***Chilocorus bipustulatus*** from **Central Asia** (40–41°N), **diapause** is **induced** by a photophase shorter than 15 hours and at 20°C 100% beetles enter diapause. At 24°C, however, diapause is completely prevented in all females in spite of the short day (9 or 11 hours). Spontaneous **termination of diapause** does not occur when the beetles are maintained at diapause-inducing conditions. An increase in temperature or a prolongation of photophase beyond the above-mentioned thresholds terminates diapause very quickly: oviposition then takes place within a few days. It is just as easy to terminate diapause 7–10 days after its induction as it is after 3 months. By contrast, transfer of the ovipositing beetles from long days to diapause-inducing conditions gradually suppresses oviposition, while a return to long days restores it. Alternatively, after an exposure to +8°C for 30–40 days, the beetles mature even in short days. Thus, sensitivity to photoperiod could be nullified by an exposure to cold (Zaslavskii & Bogdanova 1965).

Table 6.12 Effect of photoperiod and temperature on oviposition in *Chilocorus bipustulatus* in Israel (Tadmor & Applebaum 1971).

Photophase (h/24h) and temperature (°C)				Ovipositung females (%)	Mean pre-oviposition period (days)	Number of replicates
larva and pupa		adult				
24	28°	10	8°/20°*	46	21	11
24	28°	10	18°	60	17	10
10	18°	10	18°	1	21	11
24	28°	10	22°	100	10	7
24	28°	14	18°	93	14	14

*8°C during scotophase, 20°C during photophase.

The populations of ***Chilocorus bipustulatus*** in **Israel** (32–33°N), in the eastern Mediterranean area, differ from those of central Asia in their diapause threshold both for photoperiod and temperature (Tadmor & Applebaum 1971). A photophase of 14 hours prevents diapause, whereas a shorter photophase (10 hours) tends to induce it (Table 6.12). Thus, consistent with the findings of Danilevskii (1965), the **critical photoperiod** is about 2 hours shorter in populations from Israel than from central Asia. The effect of the short photophase is modified by temperature: at 22°C it is completely nullified so that all females lay eggs. A cumulative **sensitivity to diapause induction** is exhibited both in the pre-imaginal stages and in the adults.

There is contradiction between earlier and later reports from the field conditions about the summer occurrence and biocontrol efficiency of *Chil. bipustulatus* in Israel. Early reports concluded that *Chil. bipustulatus* was common in spring and extremely scarce in summer, and was therefore an inefficient predator of scale insects (Hecht 1936; Bodenheimer 1957). The high mortality in summer was supposed to have been caused by a dry hot wind from the desert. Later findings show, however, that *Chil. bipustulatus* is rather abundant in the summer (the population may even peak in early summer) and plays an important role in retarding the build-up of scales in this period (Nadel & Biron 1964; Avidov & Rosen 1965; Rosen & Gerson 1965; Kehat 1968; Ben-Dov & Rosen 1969). This has led to speculation (Hodek 1967) that the difference between the earlier and later reports has perhaps resulted from the improved environmental conditions due to irrigation, as the same difference also exists between older (Bodenheimer 1957) and more recent reports (Plaut 1965) on the abundance of *Stethorus pusillus*. In citrus groves, M. Kehat, S. Greenberg and D. Gordon (unpublished) found there is a considerable decline in numbers of *Chil. bipustulatus* females with well-developed ovaries during July, and also from October to December. When the non-reproductive females were transferred to the laboratory (28°C) and provided with coccids, their ovaries matured in both these seasons. The failure to induce diapause at 16 combinations of light conditions was apparently due to a high temperature of 28°C (Tadmor & Applebaum 1971). An even higher temperature (35°C) additionally decreases oviposition and survival.

In the **central Asian** populations of ***Chilocorus geminus*** (Tashkent, Uzbekistan, 40–41°N), induction and termination of diapause is very similar to that in *Chil. bipustulatus*. The only difference is a higher temperature threshold: as much as 26°C is needed to prevent diapause in short days (Zaslavskii & Bogdanova 1965).

6.2.14 *Stethorus punctum picipes* and *S. japonicus*

The **South Californian populations** of *S. p. picipes*, a predator on spider mites, have a weak facultative diapause which can be quickly terminated by an increase in temperature and/or photoperiod, and induced again by the reverse changes (McMurtry et al. 1974); it may be a mere quiescence. Long days (16L:8D) at 21–22°C stimulated almost all females to oviposit, but about half the females also oviposited in short days (10L:14D). An increase in temperature by about 5 to 26.7°C enabled oviposition by almost all females. In the mild conditions near the ocean the females also reproduce in winter, when they can find prey. All developmental stages were found in mid-winter on mite-infested plants, but on mite-free oaks the females had small or shrivelled ovarioles (19 of 21 females).

In the Ibaraki region (central Japan), diapause of ***Stethorus japonicus*** is induced at 18°C under photoperiods <13L:11D, but at 14L:10D and 16L:8D it is prevented in 60% of females. Active adults appear on trees from June, and the photoperiod 13L:11D occurs in the region in mid- to late September. Thus the 5–7 generations per year, estimated by Mori et al. (2005), appears unrealistically high.

6.2.15 *Scymnus (Neopullus) sinuanodulus*

The first generation (the offspring of the diapausing one) of this predator on adelgids does not oviposit until the following spring (Lu & Montgomery 2001). Although the authors deny the existence of diapause, it evidently is entered. Oviposition is achieved in autumn after an exposure to low temperature (5–10°C) for 1.5 months.

6.2.16 Ecophysiological regulation of diapause in coccinellids

The life history of coccinellids depends on regional climatic conditions (Hagen 1962; Fig. 6.7). It is not easy to 'distil' a general view on the regulation of diapause and voltinism from the very diverse evidence presented in this chapter. The evidence is very varied and defies meaningful generalizations for several reasons. (i) The reported evidence still provides just a small proportion of knowledge that would be needed; even for the frequently studied species the **analysis is incomplete**. Unfortunately, research activity in this field has received little attention in the last 10–15 years, particularly when compared with other areas of coccinellid study. (ii) It is difficult to make comparisons between species, because the direction of **research** has varied **arbitrarily**, as biased by conditions and the different inclinations of the researchers. Thus, for example, we lack detailed studies on the effect of photoperiod on *Hip. convergens*, while for both subspecies of *C. septempunctata* such studies are available. The varied experimental approach is probably the reason why particular regulatory environmental factors have become attached to individual species. Complex relationships cannot be well understood, when the results of studies are **biased by individual experimental plans**. (iii) **Diversity** of responses is **inherent to diapause** because of its **adaptive role** (6.1); thus diapause mechanisms inevitably resist an easy generalisation. (iv) In the case of aphidophagous coccinellids, the selection pressure of the **unpredictable** intermittent availability of **aphid prey** naturally leads to a certain convergence, in the direction to a **greater plasticity** of diapause mechanisms within this guild. Plastic responses to anthropogenic environmental changes have been observed both in the field (irrigation for *Hip. convergens* in California or in the coccidophagous *Chil. bipustulatus* in Israel as well) and in experiments (surplus of aphid prey, and perhaps the shading of outdoor cages for *Cer. undecimnotata* in Greece).

Despite the above constraints, some species have been shown to respond to a **complex of environmental cues**, though these factors may be differentially operational in different environmental situations or life-cycle phases. Thus, for example, *Cer. undecimnotata* responds to at least both the photoperiod and food. We may assume that, superimposed on the **basic photoperiodic response** (based on the annual astronomically precise repetition of daylength), are the less rigid reactions to less predictable environmental changes in food availability and quality, and other factors such as temperature, humidity and population density. The archetypal **primary nutritive factor** seems to be 'prepared to enter the game' under unpredicted events of prey abundance – thanks to phenotypic plasticity.

We might envisage a scenario which **combines plasticity with resilience**. One aspect of the life-cycle strategies is the 'safety' ('insurance') factor, the univoltine trait, permanently perpetuated in the gene pool and maintained (i.e. not selected out) in spite of any momentary favourable conditions that are unreliable in the long run. However, the polygenes 'watch' for changes in the environment. If there is a promising improvement they 'open the gate' for multivoltine development, more or less appropriate to the kind of improvement. The system appears resilient by **maintaining** the **univoltine trait** quite intensively.

This scenario is adequate for *C. septempunctata* and species or populations with a similar life history living

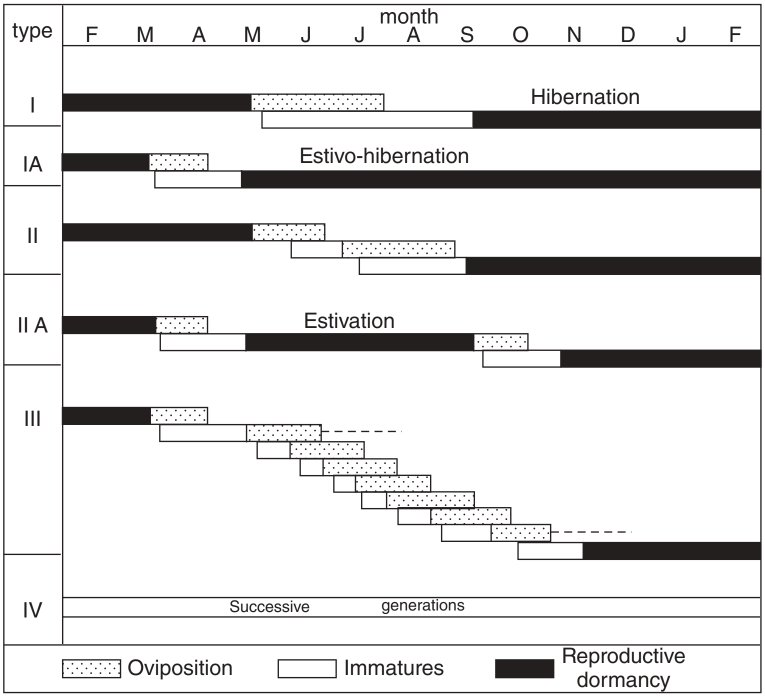

Figure 6.7 Types of voltinism among coccinellids in the northern hemisphere (from Hagen 1962).

in temperate regions/climate. In coccinellids with different prey or from different climatic areas, the regulation of voltinism can differ, as e.g. in tropical areas or in coccidophaga.

6.3 BEHAVIOUR PATTERNS RELATED TO DIAPAUSE

Coccinellids induced to diapause change their behaviour. Some species make lengthy **migratory flights** and form large **aggregations** in a dormant state, in which they may remain for 9 months. This behaviour is most pronounced in *Hip. convergens* (6.3.2.7), *Cer. undecimnotata* (6.3.2.1) and *Har. axyridis* (6.3.2.2). In general, these adaptations of migration and aggregation have developed **most often in aphidophagous** coccinellids of the tribes Coccinellini, Hippodamiini and Anisostictini. Ample descriptive literature exists on such behaviour of coccinellids, while studies on causal relationships are rarer.

What is **required** is a series of **comparative experimental studies** on migration and aggregation behaviour both in the laboratory and field. Because dormancy behaviour is not the same in different species of coccinellids, there is a danger of misleading generalizations based on only a few well-documented cases. Therefore specific variations in individual species will be discussed after a general description of the typical behavioural phases.

6.3.1 Phases of dormancy behaviour

6.3.1.1 Pre-diapause

Most coccinellid adults accumulate energy reserves for hibernation (6.4.1.1, 6.4.2) before migrating to hibernation sites (e.g. *C. septempunctata*, 6.3.2.3), while others continue to feed after the main phase of migration (e.g. *Hip. convergens*, 6.3.2.7).

In *C. septempunctata,* diapause is determined in late larval and early imaginal life (6.2.1). The adults in which the 'points' have been set in the direction of diapause feed voraciously and the ingested food is used for building up **large metabolic reserves** in the fat body. These reserves are mainly fat and glycogen, and serve as a source of energy during the long time without food that follows (6.3.2.1, 6.3.2.3). It can be assumed that it is this accumulation of sufficient reserves that represents the stimulus for the beginning of diapause.

In *Hip. convergens* it is presumed that diapause is induced mainly by an absence of aphid food (Hagen 1962; 6.2.7). When aphids are lacking, the coccinellids feed on **alternative plant food** (nectar of flowers or pollen; Chapter 5.2.9) and accumulate reserves from this food. They migrate into the mountain forests, without having built up any great amount of fat. There they feed further, deposit sufficient fat for aestivo-hibernation and move to the final overwintering sites (Hagen 1962).

The assumption that *Cer. undecimnotata* adults do not feed before dormancy and manage with the reserves accumulated by the larva (Yakhontov 1962) has subsequently been rejected (6.2.8).

6.3.1.2 Migration

There is some controversy regarding the use of the term 'migration'. Hagen (1962) argues that only the flight toward aggregational sites can be considered as migration since it is **directional** and under partial control of the beetle. The disbanding of aggregations is a simple **dispersal** flight, as it is not directional. According to Johnson's (1969) concept, both flights are migration, as the direction of both displacements is greatly affected by wind and only at the concluding phase of migration to dormancy sites do the beetles control their direction in relation to visual or other cues. Although this disagreement seems only semantic, it actually goes to the heart of the problem: do coccinellids fly to hibernation sites by a directional flight, or are they brought there passively by wind currents? There is some circumstantial evidence for both viewpoints.

The **aggregations** for dormancy are often formed **at prominent features** of the landscape. For those coccinellids that make lengthy migratory flights (e.g. *Cer. undecimnotata* or *Har. axyridis*), these features may be summits of hills, large rocks or high buildings. They may, however, also be a forest edge, a terrain wave, a shrub, a tree, or a post in a flat landscape for coccinellids that aggregate within the breeding area but usually in a different habitat or microhabitat (e.g. *C. septempunctata*). These sites are **the same year after year** if the relief remains the same or change if the relief is changed. The creation or removal of triangulation posts, fences and huts provides unintentional experiments which show that the aggregation sites of *Cer. undecimnotata* can change (Hodek 1996).

It seems improbable that coccinellids would always be transported passively to the same places, or that wind currents would be so drastically changed, for example by the erection of a post of 15 cm or less in diameter, that the coccinellids are carried elsewhere. Also direct observations of beetles landing on the top of a hill (in Raná, northern Bohemia) showed that *Cer. undecimnotata* landed actively, e.g. on a shifting person who at that moment was the most prominent object (Hodek 1996). Any air currents existing were too weak to be noticed by the observer. Some authors have even observed that coccinellid adults move against the prevailing wind, but in the direction of mountain peaks (Mani 1962).

Obata (1986) observed that *Har. axyridis* approaching the hibernation site **changed direction in flight**; she regarded their landing as an active response (6.3.2.2). Therefore an analogy has been sought between the aggregation of coccinellids at prominent objects and the habit of flying around hill tops shown by ants and other insects (Alcock 1987) or the flight to feeding sites on trees by *Melolontha*. Such **hypsotactic orientation** has also been widely accepted in ladybirds (Hodek 1960; Hagen 1962; Nalepa et al. 2000, 2005).

Coccinellids have also been observed to make use of **air-currents**, especially in mountain valleys. Savoiskaya (1966) reported that, in the Zailiiskii Alatau mountains of Kazakhstan, the coccinellids fly up the valleys to their hibernation places with the help of a steady breeze blowing up in the daytime. Also *Hip. convergens* seems to be transported to the mountains and back to the valley by air currents (Hagen 1962, 1966; Fig. 6.8).

Individual species differ in migration behaviour. Hagen (1962) considered *Col. maculata* a **'climatotactic' aggregator**. In many species behavioural responses of both types most probably apply (Hodek 1967), but one type may dominate. Johnson (1969) judges that orientation of a migrant for some perceived but far-distant hibernation site is not a tenable concept, but a 1.5 km distant mountain was experimentally

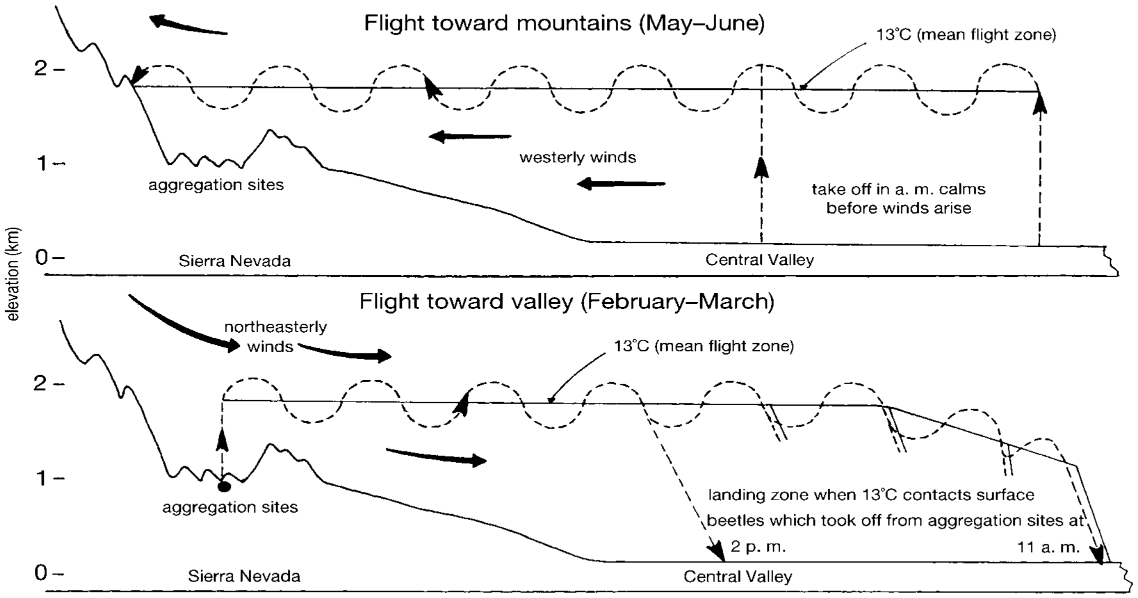

Figure 6.8 Suspected temperature-controlled flight oscillations which *Hippodamia convergens* undergoes in its migratory flights (from Hagen 1962).

established to be a visual cue for migrating *Cer. undecimnotata* (Iperti & Buscarlet 1972, 1986). In the long distance migrations which Hagen (1962, 1966) hypothesized for *Hip. convergens* (6.3.2.7; Fig. 6.8) it is only towards the end of these long migrations that the hypsotactic response may be shown and that a visually oriented directional flight can guide the coccinellids to a prominent feature. Hypsotaxis may also apply to dormancy aggregations of *Hip. convergens* on mountain tops (e.g. Stewart et al. 1967, 6.3.2.7).

6.3.1.3 Flight and methods for its study

The **tendency to migratory flight** has been studied and **quantified** in several ways. Solbreck (1974) and Rankin and Rankin (1980) used the method of **tethered flight,** respectively for *Col. maculata* and *Hip. convergens*, although details were not identical (see below). Rankin and Rankin (1980) used a low speed fan to **stimulate** the coccinellids to fly. The recorded absolute values for the flight durations are thus different. While in *Hip. convergens* flights >30 minutes were considered to be migratory (6.3.2.7), less than 10% of *Col. maculata* adults flew >10 minutes and this only occurred during the most suitable period in mid-May, and only aproximately 20% flew for about 1 minute.

The changes in flight behaviour in the second phase of dormancy (December–May) were studied by Solbreck (1974) in *Col. maculata*, also using the tethered flight technique but without additional stimuli other than loss of tarsal contact. A 30 second duration of flight was used as the best criterion for comparing flight tendency of beetles sampled at different dates or exposed to various temperatures and photoperiods before the flight tests. An important ‘maturation’ of flight behaviour, i.e. an increase in the percentage of individuals flying for 30 seconds or more, was found between mid-April and mid-May, i.e. in the season of dispersal from hibernation sites. An increase in temperature strongly increased the rate of the flight maturation process above a threshold somewhere between 15 and 19°C. Short photoperiods significantly delayed the process in December, but not in late March or even later. Diapause development was evidently not yet completed in December.

The much poorer flight performance of beetles of post-diapause *Hip. convergens* **parasitized** by the braconid *Dinocampus coccinellae* (Chapter 8.3.2.1) was recorded with tethered beetles in a **recording flight mill** (Ruzicka 1984, Ruzicka & Hagen 1985, 1986). The durations recorded for non-parasitized adults, collected before dispersal from hibernation sites, corresponded with Rankin and Rankin's (1980) data for migratory flight, i.e. 45–50 minutes. The speed of flight recorded on the mill averaged 60–70 m/minute (Ruzicka 1984).

Another method for quantifying flight behaviour is to record the incidence of **spontaneous take-offs**. This approach was used with *Myrrha octodecimguttata* (Pulliainen 1964) and *C. septempunctata* (Zaslavsky & Semyanov 1983, Okuda et al. 1986, Okuda & Hodek 1989, Honěk 1990, Nedvěd et al. 2001). This method is **only qualitative**, it simply divides the sample into 'fliers' and 'non-fliers', but does not measure the duration of the flight. Thus we cannot estimate whether the flight is migratory or just trivial. Recording take-off is suitable for comparing samples from different habitats, of different ages or phases of the life cycle. The experimental conditions must of course be identical. The importance of such conditions is shown by two studies on *C. septempunctata*. Very high **take-off frequencies** were recorded for *C. septempunctata* by Honěk (1990) when at 30°C the beetles were **stimulated to fly** by a mild air-current applied for 10 seconds, at the start and in the middle of a 3 minute test period. Under such circumstances, 60% take-offs were recorded in December in the sample from the hibernation site. When the beetles of the same species and region were left to **take-off spontaneously**, without any stimulation, at 25°C and less intense illumination (Okuda & Hodek 1983, Okuda et al. 1986, Okuda & Hodek 1989), take-off frequencies were much lower and more appropriate for the relevant phases of the life cycle. Thus from late July to mid-August, after arrival at their hibernation sites, only 10–20% took off. In spring, before the dispersal from hibernation sites, the incidence of take-off ranged from 30–70%, while after dispersal to the fields it was 20–50%.

Another interesting method for monitoring the tendency to migratory flight (Khrolinsky 1964) was used with *C. septempunctata* (Semyanov 1978a, Zaslavsky & Semyanov 1983, 1986). Within 20 minutes of a short narcosis with ether, the beetles show their urge to fly by opening their elytra and spreading their wings. In outdoor beetles this response coincided well with the periods of migration from and to hibernation sites. A certain doubt about the reliability of the 'wing spreading response' as a criterion of migratory flight might arise from its occurrence in all young beetles, irrespective of diapause-inducing or preventing photoperiods.

The discovery of **regeneration of flight muscles** in *C. septempunctata* (Okuda et al. 1986, Okuda & Hodek 1989; 6.3.2.3, 6.4.1.4; Fig. 6.12) in October in the hibernation site (seven months before dispersal from there) conflicts with the general assumption that ladybirds do not prepare for dispersal until as late as when they end dormancy in spring under the influence of increasing temperatures. Later more detailed 2-year studies on dormant beetles of the Czech *C. septempunctata* population confirmed the unexpected increases in **respiration** in the 2 years: rising from about 450 to 750 µl/g/h O_2 from late August to mid-September 1994 and from 750 to 1050 µl/g/h O_2 from mid-September to 10 October 1995. In 1995 it was also found that the volume of **flight muscles** gradually increased from 1.8 µl in early September to 2.15 µl on 23 October (the last record) (Nedvěd et al. 2001). Thus changes in both the volume of flight muscles and the respiration rate indicate a very **early tendency to re-activation**, about two months before diapause is completed in mid-winter (Hodek & Ruzicka 1979). Such an apparently non-adaptive event remains an enigma as the greater muscles could consume more reserves during any later periods of temporary increase in temperature. We might hypothesize that the unexpected findings on respiration and flight muscles (Okuda et al. 1986, Okuda & Hodek 1989, Nedvěd et al. 2001) are related to the bivoltine cycles of some Mediterranean populations with separated hibernation and aestivation periods (6.2.1.4). After a hot and dry summer a regrowth of vegetation and aphid populations, thanks to higher humidity, may enable reproduction. However, no detailed study reports such an intercalated reproductive period in the field. There are just indications of such a possibility from the coastal plain of Israel (Bodenheimer 1943) and from one locality on the French Riviera (Iperti 1966a).

Iperti (1986) studied the **migration flight**, particularly the **take-off**, in a complex manner by relating the migration behaviour to measured temperature and wind speed and by marking the insects. The effect of environmental conditions on take-off can only be understood if the individual factors are considered as a complex by the use of Richardson's index of air-turbulence (Iperti 1986), which indicates the relation between the relative influence of the mechanical and thermal energy of air. **Temperature and wind speed** were recorded at the same two heights, 0.25 m and 1.25 m, in a study of *Cer. undecimnotata* (Iperti et al. 1983, 1988, Iperti 1986). A decrease in Richardson's index was positively correlated with a decrease in the incidence of females taking-off for a migratory flight. **Marking with iridium** (the stable isotope 191), an element normally absent from the body of coccinellids, made it possible to show that the released *Cer. undecimnotata* adults directed their flight from a place on the plain (at 395 m) to a 732 m hill 1.5 km away. In samples of beetles from the hill the isotope 191 was modified in the lab into 192 by irradiation and then detected by gamma-spectroscopy (Iperti & Buscarlet 1972, 1986, Iperti & Rolley 1973).

Data on coccinellids trapped during four seasons (1992–95) in **window traps** mounted on a 15 m high tower in New Brunswick, Canada, give an interesting picture of height and **seasonal distribution of flights** for several abundant species (Table 6.13). The median height at which *C. septempunctata* (*n* = 349), *Hip. convergens* (*n* = 279) and *A. bipunctata* (*n* = 551) were trapped was 3.8 m, 0.8 m and 5.3 m, respectively (Boiteau et al. 1999). *Hippodamia convergens* was not caught in flight till as late as August, while *A. bipunctata* was already flying in spring; maximum catches were recorded for *A. bipunctata* in July and for *C. septempunctata* about a month later (Boiteau et al. 1999; Table 6.14). Both the heights and dates of these catches may indicate trivial rather than long-distance flights.

Observations in Hungary showed the different character of pre- and post-hibernation movements in *C. septempunctata* in terms of the **height of flight**. In a year when meteorological conditions were favourable for flight, negligible numbers of beetles were caught in spring by Malaise traps at 12.5–14.5 m and 25–27 m, but high numbers were trapped in late summer (Sarospataki & Marko 1995). It is conceivable that the long distance migratory flight (in contrast to gradual dispersal) operates at a greater height than the dispersion flight after hibernation. Beetles caught at shrub level apparently represent individuals which hibernate near to their breeding sites.

In the course of 2000–2002, *Har. axyridis* was the most abundant species (*n* = 325) caught in **blacklight traps** (Koch & Hutchison 2003). The second most abundant was *Hip. tredecimpunctata.* The other species were very rare: *C. septempunctata* (2 individuals), *Col. maculata* (1), *Cycloneda munda* (1). Non-specified coccinellids were reported to be significantly more attracted (*n* = 30 samples) to blacklight fluorescent lamps (mean number of individuals = 2.46) than to black light blue (1.56) or to cool light (0.36). Blacklight and 'blacklight–blue' lamps have a major peak in the ultraviolet region at around 365 nm. The black light had other peaks in the visible range around 430 and 540 nm (Nabli et al. 1999).

In relation to the **adaptive aspects of dormancy behaviour,** Honěk (1989) stresses the fact that it is often warm sites that are chosen for hibernation (usually the south, southwest, or west aspect) and assumes that an appropriate temperature/humidity relationship and sufficient aeration may **prevent the spread of** diseases (particularly **mycosis**) during winter. In addition to the hypsotactic visual orientation, he suspects that *C. septempunctata* also perceive the overall temperature of the site on warm days. The preference for north-facing situations on trees, reported from England for *Chil. bipustulatus* (Majerus & Kearns 1989), is a rare exception.

Semyanov (1965b) states that the tendency to hibernate on at least slightly elevated ground may have a survival advantage in lowland regions when extensive parts of the **plains** are **flooded**. Many other authors have speculated on the adaptive significance of hibernation in the mountains and as aggregations (summarized in Hodek 1960). It is quite possible that mass hibernation facilitates **contact** with beetles of the **opposite gender** which will be important for less abundant species and leads to crossing between beetles that have developed under different climatic conditions. In regions with warm climates, hibernation in high mountains may **retard** the beginning of **spring activity** of the beetles till aphids in the plain have multiplied sufficiently. A similar function may be attributed to hibernation among the litter in plains, under mosses and in other humid microhabitats where cooling is caused by evaporation.

6.3.1.4 Aggregations

Aggregating is a specific behavioural feature connected with dormancy in many Coccinellidae. Coccinellids are led to form aggregations when brought passively (wind currents) and actively (hypsotaxis; 6.3.1.2) repeatedly to the same dormancy locality and habitat (e.g. a hill or a line of high trees). After arriving at the site, the beetles are led to specific portions of habitats by their responses to physical factors (**hydrotaxis**, **thermotaxis**), and **negative phototaxis**, **geotaxis** and

Table 6.13 Weekly vertical flight frequency distribution for three coccinellid species (week 1, 17–23 May), New Brunswick, Canada (Boiteau et al. 1999, modified).

	Week																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Adalia bipunctata																				
Height 0.8–6.8 m	---- 8 ----		5	13	--- 18 ----		58	40	28	27	18	------- 14 --------			----------- 10 ------------				0	0
Height 8.8–14.3 m	--- 16 ----		7	9	--- 16 ----		26	17	8	10	5	-------- 4 --------			------------ 5 ------------				0	0
Coccinella septempunctata																				
Height 0.8–6.8 m	10	---- 9 ----		16	--- 10 ----		--- 29 ----		16	38	39	---------------------- 18 ------------------------							-	-
Height 8.8–14.3 m	5	---- 4 ----		9	---- 8 ----		---- 4 ----		4	6	17	----------------------- 2 ------------------------							-	-
Hippodamia convergens																				
Height 0.8–6.8 m	0	0	0	0	0	--------------- 8 ---------------					53	77	0	74	------------------- 12 --------------------					
Height 8.8–14.3 m	0	0	0	0	0	--------------- 0 ---------------					2	2	0	1	-------------------- 0 --------------------					

Data are from 1992 for *Adalia bipunctata* and *Coccinella septempunctata* and from 1995 for *Hippodamia convergens*.

Table 6.14 Seasonal distribution of coccinellid species trapped between 1992 and 1995 with annual catch greater than 40 (week 1, 17–23 May), New Brunswick, Canada (Boiteau et al. 1999, modified).

	No. of individuals per year					Week									
Species	**1992**	**1993**	**1994**	**1995**	**Height of 50% capture (m)**	**1–2**	**3–4**	**5–6**	**7–8**	**9–10**	**11–12**	**13–14**	**15–16**	**17–18**	**19–20**
Hyperaspis bigeminata	7	7	10	47	5.3	0	46	18	0	0	3	1	2	1	0
Anatis mali	27	1	9	40	5.3	1	36	9	3	17	7	3	0	0	1
Coccinella septempunctata	244	32	20	53	3.8	20	48	40	43	82	91	12	3	6	2
Adalia bipunctata	362	69	51	32	5.3	25	64	116	182	109	43	12	10	4	6
Hippodamia convergens	5	15	30	229	0.8	0	2	2	1	6	134	76	10	21	27

Less abundant species (i.e. ≤40) are as follows: *Propylea quatuordecimpunctata*, *Hippodamia tredecimpunctata*, *Myzia pullata randalli*, *Coccinella hieroglyphica kirbyi*, *Hyperaspis undulata*, *Brachyacantha ursina*, *Hippodamia parenthesis*, *Anisosticta bitriangularis*, *Chilocorus stigma*, *Mulsantina hudsonica*, *Scymnus brullei*, *Coccinella trifasciata perplexa*, *Calvia quatuordecimguttata*, *Psyllobora vigintimaculata* and *Harmonia axyridis.*

thigmotaxis cause them to hide in microhabitats such as the space under a stone, the crevices in a rock, in a grass tussock, or in tree bark. For example, the beetles hibernating in litter are apparently guided by negative phototaxis and positive geotaxis (possibly also positive hydrotaxis) to hide on or near the ground after a period of sitting on the plants. During this **period of 'waiting'** their behavioural patterns gradually change from those which led the beetles to the hibernation habitat during migration. Hydrotactic responses prevail in climatotactic species such as *Hip. convergens* and *Col. maculata* (Hagen 1962; 6.3.1.2). In aestivating *C. novemnotata* (McMullen 1967a) and hibernating *Cer. undecimnotata*, ventilated crevices are preferred (Hodek 1960, Yakhontov 1962, Iperti 1966a) as they apparently **reduce the danger of mycosis** (Iperti 1964, 1966b) (Chapter 8.4.3). Honěk et al. (2007) first studied for hibernation aggregation in ***Hip. variegata***.

Pulliainen (1963, 1964) studied the responses of ***Myrrha octodecimguttata*** collected in bark crevices in the final period of dormancy and re-activated by a higher temperature in the laboratory. The beetles showed a strong hydro-negative reaction, which only changed after a prolonged desiccation. They reacted indifferently to long-wave light, and photo-negatively to short-wave light. After desiccation, they became strongly photo-negative to long-wave light also. The optimum relative humidity was about 30–40%. Novák (1966) found that the geotactic reaction was slightly positive in dormant *C. septempunctata* collected when 'waiting' on trees before they hid in the litter. In humid environments, the response changed to be slightly geo-negative.

In the laboratory, the formation of clusters of *Hip. convergens* adults increased with a decrease in temperature from 35°C to 15°C. Temperature also affected the response to light: the beetles became photo-negative when the temperature dropped below 5°C (Copp 1983). These experiments were performed from January to March, i.e. relatively late in dormancy (August–April) when diapause had most probably been terminated (6.1).

The direct stimuli which result in aggregation may – apart from thigmotaxis – also include **chemotaxis**, i.e. **attraction by semiochemicals** (Al Abassi et al. 1998; Chapter 9.7). The specific odour of coccinellids is penetratingly noticeable to humans, and speculation has been published on the role of pheromones in the formation of aggregations. However, the evidence is still limited. Savoiskaya (1966) assumed an aggregative function for odour, based on her observations on the genus *Adalia*. It may be that a pheromone attracts later migrants to those beetles which have arrived earlier. Yakhontov (1962), Majerus and Kearns (1989) and Majerus (1994) supposed that dead beetles remaining from the previous hibernation had an attraction, but this has been refuted by G. Iperti (unpublished). Indeed, Novák (1965) has proved experimentally that dead beetles are more likely to act as a repellent than as an attractant.

Copp (1983) reports the ability of ***Hip. convergens*** adults to locate clusters of living conspecifics by **olfactory cues** in special test chamber in which visual cues and direct contact were eliminated (Table 6.15). The volatile sesquiterpene (-)-beta-caryophylene, suspected to act as an aggregation semiochemical, attracted both genders of ***Har. axyridis*** in a bioassay, while only males responded in a four-arm olfactometer (Verheggen et al. 2007).

In coccinellids, which are distasteful prey with aposematic colouration, gregariousness may have

Table 6.15 Distribution of adults of *Hippodamia convergens* in the arena above a chamber that contains a beetle cluster in one quadrant (Copp 1983).

		Without CO_2 absorbent		With CO_2 absorbent	
Quadrant of arena	**Contents in the quadrant below**	**total no.of beetles**	**mean ± SD**	**total no.of beetles**	**mean ± SD**
1	beetle cluster	162	7.7 ± 1.9	53	6.6 ± 1.4
2	blank	15	0.7 ± 0.9	11	1.4 ± 1.6
3	blank	20	0.9 ± 1.4	5	0.6 ± 0.5
4	blank	20	0.9 ± 1.1	11	1.4 ± 1.2

Ten adults were tested in one replicate; they were counted after 48 hours.

Table 6.16 The mean numbers (*n*) per m^2 in autumn litter samples and spring emergence samples, the proportion of living beetles in the autumn samples, and the overwintering survival (density spring emergents/density in autumn), for *Hippodamia tredecimpunctata* (H13), *Coccinella septempunctata* (C7), *Coccinella transversoguttata* (CT), *Hippodamia convergens* (HC), and *Hippodamia parenthesis* (HP), and all coccinellids in the beach-ridge forest at the Delta Marsh Field Station, Manitoba, Canada (Turnock & Wise 2004).

Species		1992 Autumn	1993 Spring	1993 Autumn	1994 Spring	1995 Spring
H13	n/m^2	47.3	17.5	65.6	9.1	27.9
	Survival	0.95	0.37	0.97	0.14	—
	n	355	54	492	28	86
C7	n/m^2	31.3	0.65	45.3	2.3	1.9
	Survival	0.72	0.02	0.73	0.05	—
	n	235	2	340	7	6
CT	n/m^2	6.9	0.32	8.5	0	0
	Survival	0.37	0.05	0.16	0	—
	n	52	1	64	0	0
HC	n/m^2	101	0	2.1	0	0
	Survival	0.38	0	0.5	0	—
	n	8	0	16	0	0
HP	n/m^2	0.4	0	0.13	0	0
	Survival	0.67	0	1	0	—
	n	3	0	1	0	0
Total	n/m^2	87.1	18.5	122.4	11.7	30.5
	Survival	0.81	0.21	0.81	0.1	—
	n	653	57	913	36*	94*

*Includes one specimen of *Anisosticta bitriangularis* in 1994 and two specimens of *Calvia quatuordecimguttata* in 1995.

evolved as a means of **defence against predation**. Above a certain minimum group size, group members have a lower rate of death from predation than solitary individuals (Sillén-Tullberg & Leimar 1988).

Both **monospecific** and **heterospecific aggregations** of coccinellids may be formed. As a general rule, only monospecific clusters are formed whenever several species hibernate in the same habitat (McMullen 1967a), though the presence of a few adults of other species has been reported. Only twice have mixed clusters of *Cer. undecimnotata* with *A. bipunctata* been observed. It is more usual to find heterospecific aggregations of *C. septempunctata* with *Cer. undecimnotata* in grass tussocks on the tops of hills (Hodek 1960), especially where rocks are missing. Pulliainen (1966) has reported that *Scymnus suturalis* and *Aphidecta obliterata* were admixed to aggregations of *Myrrha octodecimguttata*. Kuznetsov (1977) found multispecies aggregations most often when *Har. axyridis* and sometimes also *Oenopia* (= *Synharmonia*) *conglobata* were together with *Aiolocaria hexaspilota (= mirabilis)*, as well as other insects. Heterospecific aggregations of overwintering coccinellids (Table 6.16) were found in leaf litter in late October in a beach-ridge forest on the southern shore of Lake Manitoba (Manitoba, Canada) (Turnock & Wise 2004).

Some of the mass aggregations, e.g. those washed up on beaches (Lee 1980a) are, however, not directly related to diapause, but originally produced by hunger and thirst when the emergence of large numbers of ladybirds coincides with the disappearance of aphids in the landscape (Chapter 5.4.1.5).

Transmission between beetles of the ectoparasitic **fungus** *Hesperomyces virescens* between beetles occurred during the hibernation of *Har. axyridis* in aggregations. It is both the social contact in multi-layered piles in aggregations and mating that enables such transmission in the winter (Nalepa & Weir 2007; Chapter 8.4.3).

6.3.1.5 Emergence from dormancy sites

At the end of dormancy, the beetles gradually change their behaviour. The inactivity in the hiding places changes to **slight mobility**, sometimes already long before dispersal. This final stage is conspicuous and has often been observed in coccinellids hibernating in litter at forest edges. Here coccinellids stay for a prolonged period on the trees (especially on young pines) in the dormancy site **before dispersal** (e.g. Bielawski 1961; 6.3.2.3). During this closing period of dormancy, the coccinellids have often been observed **mating**. Mating was verified by dissection of the spermathecae before dispersal from the hibernation sites of *Cer. undecimnotata* and *C. septempunctata* (Hodek & Landa 1971, Hodek & Ceryngier 2000, Ceryngier et al. 2004) and of *Col. maculata* (Solbreck 1974). About half of *C. septempunctata* females already had sperm in their spermathecae in September when they were in early diapause in their hibernation site (Hodek & Ceryngier 2000, Ceryngier et al. 2004; 6.4.1.2).

Dispersal from dormancy sites continues **over several weeks** (Hodek 1960, Bielawski 1961, Savoiskaya 1965, McMullen 1967a). The onset and progress of dispersal is regulated by the ambient temperature, as the second phase of dormancy is simple quiescence (6.1). Usually a prolonged **increase of average temperature** over 10°C is required to induce the beetles to emerge (Hodek 1960). In the laboratory, the distance walked by diapausing *C. septempunctata* was recorded; the best correlation for mobility was with a previous exposure of 10–15 days to the increased temperatures (Ruzicka & Kindlmann 1991).

Different species, even if they have hibernated at the same site, **do not disperse simultaneously**. In Bohemia (western Czech Republic) *A. bipunctata* is the earliest species, followed by *C. quinquepunctata*, *P. quatuordecimpunctata* and *Tytthaspis sedecimpunctata*, then by *C. septempunctata* and *Hip. variegata*. while *Cer. undecimnotata* leaves very late (Hodek 1960). A similar, but not identical picture was reported by Bielawski (1961) at a hibernation site under young pines in Poland (Warszawa–Bielany). *Adalia bipunctata*, *A. decempunctata* and *C. quinquepunctata* leave about a month earlier than *C. septempunctata* and *P. quatuordecimpunctata*. Whereas these two observations agree that *A. bipunctata* is an early and *C. septempunctata* a late disperser, Banks (1955) reported the opposite observation from England: *C. septempunctata* appeared on nettles one month earlier than *A. bipunctata* and *P. quatuordecimpunctata*. However, for *C. septempunctata* the nettles were probably the first habitat visited after dormancy, while *A. bipunctata* and *P. quatuordecimpunctata* (due to their habitat preference; Chapter 4) had previously spent some time in another habitat of shrubs or trees.

The flight of coccinellids from hibernation sites to breeding habitats is considered a **dispersal flight** (Hodek et al. 1993; 6.3.1.3). It is not a long-distance flight, but usually a **step-wise process**. Beetles of several species have been observed to fly from the dormancy site only to adjacent or nearby fields at first (Hodek 1960, Okuda & Hodek 1983). If suitable aphids (Chapter 5.2.2) are there in sufficient quantity, the coccinellids will only gradually disperse further afield. In the dispersing beetles, the previous great depletion of reserves during dormancy would most probably cause hunger; thus the dispersal flight may gradually change into the **normal, trivial (appetitive) flight** (Hodek et al. 1993).

6.3.2 Behaviour of individual species

For a detailed survey on behaviour of ladybirds related to phases of dormancy and the difference between climatotactic and hypsotactic aggregators, see Hagen's (1962) classic review. A comprehensive survey of varied hibernation habitats of ladybirds in Britain is on pp. 158–159 in Majerus's (1994) book.

6.3.2.1 *Ceratomegilla (=Semiadalia) undecimnotata*

The migratory and aggregation behaviour of this species is similar in the three regions of central Asia (Dobzhansky 1925, Radzievskaya 1939, Yakhontov 1966), the Czech and Slovak Republics at the northern limit of the distribution area (Hodek 1960, 1967) and southeastern France (Iperti 1966b). The dormancy sites are situated **at prominent features on hills or mountains** (large rocks, heaps of stones, shrubs or other plants, or artificial structures, such as posts, triangulation points, but only very rarely buildings or their ruins). It is assumed that such sites are chosen as the result of **hypsotactic responses** (6.3.1.2). *Ceratomegilla undecimnotata* prefer rock cracks, especially those exposed to wind, where their mortality is reduced. In the absence of such cracks, the beetles hibernate at the base of bushes, but then there is a great danger of mycosis. The petrography of the site is not crucial, hibernation quarters can be found both on limestone and on igneous rocks (e.g. basalt). The **altitude** of the site above sea level varies: in central Asia it is much higher (2000 m) than in the Czech Republic and Slovakia (400–900 m) or in France (usually above 700 m). The majority of dormancy sites in these regions are situated on a **southwest aspect**. According to Iperti (1966b) the attraction of this aspect may be the afternoon insolation of these sites (6.2.8).

The coccinellids arrive at the hibernation places over a period of several weeks on warm calm days and leave similarly. At the onset and end of hibernation they creep around close to the shelter during the day, forming numerous small clusters during the night or colder spells. For the cold part of the period of dormancy they aggregate in a few large clusters. Their spring **emergence** depends on the **ambient temperature**. In the Louny-hills (northern Bohemia) emergence occurs after a period of mean temperature at 12–14.5°C (Hodek 1960). Yakhontov (1962) assumed that a certain degree of gonad maturation is the signal for emergence. In Bohemia, however, females leaving the hibernation sites had ovaries at different stages of maturation. The females leaving the hibernation sites early lack the stage of the first oocyte. If they disperse later, this stage has already been attained at the dormancy site. In males, the tissue of the testicular follicles is already fully active 2–4 weeks before emergence and the males fertilize the females at the dormancy site (Hodek & Landa 1971, Hodek & Ceryngier 2000, Ceryngier et al. 2004; 6.4.1.3). Year after year, *Cer. undecimnotata* uses the same dormancy sites if the silhouette of the hill has not changed.

6.3.2.2 Hypsotactic species *Harmonia axyridis*, *Har. conformis* and *Aiolocaria hexaspilota (=mirabilis)*

Harmonia axyridis has dormancy behaviour similar to that of *Cer. undecimnotata*. After a short report from east Siberia (Telenga & Bogunova 1936), the first detailed study was undertaken in **Japan**, near Kyoto. All 14 hibernation sites visited by Obata (1986) were situated near **prominent features** of a whitish or light colour. When the beetles were offered black, red, green, yellow and white boards near one of the aggregation sites below a large rock at the top of Mt. Shiroyama, they landed mostly on the white boards. The white board was more attractive when the sun shone brightly. Obata (1986) and Obata et al. (1986) suppose that the whitish or light colour may be an indication of a dry site (but see Nalepa et al. 2005 below). Obata's observation that *Har. axyridis* adults changed flight direction towards the white board from some distance is important; thus their landing appears to be directional and visually oriented (Obata 1986).

After its successful introduction to the USA and Canada, and the subsequent increase in population, *Har. axyridis* has become a **nuisance by entering buildings** in search of dormancy shelter (Koch & Galvan 2008). Sometimes the beetles **also** use **partly open structures**. Since 1993 repeated winter aggregations of thousands of *Har. axyridis* have been observed in a concrete observation tower (20 m high) built on the crest of a ridge (at 326 m) in south-central Pennsylvania. During very cold winters (e.g. 1994) a majority of the beetles died (Schaefer 2003; see 6.4.4).

In habitations, *Har. axyridis* are totally unwelcome. They irritate people just by their mass presence, but also provoke allergic reactions. This problem has initiated an increased interest in the ethological aspects of their hibernation with the practical aim of preventing beetles entering buildings. A series of studies in the USA focussed on the **orientation** of *Har. axyridis* to their dormancy sites. Nalepa et al. (2000) **contested distant orientation** of arriving beetles **to** the **conspecifics** present in these sites. They found little evidence for volatile aggregation pheromones, but did not exclude contact chemoreception with those conspecifics that had arrived earlier. Thus the suggestion that pheromones are important for the orientation of the beetles remains open (9.7).

Important progress in our understanding of the behaviour of *Har. axyridis,* when searching for dormancy sites, has come from a field study in which black stripes on white backgrounds but with different degrees of contrast were offered to **alighting ladybirds**. The beetles significantly **preferred** the targets with **contrast** (Nalepa et al. 2005). This showed that *Har. axyridis* is **not attracted to white colours per se**.

This pre-dormancy flight occurred when the temperature rose above 21°C with peaks between 14.00

and 16.00 hours, shifting to earlier hours as the season advanced (Nalepa et al. 2005). In Monticello, Florida, *Har. axyridis* adults arrived at the hibernation sites as late as mid-November to early December, due to the extended summer season (Riddick et al. 2000). This indicates a multivoltine life cycle (6.2.9).

To deter the invasion of homes by *Har. axyridis*, menthol or camphor were used in Maryland and Florida to treat the crevices through which the beetles enter houses. Camphor was more efficient than menthol, but both substances evaporated rather quickly (Riddick et al. 2000).

The dormancy behaviour in eastern Australia of the introduced species ***Harmonia conformis*** has also been described as **hypsotactic** (Hales et al. 1986). Both in summer and winter the aggregating coccinellids were found on mountain tops (800–1000 m) in crevices of radio/TV towers and telegraph/beacon poles, till 22° N (in Queensland). On one occasion a 1 m × 1 m × 0.5 m box was completely filled with dormant *Har. conformis*.

Aiolocaria hexaspilota, a univoltine predator of chrysomelids which lives in the Ussuri region of **east Siberia**, also uses **cracks** in **rocks on bare hills** as dormancy sites (Kuznetsov 1977). From late August to early September the beetles fly first from the forests to their edges and land on well-insolated trees. Only later, after the first spells of frost, when the average temperature falls to around 12°C, do the beetles migrate to well-insolated slopes of rocky hills or also to buildings. Exceptionally, the earliest beetles may arrive in late August but usually the migration occurs from late September to early October (Table 6.17). On the hills, the aggregation sites are situated at any altitude, on the southern, southwestern or western slopes and the site is determined by the presence of rocks. Clusters from 5–20 to several hundred individuals occur in the crevices which may be as deep as 2 m. In such crevices the temperature is much higher than ambient: even if the temperature falls to as low as −32°C, it does not decrease below −6°C at 50 cm deep in a crevice. In the spring the adults mate and disperse in May at an average temperature of 10–12°C.

Table 6.17 Arrival of adults of *Aiolocaria hexaspilota* to their dormancy sites on a rocky hill in the Ussuri region of east Siberia (monitored area 0.25 m^2, time of counting 20 min) (Kuznetsov 1977).

Date	Number of beetles counted at given hours					
1970	8	10	12	14	16	18
28 Sept.	0	8	32	25	21	1
29 Sept.	0	19	38	69	29	5
30 Sept.	0	21	15	87	20	6
2 Oct.	0	17	27	80	19	13
3 Oct.	0	7	18	41	8	4
6 Oct.	0	2	24	31	2	0
9 Oct.	0	0	1	3	0	0

6.3.2.3 *Coccinella septempunctata* and other species dormant in the litter

Attention should be drawn to the fact that old references to hibernation of *C. septempunctata*, which describe mass assemblages between rocks on mountain summits (for earlier references see Hodek 1960) may refer to *Cer. undecimnotata*. The seven-spotted form of *Cer. undecimnotata* is often confused with *C. septempunctata*, even by professional entomologists. G. Iperti (unpublished) has verified that such confusion occurred in Fabre's (1879) data from Mont Ventoux and St. Amand (France). The same confusion has probably occurred elsewhere.

Dormancy behaviour of *C. septempunctata*, in contrast to that of *Cer. undecimnotata*, is heterogeneous even within the same geographical area. The only safe generalization is that *C. septempunctata* always hibernates **on the ground**: under stones, in litter, in holes in the soil surface, near the base of plants in grass tussocks, but never in the cracks of tree bark or walls – in contrast to *A. bipunctata* (6.3.2.5). The species mostly forms only small- or medium-sized aggregations, not exceeding tens of beetles. In lowlands the usual dormancy sites of *C. septempunctata* are situated at forest edges, in clearings or in windbreaks. If no such site is close by, *C. septempunctata* hibernates near isolated shrubs or other plants, or on slight unevennesses of the terrain. No special differences seem to occur between individual countries (Hodek 1996).

The flight of *C. septempunctata* to dormancy sites is often step-wise, particularly when the beetles have not completed feeding and continue to forage on the way (Nedvěd et al. 2001). Such pre-hibernation roving of *C. septempunctata* adults was monitored in a detailed way over 5 years (1998–2002) in **central Italy** (Ricci et al. 2005). After the field crops matured and the aphids there had disappeared, the beetles moved to wild plants in increasingly elevated habitats: to about

1250 m in early July and later up to 1800 m. Their summer feeding on several species of aphids, pollen and spores of fungi was observed and quantified by gut analysis. In the period between mid-June and mid-July, less than 40% of adults had aphids in their gut. This percentage increased again later. Also in central Italy, individual matings may be observed during summer, which provide sperm for storage by the females during winter – similar to findings in central Europe (6.4.1.2, 6.4.1.3). In August some adults stop feeding, enter diapause and aggregate under stones on mountain tops or in plants, e.g. among the leaves of *Verbascum thapsus* (Ricci et al. 2005).

After their arrival at the dormancy site, *C. septempunctata* remain clustered on the plants close to their subsequent hiding places (during August and September in **Bohemia**) and only hide gradually as the air becomes cooler. Very often they can be found at the ends of twigs of young pines (Hodek 1960, Bielawski 1961, Semyanov 1965a,b, Klausnitzer 1967) or between the ear spathes of maize plants left after harvest (Hemptinne 1988). In a similar way they stay on the vegetation near the hibernation site before their dispersal in spring.

The largest aggregations of dormant beetles have been found in mountains or on hills (Hodek et al. 1977, 1989, Honěk 1989, Hodek & Okuda 1993, Honěk et al. 2007). The populations from **montane** and lowland dormancy **sites** differ. **Intensity of diapause** (as measured by duration of oviposition delay; 6.1) was much **greater** in individuals from 1500 m (peak of Mt. Cousson) than in the beetles sampled from a nearby hill of about 600 m (both sites in the region of Digne, France) (Hodek et al. 1977; 6.2.1.2). The coccinellids from about 1400–1500 m (peaks of the Giant Mountains = Krkonose, northern Bohemia) were much larger than those in lowland hibernation sites at forest edges, at about 300–400 m (Honěk 1989). *Coccinella septempunctata* has a plastic life cycle (6.2.1; Tables 6.1 and 6.3) which is again reflected by the above differences. Presumably the beetles entering diapause earliest in the season are those individuals with a strong tendency to a univoltine life cycle. Honěk (1989) and Honěk et al. (2007) assume that larger individuals which develop earlier in the breeding season have a better ability to complete long range flight. Thus the larger individuals with deeper diapause occupy the dormancy sites in mountains.

Winter **survival** of *C. septempunctata* was recorded in artificial hibernacula from early November 1993 to early April 1995 in Bohemia. Hill top populations from the Giant Mountains (Krkonose, 1480 m) and Rana (400 m) were compared with those from the lowland (300–360 m). One particular site at 1100 m was not on a hill top but in a meadow. An interesting difference was found between the two altitudes. A high proportion in the meadow of small individuals, less capable of long flight, indicated poor nutrition of larvae (and/or pre-hibernation adults) and presence of local ladybirds. In this sample medium sized individuals had only 1.7% survival, while in the high altitude sample the survival was 83.3% in medium sized males and 86.7% in medium sized females (Zhou et al. 1995, Honěk 1997). As the cause of mortality was not studied, it cannot be excluded that the high altitude hill top sample was less prone to fungal infection (Chapter 8.4.3) and this contributed to higher survival.

Of the common coccinellids, ***C. quinquepunctata*** has a hibernation behaviour similar to that of *C. septempunctata*. However, it is found far more often than the latter on young pine trees at forest edges (Hodek 1960, Bielawski 1961, Semyanov 1965a, b, Klausnitzer 1967). It is reported from shingle banks (Majerus 1994), and can also be found among small stones on hills (e.g. the Louny-hills in north Bohemia) used as hibernation quarters by *Cer. undecimnotata* and *C. septempunctata* (Hodek 1960 and unpublished). *Coccinella quinquepunctata* and *P. quatuordecimpunctata* were the most abundant species hidden in pine cones during hibernation (Ruzicka & Vostrel 1985) on the same hills.

Some **other species** are usually present in similar hibernation sites on the plain (the litter or upper soil layer at forest edges) (Hodek 1960, Bielawski 1961, Semyanov 1965a, b, Klausnitzer 1967, Novák & Grenarová 1967). Such species are *Coccinula quatuordecimpustulata*, *P. quatuordecimpunctata*, *Hip. variegata* (in dry places), other *Hippodamia* spp. (if moist habitats are nearby), the phytophagous *Subcoccinella vigintiquatuorpunctata* and the mycophagous *Psyllobora* (= *Thea*) *vigintiduopunctata*, especially in rather moist places. *Tytthaspis sedecimpunctata* formed the largest aggregation (over 10,000 individuals) ever found in Britain (Majerus & Kearns 1989). Evans (1936) found a dormancy aggregation of ***Tytthaspis sedecimpunctata*** on a wooden post in Berkshire, England in December. Legay & De Reggi (1962) found aggregations (50–150 individuals per 100 m^2) of the same species (together with 1% of ***Psyllobora vigintiduopunctata***) near Lyon, France under litter at the base of trees on a small rise overtopping the surrounding fields by only 20 m. Whereas in central Europe *T. sedecimpunctata* is usually found in dry places, in central Italy its usual hibernation sites are the wild vegetation of ditches and embankments. In central Italy this habitat is also used for hibernation by ***Rhyzobius litura*** which, however, remains there also during the breeding period; at least 90% of the apterous population do so (Ricci 1986).

The Australian species ***C. leonina*** was found in similar dormancy sites as *C. septempunctata* both in summer and winter: in soil cracks, near the roots of herbaceous plants, under pieces of cloth, and once at the top of a hill close to its feeding habitats (Hales et al. 1986; also 6.2.10). In addition to the above species, Savoiskaya (1983, p.174) reported from Kazakhstan that *Vibidia duodecimguttata*, *Bulaea lichatschovi*, *Exochomus flavipes* and *Parexochomus melanocephalus,* and from Siberia that *C. hieroglyphica* and *Hip. septemmaculata,* also hibernated in litter or in the upper soil layers.

Besides the above species for which litter in the widest sense typically provides shelter, *Calvia quatuordecimguttata* and *Exochomus quadripustulatus* sometimes also occur in litter. These two species are equally abundant in bark crevices. Litter also serves as the dormancy site for *Stethorus pusillus* (Putman 1955, Berker 1958, Savoiskaya 1983) and *S. punctum punctum* (Felland & Hull 1996), although some authors report *Stethorus* spp. hibernating in the bark (Moter 1959).

Hibernation behaviour of the abundant ***P. quatuordecimpunctata*** was not well known until the detailed study on populations from an agricultural but extensively wooded area of the Province of Hainaut in western Belgium (Hemptinne 1988). Hemptinne compared litter samples from the forest edge and 20 m into the forest, and found that *P. quatuordecimpunctata* preferred the inner site for hibernation, with 1.11 beetles/m^2 at the edge compared with 1.41 beetles/m^2 inside the wood. By contrast, *C. septempunctata* preferred the edge (0.98 beetles/m^2) to the interior of the wood (0.78 beetles/m^2), and an orientation facing south or west. The compass orientation was not important for *P. quatuordecimpunctata*. The well-known preference for higher elevations was again shown by *C. septempunctata* but was less obvious with *P. quatuordecimpunctata*. In Poland *P. quatuordecimpunctata* hibernates more often in pine than in oak litter (Bielawski 1961). For England, Majerus (1994) reports a diversity of hibernation sites near to the ground, and small groups of 2–3 *P. quatuordecimpunctata* are found.

6.3.2.4 *Coleomegilla maculata*

Moist places are preferred by *Col. maculata* (Hodson 1937, Solbreck 1974, Benton & Crump 1979, Jean et al. 1990, Roach & Thomas 1991). The vertical distribution of active adults on the plants and their susceptibility to desiccation also indicate a preference for higher humidity (eg. Ewert & Chiang 1966; Chapter 4). The migration to dormancy sites occurs at low level and the aggregations are found at or near the edges of open fields at the base of dominant trees (Parker et al. 1977, Benton & Crump 1979). Three dormancy sites, described by the latter authors in New York State, were at the base of willow trees and a fourth was at the base of a large poplar. In one case the aggregation was located near a small pond. Also a large aggregation near Montréal (D. Coderre, unpublished) was situated below a large willow tree near a small reservoir. In Vermont, USA, the attractive trees were mostly maples (Parker et al. 1977) and pecans in the upper coastal plain of South Carolina (Roach & Thomas 1991). All these sites were repeatedly visited by the beetles year after year. In *Col. maculata* (similarly to *Hip. convergens*) the orientation to the dormancy sites is mainly by **climatotaxis**, and it is most probable that one of the major factors is humidity. During the influx, the beetles in New York alighted on undergrowth vegetation, including wild raspberry (*Rubus occidentalis*) and sensitive fern (*Onoclea sensibilis*). With drops in temperature, the beetles moved down to the ground. According to Roach and Thomas (1991) the hibernation sites are characterized by a deep layer of largely decomposed organic matter. In the mid-Atlantic states of the USA, *Col. maculata* overwintered in the greatest numbers in leaf litter in windbreaks that were adjacent to maize fields. This type of overwintering site was preferred to forest edges adjacent to maize (Nault & Kennedy 2000).

While the migration to hibernation sites lasts only 1–2 weeks, the spring dispersal is much longer (Solbreck 1974, Benton & Crump 1981). Adults that had overwintered were observed to feed on pollen at or near the dormancy site, on *Populus*, dandelions or cowslip (*Caltha palustris*) (see also 6.3.1.3).

6.3.2.5 *Adalia bipunctata*

This coccinellid is known in early dormancy for frequent invasions of buildings, where it appears in cracks in walls, in lofts, or behind windows and even in rooms (e.g. Hawkes 1920, Semyanov 1970). However, recently it has been replaced by *Har. axyridis* in the USA and western Europe (6.3.2.2). It can be assumed that the coccinellids are brought to buildings by their hypsotactic behaviour. Such behaviour is also indicated by a small aggregation of *A. bipunctata* (20–30 individuals) found close to a large aggregation of *Cer. undecimnotata* on a hill top (I. Hodek, unpublished).

However, a fair proportion of *A. bipunctata* apparently does not leave the orchard, park or forest habitats, and hibernate together with *Chil. bipustulatus* and *Stethorus pusillus*, either in crevices in tree bark or even in paper bands around trees (Speyer 1934).

Mass hibernation by *A. bipunctata* in the thick bark of old Tyan-shan spruces (*Picea schrenkiana*) is described by Savoiskaya (1983) from Kazakhstan. Every autumn, the beetles migrate in masses to the same trees in mountain valleys, flying up the valley; a rate of 50–55 beetles/min was observed in late September. It was noticed that they turned back sharply just behind the tree (evidently pushed there by air turbulence), swarmed in the wind shelter and dropped rapidly onto the trunk. Up to 6000 beetles were found on one tree. Similar sites on *Picea* were also used by ***A. bipunctata*** *(=fasciatopunctata)* and by ***Oenopia conglobata***. The latter species was most abundant up to 1500 m, while *Adalia* species preferred higher altitudes. Winter aggregations of *O. conglobata* and ***Halyzia tschitscherini*** were found in the bark crevices of poplars in the valleys of montane rivers in Kazakhstan (Savoiskaya 1983). Aggregations of up to 200 *A. bipunctata* adults were observed by Smee (1922) on elm trees (*Ulmus*) in England (as many as 1000 beetles on one tree). Smee was the first to succeed in attracting coccinellids to cages containing tree bark, i.e. artificial hibernacula. In Belgium, *A. bipunctata* often overwinters in poplar plantations with a preference for the trees on the southern edge. Most of the aggregations (mean size, 2–6 individuals; range, 1–36) are situated in bark crevices on the south-west sides of the trees (Hemptinne 1985, Hemptinne & Naisse 1988).

Adalia bipunctata, being a multivoltine species, enters diapause relatively late (in early October in Germany; Speyer 1934) and emerges very early in spring (in March in Belgium; Hemptinne 1985). *Adalia bipunctata* is often found together with *Chil. bipustulatus* (Speyer 1934, Bielawski 1961, Savoiskaya 1965).

6.3.2.6 *Myrrha octodecimguttata* and other forest species which hibernate in bark crevices

Bark crevices of Scots pine and other conifers are reported as hibernation sites for *Har. quadripunctata*, *M. octodecimguttata* and *Aphidecta obliterata* (Majerus 1994). According to this author, the dormancy sites of ***Myzia oblongoguttata*** and ***Anatis ocellata*** are unknown in England. Not much is known about the hibernation of other coccinellid species of coniferous forests. According to recent observations, they do not seem to leave their breeding habitat.

Myrrha octodecimguttata, which lives and breeds in the crowns of pine trees in Germany (Klausnitzer 1968), hibernates in bark crevices of old pines; this has been established also for Poland by Bielawski (1961), for Finland by Pulliainen (1963, 1964, 1966) and for England by Majerus (1994). Pulliainen observed that the coccinellids preferred to hibernate in the lowest 10 cm of the trunk (93% of beetles) and the south and east sides of the tree (62%). On these sides the tendency to aggregate was most pronounced; the aggregations averaged 3–8 individuals (maximum 14).

Bark crevices of old pines are used as dormancy sites by many other forest species (but 6.3.2.5) including: ***Scymnus suturalis*** (Delucchi 1954, Pulliainen 1966, while Bielawski 1961 found this species more frequently in the bark crevices of deciduous trees), ***Scymnus nigrinus*** (Bielawski 1961), and ***Aphidecta obliterata*** (Pulliainen 1966, Parry 1980). ***Exochomus quadripustulatus*** uses bark crevices and litter with about equal frequency for its dormancy site (Bielawski 1961, Klausnitzer 1967). Other conifers than pines may also be visited, e.g. cedar by *Hip. tredecimpunctata* (Thomas 1932). Some coccinellids prefer the bark crevices of deciduous trees, e.g. *Nephus quadrimaculatus* prefers chestnuts (Bielawski 1961), and *Oenopia conglobata* and *Har. quadripunctata* chestnut and poplar (Bielawski 1961; I. Hodek, unpublished).

6.3.2.7 *Hippodamia convergens*

The progeny of diapausing beetles emerge from pupae in early May. If there are not enough aphids nearby, they migrate long distances towards the mountains (in northern California to the Sierra Nevada). Hagen (1962, 1966) proposed a hypothesis to explain the mechanism of migration (Fig. 6.8; 6.2.7). Migration is initiated by take-off during the calm of morning: the subsequent vertical upward primary flight is apparently assisted by convection currents. This goes on as long as adequate warm temperature permits the beetles to fly. At the lower temperature threshold of about 11–13°C flight is inhibited and the beetles fall about 300 m into a warmer layer of air (13–18°C) where flight is resumed. The beetles which are thought to undergo these vertical oscillations are simultaneously blown sideways. Each day during the late morning in May and June, westerly **winds carry the beetles** toward the mountains (Fig. 6.8). The migration is terminated when the air currents contact the mountains (Hagen 1962).

If aphids are found near the landing place, the beetles produce eggs there. Usually, however, they feed on a non-insect diet (e.g. pollen) and build up reserves. After a week or so the coccinellids exhibit **secondary, directional flight** near the ground, flying up and down mountain creeks in search for **summer aggregation** sites. There the adults remain rather inactive until October, when they become mobile again, presumably disturbed by the first rains. The **tertiary flight** during warm periods usually leads them to lower parts of the creek where new, larger **overwintering aggregations** are formed (Hagen 1962).

Hippodamia convergens adults are guided in their search for the aggregation sites by a series of factors. In contrast to *Cer. undecimnotata*, they do not always respond to prominent objects. Sometimes assemblages of *Hip. convergens* are found at mountain tops (Douglass 1930, Throne 1935, Sherman 1938, Stewart et al. 1967), but according to Hagen's (1962) hypothesis they reach summits in a semi-passive way. **Moisture and light** are considered to be the most important factors influencing **selection of aggregation sites**. At first the coccinellids assemble on bushes and trees in large clearings near creeks. After several days they move close to the creeks, and settle down along the banks, often in spots exposed to afternoon sunlight. Thus the aggregations are formed in similar places year after year, but also new clearings are occupied. The beetles exhibit a marked preference for litter with 20% moisture (Hodson 1937). Hagen (1962) assumed that *Hip. convergens* **drinks water** in order to maintain a constant water content and that a physiological requirement for free water may be the basic characteristic of **climatotactic aggregators** *(Hip. convergens, Col. maculata)* in contrast to hypsotactic species such as *Cer. undecimnotata* (6.3.2.1).

On the eastern edge of the Rocky Mountains, massive aggregations of *Hip. convergens* (mixed with the curculionid *Hypera postica*) were found below stones about 1 m above the edge of the water of an irrigation reservoir in mid-March. Most ladybirds dispersed before the end of March (Simpson & Welborn 1975). This finding might also indicate the preference for moist habitats in dormant *Hip. convergens*.

Large winter colonies (from one, about 40 million beetles have been collected) are formed by many noncontiguous small aggregations under leaves, at the bases of bushes, and on tree trunks. At higher altitudes, snow often covers *Hip. convergens* aggregations for about three months. The majority, however, hibernate near the snow line in the Sierra Nevada, and during most of the winter they are not covered with snow (Hagen 1962).

The beetles aggregate in canyons and are thus in shade. When they become heated to above 14°C in the early spring, they take off vertically and are eventually caught up by winds (blowing in the opposite direction to that in summer) which carry them over ridges that lie to the west between the aggregation sites and the plains. The fall in air temperature in the evening brings the beetles to the ground, terminating the flight. Not all coccinellids, however, get so far. The beetles that overwinter on the highest peaks of the Sierra are found after dispersal to have reached only the mountain valleys just below these peaks (Hagen 1962).

Hagen's findings were complemented by **laboratory experiments** using tethered flight (Rankin & Rankin 1980). Individual coccinellids were suspended from their pronotum by a toothpick attached with melted wax and were **stimulated to fly** by a low-speed fan; the duration of flight was measured. **Long tethered flight** (>30 minutes) was considered a good **indication of migratory behaviour** (6.3.1.3). *Hippodamia convergens* was found to be a typical migrant: the long flights are post-teneral and pre-reproductive in females, and are associated with adult diapause. Starvation greatly enhances migratory behaviour and, under optimal feeding conditions, short daylength has the same effect (Rankin & Rankin 1980). Optimal feeding for 7 days induces maturation of ovaries and greatly reduces the tendency to long tethered flight.

To monitor the **tendency to long flights in the field**, active beetles were collected in aphid-infested fields from March to June and diapausing beetles were sampled on the top of a mountain from July to December (Fig. 6.9). The adults, newly arrived at the fields in

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Figure 6.9 Flight activity of *Hippodamia convergens* and monthly precipitation in 1977 (dashed line) (from Rankin & Rankin 1980). The adults were collected in fields around Austin (open circles, solid line) and on top of Mt Locke, Davis Mts, W. Texas (closed circles).

March, had a relatively high proportion (50%) of individuals with a migratory tendency, which decreased to about 20–30% in the next two months. By late May and June, about 60% of coccinellids again displayed long tethered flight. The beetles behaved similarly when sampled in the field (before migration) and at the mountain top, early after their arrival at the dormancy site. The incidence of migratory tendency then dropped to about 20% in August and to less than 10% in the last months of the year (Fig. 6.9; Rankin & Rankin 1980).

6.3.2.8 *Hippodamia quinquesignata*

The location of hibernation sites by *Hip. quinquesignata*, as described by Edwards (1957) and Harper and Lilly (1982), seems also to be **hypsotactic** as in *Cer. undecimnotata*. At least all aggregations described were found in the mountains at altitudes ranging between 1677 m and 3354 m in the USA (Edwards 1957) and 1250–2744 m in Alberta, Canada (Harper & Lilly 1982). *Hippodamia quinquesignata* uses the same aggregation sites each year. They are usually located on upper, exposed slopes (often west-facing) with sparse vegetation and covered with rocks. The beetles are found under rocks and debris, in crevices in rocks, logs or pine cones, or at the base of junipers. Sometimes ***Hip. caseyi*** is admixed in small numbers.

In spring, mating occurs before dispersal. In California, however, *Hip. quinquesignata* does not migrate long distances and spends its **facultative** summer **diapause**, as well as its winter diapause, at the edges of fields. Thus the adults can easily **respond to** later increases in **aphid numbers** by producing summer and autumn generations (Hagen 1962, Neuenschwander et al. 1975).

6.3.2.9 Hibernation of mycophagous and phytophagous species

Hibernation in aggregations is most common in entomophagous coccinellids but also two mycophagous species have been reported hibernating in aggregations in litter. In mid-April Ruscinsky (1933) found four large aggregations of ***Vibidia duodecimguttata*** among fallen leaves at the base of trees on top of a hill in erstwhile Rumania (Bessarabia). Each aggregation covered about 1 m^2 and consisted of some 2500 individuals. ***Psyllobora* (=*Thea*) *vigintiduopunctata*** was often found in moist litter at forest edges visited by *C. septempunctata* and related species (Hodek 1960, Bielawski 1961) (6.3.2.3).

Aggregations have also been observed in **phytophagous coccinellids** in the subfamily Epilachninae. In Africa, Poulton (1936) observed aestivation aggregations of immobile ***Epilachna dregei***, once in Bechuanaland and once in northern Uganda on prominent features (a hill, a termite-hill) in mid-July, i.e. in the middle of the dry season. In February, Kapur (1954) found four aggregations of several thousand individuals of ***Aphidentula bisquadripunctata*** *(=Epilachna)* at an altitude of about 400 m in India (Chota Nagpur, Bihar) at the base of grass c. 0.7 m high, in the vicinity of an almost dry brook. The coccinellids were in diapause (empty guts, a large fat body, unripe ovaries). In both *Epilachna* spp. observed, hypsotactic aggregation may have been involved. Ghabn (1951) mentions winter migrations of ***E. 'chrysomelina'*** from the fields into the surrounding desert in Egypt without, however, describing the hibernation sites.

6.4 ANATOMICAL AND PHYSIOLOGICAL CHANGES RELATED TO DORMANCY

6.4.1 Anatomical state

6.4.1.1 Fat body and digestive tract

A greatly enlarged fat body and voided digestive tract are the conspicuous features of diapausing coccinellids of both sexes. The characteristics of midguts in *C. novemnotata* are typical for all coccinellid species: the midguts of dormant beetles are reduced to whitish, opaque, thick-walled tubes containing a brown fluid (McMullen 1967a).

In *C. s. brucki*, an important difference between feeding and dormant adults was found in the **proteolytic activity** and **ultrastructure of the midgut** (Morikawa et al. 1989, Sakurai et al. 1991). Trypsin activity was very low in summer and completely inhibited in January. The columnar cells of the midgut epithelium of active beetles contained abundant rough endoplasmic reticulum (RER) and the **mitochondria** showed distinct cristae. In dormant coccinellids the mitochondrial cristae were obscure and RER was rarely found. These differences are evidently due to feeding versus absence of feeding; they are identical in the aestival diapause and winter quiescence (6.2.2).

6.4.1.2 Ovary, spermatheca

The **developmental progress** of ovaries was quantified in *Har. axyridis* on a six-stage scale (Obata 1988a, b, Osawa 2005): (1) no oocytes; (2) one oocyte; (3) two whitish oocytes; (4) two oocytes, with the basal oocyte filled with whitish yellow yolk; (5) two oocytes, with the basal oocyte with yellow yolk; (6) the basal oocyte grown to the maximum size. Stages 5 and 6 are considered as matured ovarioles (Osawa 2005). The next reproductive state is represented by the eggs present in the calyx of the ovary. Stages 3 and 4 were considered as one stage in a five-stage scale for *C. septempunctata* (Okuda et al. 1986, Okuda & Hodek 1989).

As coccinellids, particularly the aphidophagous species, often encounter a scarcity or absence of suitable prey (Chapter 5.3.3), oosorptive ovaries may also be found. **Oosorption** occurs during the intermediate developmental stage of ovarioles (Osawa 2005). The survival of females is maintained by egg resorption because of the possibility of future oviposition when aphid resources again become available, often after long- or short-distance movement (6.3.1.2). Oosorption also takes place when diapause is induced in reproducing females, i.e. 'secondarily'. Egg resorption in the late reproductive season and due to bad condition of host plants has been recorded in several studies on the *Henosepilachna* complex. When the conditions improved, oviposition was resumed (Ohgushi 1996 and the references therein). Osawa (2005) gives two main characteristics of an oosorptive oocyte: (i) dark yellow or orange colour, (ii) modified shape (see Osawa 2005, p. 504, figure 2 therein).

In a European study of **gonads during dormancy** in four coccinellid species, Osawa's stages 1–3 (and the stage during which resorption occurs) were recorded. However, the transparency of the oocytes was emphasized rather than their number, i.e. (1) no oocyte, (2) transparent oocytes, (3) early phase of vitellinisation (Ceryngier et al. 2004). From the four univoltine species sampled at dormancy sites (*C. septempunctata*, *C. quinquepunctata*, *C. magnifica* and *Cer. undecimnotata*), only the last-named species completely lacked any indication of reproductive activity in early dormancy in September. All females had completely inactive ovaries (stage 1) and none of them had sperm in their spermathecae (Figs 6.10, 6.11; Ceryngier et al. 2004). This finding is in agreement with older data for *Cer. undecimnotata* (Hodek & Landa 1971; Table 6.18). While all *C. septempunctata* females had inactive ovaries similarly to *Cer. undecimnotata*, 10% of the ovaries of *C. quinquepunctata* were in stage 2 and one *C. magnifica* female had resorbed oocytes. In all *Coccinella* species, some females had sperm in their spermathecae; the incidence of mated females was highest (30%) in *C. septempunctata* (Fig. 6.11; Ceryngier et al. 2004). More than 10 years earlier (Ceryngier et al. 1992) a higher percentage (50%) was recorded.

Parasitization of *C. septempunctata* by the braconid *Dinocampus coccinellae* (8.3) surprisingly increased not only the incidence of mated females (to 50% in September), but also the state of maturity of the ovaries (Ceryngier et al. 2004). Two females that contained second instar larvae of *D. coccinellae* had vitellinized oocytes, while another two parasitized by younger stages had inactive ovaries. This observation still needs a detailed experimental study to verify whether parasitization can really cause a kind of activation similar to that caused by injury (in a French population of *C. septempunctata*; Hodek et al. 1977; 6.2.1.2).

For England, Majerus (1994) reported that *C. septempunctata* only rarely mate before hibernation. In North Carolina, the females of *Har. axyridis*, collected from buildings shortly after they arrived there, mostly in October, varied greatly in the proportion that had mated: in 1993 the mean was 11.8% (range 0–25%; $n = 246$) and in 1994 it was 41.4% (range 0–70%; $n = 461$) (Nalepa et al. 1996).

In general, the **receptivity of female** coccinellids for mating is evidently not dependent on the physiological state of their ovaries. As mentioned above, in

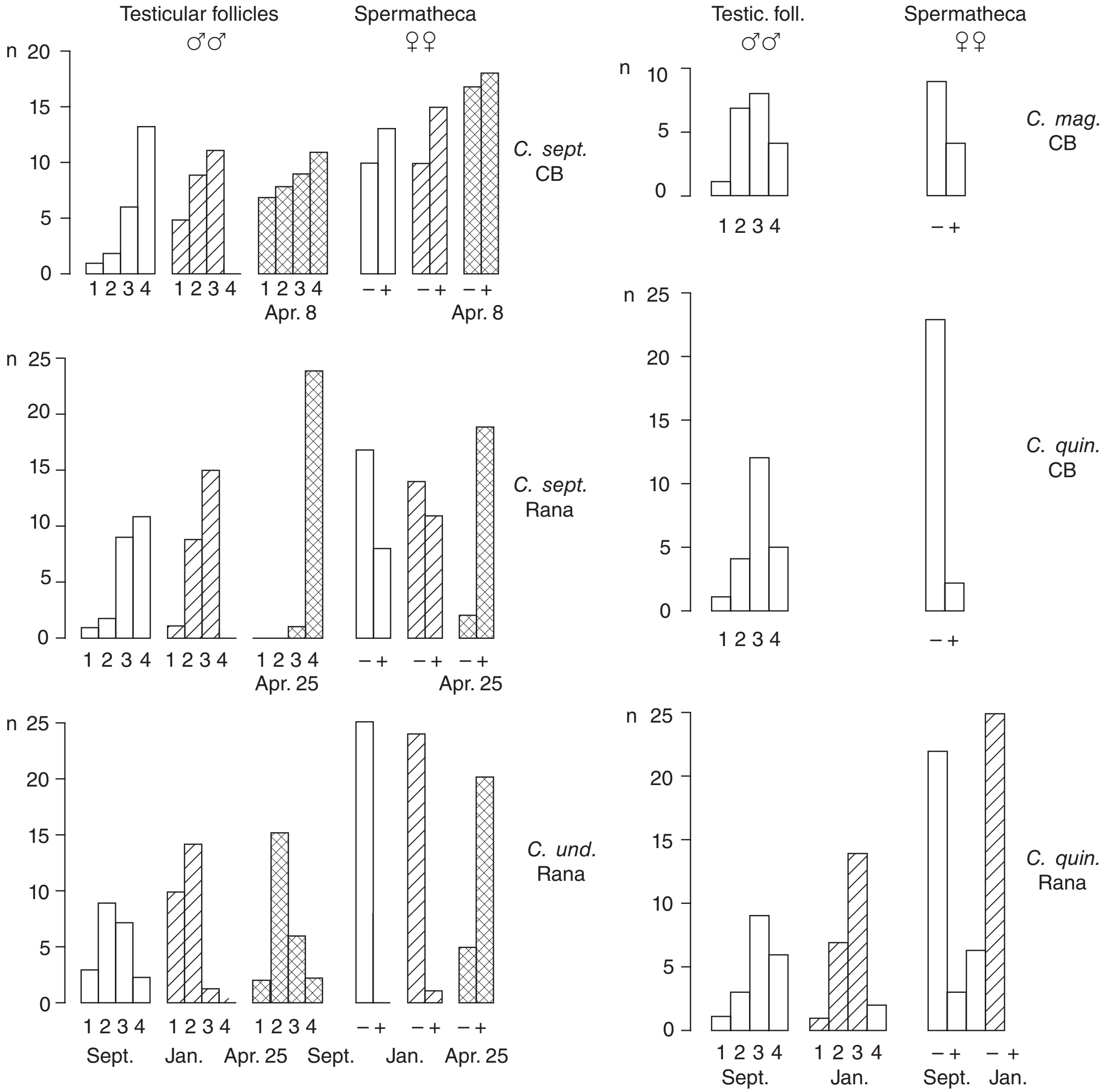

Figure 6.10 Condition of testicular follicles and spermatheca. *Coccinella septempunctata*, *Ceratomegilla undecimnotata*, *Coccinella magnifica* and *Coccinella quinquepunctata*. 1, lowest activity; 4, highest activity; –, empty; +, filled with sperm; České Budějovice (CB, southern Bohemia; Rana, northern Bohemia) (from Hodek & Ceryngier 2000).

C. septempunctata 30–50% of females entering diapause have the spermathecae full of sperm (Ceryngier et al. 1992, 2004) and the first females of *Cer. undecimnotata* contain sperm in their spermathecae 3 weeks before the ovaries mature (Hodek & Landa 1971; Table 6.18).

Also in old studies (e.g. on *P. quatuordecimpunctata*, Hariri 1966; *C. novemnotata*, McMullen 1967a) the ovaries of diapausing females are described as consisting of mere germaria.

In *A. bipunctata*, however, continual activity of the ovaries was reported from Belgium when the

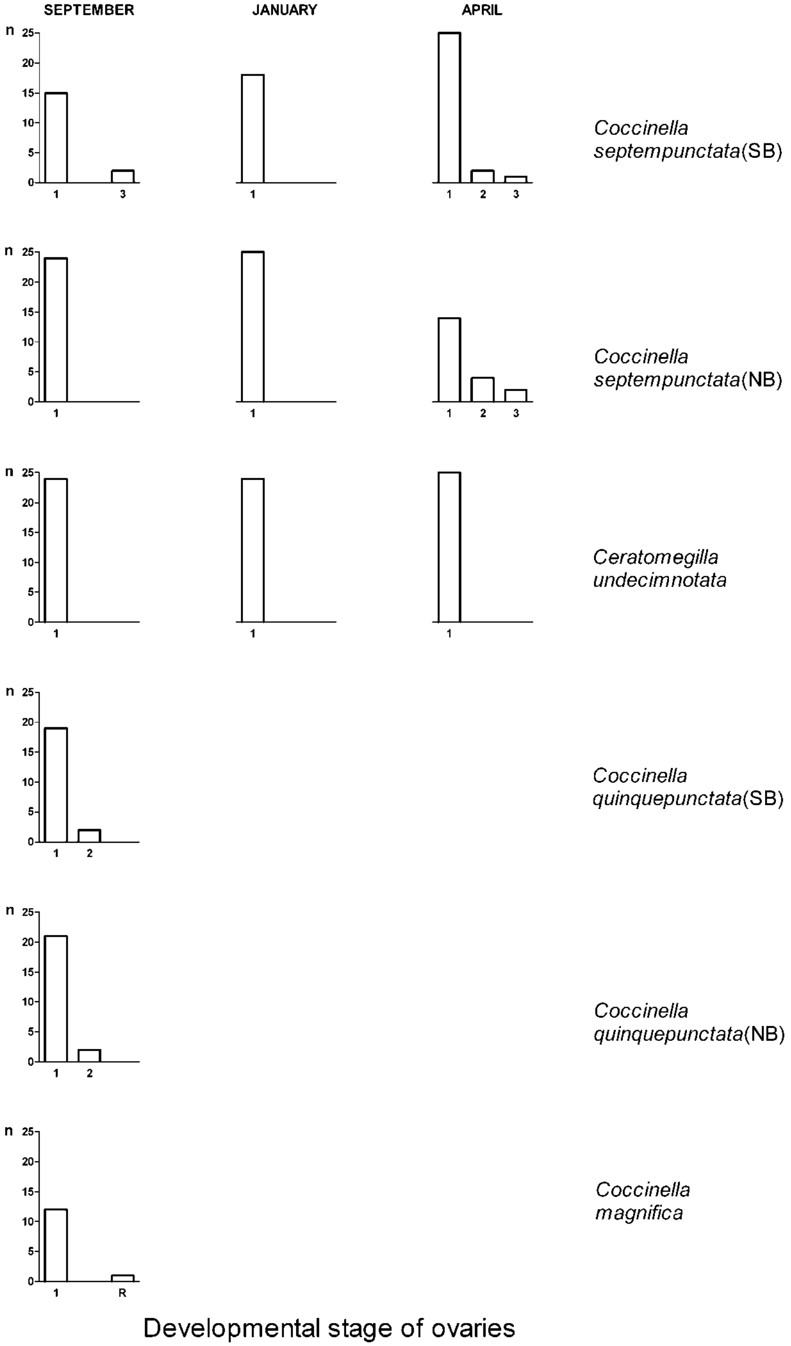

Figure 6.11 Condition of ovaries in coccinellid females. 1, no oocytes in ovarioles; 2, first, transparent oocytes; 3, small vitellinized oocytes; R, vitellinized oocytes partly resorbed. Only non-parasitized coccinellids are included. Origin of samples: southern Bohemia (SB), northern Bohemia (NB) (from Ceryngier et al. 2004).

Table 6.18 Gonads in dormant *Ceratomegilla undecimnotata* collected in Louny hills (N. Bohemia) (Hodek & Landa 1971).

Date of sampling	Ovaria		Spermatheca		Spermatocytes in testicular follicles*	
	no egg chambers	one oocyte	no sperm	with sperm	absent	present
16.12.57	20	—	20	—	20	—
11.02.58	20	—	20	—	20	—
08.04.58	30	—	30	—	20	—
23.04.58	20	—	18	2	11	9
29.04.58	20	—	12	8	7	13
05.05.58	20	—	4	16	—	20
13.05.58	10	10	—	20	—	20
19.05.58	7	13	—	20	—	20

*Vesiculae seminales always full of sperm.

differentiated and growing oocytes were resorbed and not vitellinized for most of the season: vitellinized and chorionated oocytes were only found in May. This report seems somewhat to contradict the 3–4 generation cycle reported for Belgium (Hemptinne & Naisse 1987). Usually a bivoltine life cycle has been recorded for *A. bipunctata* in cold temperate climates (e.g. Obrycki et al. 1983, Majerus 1994, Klausnitzer & Klausnitzer 1997).

In central European populations of *C. septempunctata,* which are **heterogeneous as to voltinism** (6.2.1.1), dissections of samples from summer and early autumn show both processes, differentiation and resorption. In July, about 40% of females show the start of vitellogenesis, but in the next month the oocytes begin to be resorbed, earlier in the beetles from dormancy sites and slightly later in the beetles still remaining in breeding sites (Fig. 6.12; Okuda et al. 1986). A similar result was obtained in the laboratory at 25 ± 2°C and short daylength (12L:12D): a maximum of aproximately 40% females had vitellogenic oocytes on the third day and resorption was terminated on the ninth day. Under long daylength (18L:6D) the process of resorption was delayed; it was only terminated after about 3 weeks (Okuda et al. 1986).

As late as in mid- and late **September**, a fraction of the central European populations of *C. septempunctata* may still be found actively feeding on remaining aphids. Most of these coccinellids have their guts full of digested aphids and some of them (**10–20%**) also have **vitellinized oocytes** (Hodek 1962; Table 6.1). This finding was confirmed in a recent study on populations from southern Bohemia (Ceryngier et al. 2004). It has been assumed that this heterogeneity in the maturation of ovaries found in the Czech populations is related to the partial tendency to multivoltinism (Hodek 1962; Table 6.3; 6.2.1.6, 6.2.16).

In the **bivoltine *C. s. brucki*** in central Japan the females have matured oocytes in spring and in autumn. They spend both aestivation and hibernation with previtellogenic ovaries. However, oogenesis was reported to advance gradually during hibernation (in a relatively mild winter), while it was completely suppressed in the course of aestivation (Sakurai et al. 1983, 1986). These observations indicate that, from the physiological point of view, only the aestivation is diapause (6.2.2.1).

In *Chil. renipustulatus*, the majority of females diapause with ovaries in which development is blocked at an early stage; in about 7% of the females development is stopped even earlier and follicles are completely missing (Pantyukhov 1965, 1968a). The females with no follicles have a substantially greater longevity (by 1–2 months in the laboratory) than those where some development has occurred. The situation is probably similar in *Chil. bipustulatus* and *Chil. geminus*. Zaslavskii and Bogdanova (1965) report that in some diapausing females of these two species, the development of the ovarioles is inhibited at the stage of one small follicle with some yolk deposited.

It is at the **end of dormancy** in spring, often still at dormancy sites while no food is taken, that previtellogenesis and the formation of the first oocyte occur. In *Cer. undecimnotata* (Hodek & Landa 1971; Ceryngier

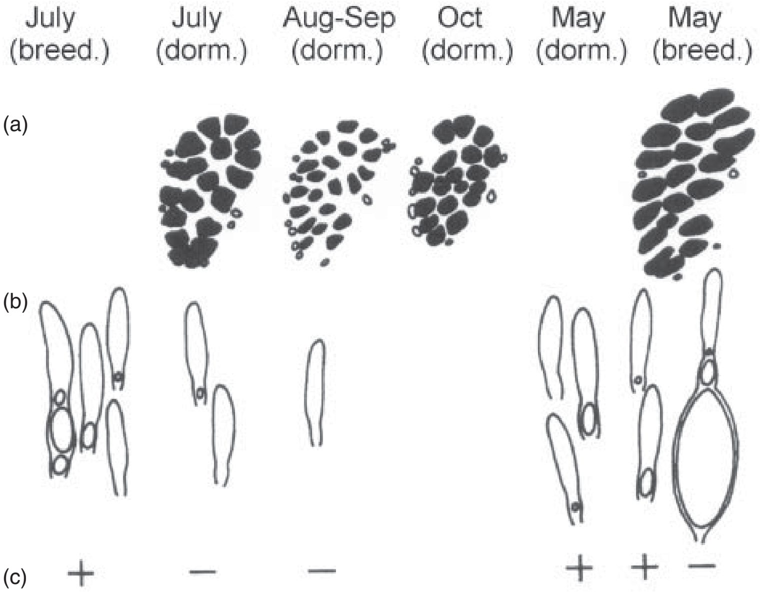

Figure 6.12 Schematic representation of changes in dorso-longitudinal flight muscle fibres (a) and ovaries (b) in relation to flight tendency (c) in *Coccinella septempunctata* adults. Breeding (breed.) and dormancy (dorm.) sites; +, flight tendency high; –, flight tendency low or absent (from Okuda et al. 1986).

et al. 2004; Table 6.18; Fig. 6.11) this happens much later, around mid-May, than in *C. septempunctata* (Okuda et al. 1986, Ceryngier et al. 2004; Fig. 6.12).

In three studied species, *C. septempunctata* and *Cer. undecimnotata* (Hodek & Landa 1971, Ceryngier et al. 2004) and *A. bipunctata* (Hemptinne & Naisse 1987), the spermathecae of at least part of the sampled females contain **spermatozoids before** the **dispersal** from the hibernation sites. *Adalia bipunctata* have empty spermathecae from the onset of dormancy until spring (Hemptinne & Naisse 1987).

The females of *S. pusillus* often live for 2 years and enter a **second dormancy**. The mature ovaries are largely resorbed and the metabolic reserves are re-accumulated in the enlarged fat body. This resorption of ovaries even occurs during the first hibernation of those females which have emerged from pupae before late July and have already oviposited in that season (Putman 1955). This latter situation may be expected to apply in every multivoltine or at least partly bivoltine life cycle, as occurs in *C. septempunctata* (Hodek 1962). It is also unlikely that a second hibernation is an exceptional event; it has been proved to occur in *P. quatuordecimpunctata* by Hariri (1966) in England, in *C. septempunctata* by Sundby (1968) in Norway, and in two species (*Har. axyridis* and *Aiolocaria hexaspilota*) introduced to central Asia from the Far East (Savoiskaya 1970a, b).

6.4.1.3 Male gonads

Contrary to what is often assumed, the activity of the tissue of coccinellid testicular follicles is not arrested when diapause is induced. Testes already mature in the pupa, and the males are ready to mate shortly after adult emergence. While the males of *C. septempunctata* acquire all other characteristics of the diapause syndrome, such as accumulation of reserves and behavioural characteristics, i.e. migration and change of taxes, they are nevertheless able to **fertilize females** just **prior to** entering **diapause**. On 22 September 1998, the males of *C. septempunctata* still had highly active testicular follicles (at stage 3.5 on average, when estimated at 4 stages), in spite of being dormant in the tussocks on the hill top (Fig. 6.10; Hodek & Ceryngier 2000). This is not the same for all coccinellid species; see *Cer. undecimnotata* in Fig. 6.10 and *A. bipunctata* (Hemptinne & Naisse 1987).

In the next 4 months of the cold period, up to 22 January, the activity of the testicular follicles in most males of *C. septempunctata* had on average regressed to stage 2.2, due to the low temperature prevailing over that period. There were loose spermatocytes and spermatids in the centre of the testicular follicles, with spermatogonies (apical cells) at the distal top and spermatodesms (sperm bundles) at the proximal end. Spermatogenesis ceases at the stage of the division of spermatogonies or of young spermatocytes. In January the average state of activity of the testicular follicles was again much lower in *Cer. undecimnotata* than in *C. septempunctata* (Fig. 6.10).

The degree of testicular activity differed between autumn samples of four species (Fig. 6.10). The least active were the testes of *Cer. undecimnotata* (mean degree of activity: 2.4) and the next were those of *C. magnifica* (2.8), *C. quinquepunctata* (3.2) and *C. septempunctata* (3.6).

In spring, the increase in ambient temperature (to about 12°C; Hodek 1960, 1973) initiates the opposite process. In April the **activity** of the **testicular follicles** is **resumed**; this occurs earlier in *C. septempunctata* than in *Cer. undecimnotata* (Fig. 6.10). In the plain near České Budějovice (South Bohemia), the progress in activity is more varied than in the hills in northern Bohemia. Spermatogenesis begins: the spermatogonies begin to divide and groups of growing spermatocytes enveloped by cyst cells are formed which quickly fill the whole follicle. In some years the tissue of the testicular follicles is fully active and spermiogenesis is complete in all males by mid-April (Hodek & Ceryngier 2000, Ceryngier et al. 2004). Such a situation was already reported earlier with photographs (Hodek & Landa 1971).

However, the males do not have to wait for the new sperm to mature before mating, as they have kept **reserves of sperm in** their **seminal vesicles**. Also, if the females disperse early, about one half of them could use sperm from their **spermathecae** for fertilizing their eggs. Mating at the dormancy sites may be advantageous in central Europe for *Cer. undecimnotata* that occurs there on the northernmost boundary of its distribution area and is rather rare, so that the probability of genders meeting away from the hibernation sites is much lower than in *C. septempunctata* (Hodek & Ceryngier 2000).

A biochemical study on the **Japanese subspecies *C. septempunctata brucki*** reported interesting results. In central Japan, this subspecies has a summer diapause and a winter quiescence (Okuda & Hodek 1983; 6.2.2). In the laboratory at 25°C and long days (i.e. conditions inducing summer diapause) Okuda (2000) found that **DNA synthesis in** the **testicular follicles** decreased within 30 days of adult life to less than half the levels recorded in pre-diapause (adult age 1 day) and post-diapause (age 120 days). Thus DNA synthesis **never ceased** completely and spermatocytes, although reduced in number, were present in diapausing males. In fact this is not particularly surprising, because the activity of the tissue of the testicular follicles is affected by the ambient temperature (Hodek & Landa 1971). The metabolic rate of Okuda's beetles at 25°C remained high since they did not experience the marked decrease in temperature of the Czech outdoor beetles. In the mild winter of central Japan temperature is rather high also in the field (Okuda 2000).

In Belgian populations of the multivoltine *A. bipunctata,* the degeneration of differentiated spermatogonia begins in September. Spermatogenesis ceases in January and February and resumes fully in May (Hemptinne & Naisse 1987).

Mating activity just **prior to dispersing** from aggregations has also been reported in California for *Hip. quinquesignata punctulata*, *Hip. sinuata* and *Hip. parenthesis* (Hagen 1962). Well-developed testes in *Hip. convergens* in dormancy sites have been reported by Stewart et al. (1967).

Similar to the findings on four Czech coccinellid populations (Ceryngier et al. 2004), Zaslavskii and Bogdanova (1965) could not find any effect of diapause-inducing conditions on the state of the testes in *Chil. bipustulatus* and *Chil. geminus*. However, gametogenesis evidently ceases very early in the life of both these species, since it was very difficult to find mitotic divisions in active or diapausing males older than 7–10 days.

The presence of spermatozoa in the spermathecae of some ***C. septempunctata*** females in autumn (Hodek & Cerkasov 1961) and particularly the fertilization of females by males kept under diapause conditions, that was recorded in *C. septempunctata* by Bonnemaison (1964) and in *Stethorus pusillus* by Putman (1955), would suggest that the activity of the **male accessory**

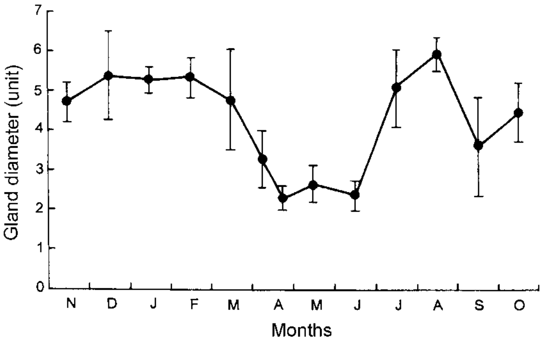

Figure 6.13 Seasonal changes in the size of male accessory glands in *Coccinella septempunctata brucki*. ($n = 10$; one unit = 25 µm) (from Sakurai et al. 1982).

glands might also be independent of diapause. However, in *C. s. brucki* the glands were much smaller in diapausing aestivating males than in quiescent hibernating ones in which they were developed (Sakurai et al. 1982; Fig. 6.13).

It has been shown experimentally with ***Cer. undecimnotata*** that the activity of the **testicular follicles** depends on the ambient temperature: under favourable temperature it may be continued or resumed, when alternative food (e.g. honey solution) is provided. At a temperature of around 12°C, the males can embark on a normal and gradual decrease in gonad activity (Hodek & Landa 1971).

In *C. septempunctata*, photoperiod did not exert any influence on the activity of the testicular follicles that was increased by exposure to 20°C, even in starved beetles. Activity of the follicles was positively affected by feeding the males on honey solution and still more by them preying on aphids, while it was lower in males parasitized by larvae of *Dinocampus coccinellae* (Ceryngier et al. 1992). This enhancing effect of feeding is in agreement with earlier findings on males of *Cer. undecimnotata* (Hodek & Landa 1971).

6.4.1.4 Flight muscles

In late summer *C. septempunctata* adults migrate to dormancy sites to return in spring to breeding sites (see also 6.3.1.2 and 6.3.1.5). Flight muscle fibres are well developed immediately after arrival at the aestivo-hibernation sites, but are gradually resorbed to about 80% of their maximum diameter by August and September. While this resorption in **early dormancy** was expected, the regeneration recorded already in October, 6 months before the dispersal flight, was rather surprising (Fig. 6.12). These histological findings (Okuda et al. 1986) were corroborated several years later by measuring changes in the volume of the flight muscles (Nedvěd et al. 2001). Changes in oxygen consumption and take-off also indicated a tendency to flight resumption a long time before the spring dispersal (Nedvěd et al. 2001). For a discussion of this unexpected phenomenon, see 6.3.1.3. The decrease in flight tendency recorded **after dispersal** to the breeding sites was not followed by flight muscle autolysis (Okuda et al. 1986). Evidently the flight muscles remain well developed as they are needed for trivial flight for foraging. Coccinellids foraging for their aphid prey, that quickly changes in abundance, often undertakes flights of relatively great distance.

In ***C. s. brucki*** from central Honshu, Japan, autolysis of flight muscles occurs **during aestivation**, while the flight muscles of hibernating adults are not autolyzed, and in this population aestivation and hibernation are separated by a period of reproduction (Okuda 1981, Sakurai et al. 1982, 1986). **Regeneration** of the flight muscles **after aestivation** is thus necessary for dispersal in this population of *C. s. brucki*. In central Europe, *C. s. septempunctata* undergoes a long uninterrupted aestivo-hibernation. It is a pity that neither the condition of flight muscles, nor flight tendency have been studied in warmer European regions, where two (or more) generations of *C. s. septempunctata* could develop.

Resorption of flight muscles during aestivation of *C. s. brucki* decreases the metabolic rate which, in spite of their diapause condition, would be high because of the high ambient temperature in summer. During hibernation, metabolism is lowered by the low ambient temperature.

6.4.2 Metabolic changes related to diapause

6.4.2.1 Lipids

Diapausing insects typically show an increased fat content (Lees 1955, Hodek & Cerkasov 1963, 1965, Tauber et al. 1986, Danks 1987, 2001). Their survival during dormancy largely depends on the amount of metabolic reserves they can accumulate before diapause.

In many coccinellids the deposition of reserves in the fat body is quite extensive, and a **hypertrophied fat body** has always been reported in diapausing coccinellids. Numerical data based on analysis of the amount of fat accumulated are, however, less common. For comparison of early findings in six coccinellid species, see the table on p. 247 in Hodek (1996).

In the laboratory, slightly less fat is accumulated in ***C. septempunctata*** than in the field (Hodek & Cerkasov 1961). Females, sampled in the hibernation sites in early October, when an important proportion of the reserves had already been utilized, still had 0.205 mg of lipids per mg total weight. Females, in which diapause was induced in the laboratory at 12L:12D and 18°C had 20% less lipids (0.164 mg).

Zhou et al. (1995) reported, also for the Czech Republic, a slightly higher content of fat in *C. septempunctata* females, sampled in mid-September in **mountains** (1420 m a.s.l.: 9.4 mg per individual) than in those from a hill (400 m a.s.l.: 8.9 mg per individual). During hibernation there was a lower decrease in fat content at the higher altitude (to 6.6 mg per individual, i.e. by about 30%) than at lower sites (to 4.9 mg per individual, i.e. a decrease of about 50%).

To monitor **changes during dormancy**, it is advisable to express lipids and glycogen in **absolute values** of weight per specimen. Then changes in individual components of body weight are independent, unlike the situation if, for example, lipids are expressed as percentage of dry or fresh weight. Whereas in reality the fat content in ***Cer. undecimnotata*** males substantially decreased from late August to late October from 6.4 to 3.6 mg per beetle, i.e. by 44%, in the relative expression, in contrast, the initial 41% of fat per dry weight only falls to 38% (Hodek & Cerkasov 1963). While the latter expression is an important drawback, some authors use it and thus their data have to be reported here in this way. Weights that follow are all given as absolute weight per individual unless stated otherwise.

In *Cer. undecimnotata* 6–7 mg of fat per female were recovered **in late August**, i.e. about 40% weight (Hodek & Cerkasov 1963). As the first sample was taken 1 month after the arrival of the beetles in the hibernation sites, it can be supposed that the initial fat content was substantially higher. *Ceratomegilla undecimnotata* has the **highest** relative **fat content** of all insect species which were analyzed before this study. In three coccinellid species, Hariri (1966) found 5–9 mg fat per beetle in the much heavier ***C. septempunctata***, 1–3 mg in ***A. bipunctata*** and 1–2 mg in ***P. quatuordecimpunctata***. Coccidophagous ***Chil. rubidus*** only has 26.5% dry weight as fat at the beginning of hibernation (Pantyukhov 1968b) and ***Chil. renipustulatus*** 25% (Pantyukhov 1968a) or 20% (Pantyukhov 1965).

Two Australian coccinellids, the aphidophagous ***Apolinus lividigaster*** and the mycophagous ***Illeis galbula***, doubled their fat content before reproductive diapause to about 20–30%. Fat deposition was associated with consumption of alternative food such as pollen (Anderson 1981).

While Jean et al. (1990) recorded only 1.9 mg of fat for ***Col. maculata*** (comparable to the values above) in November, Labrie et al. (2008) reported a surprisingly high content of lipids in this species, also in Quebec and also in November: 73.2% of dry weight in females and 61.4% in males. The values of the latter authors for ***Har. axyridis*** were in the usual range of 35.5% in females and 26.4% in males.

Rate of decrease

The decrease in the substantial fat reserves during hibernation is drastic: for example, during the 8 months of dormancy of *Cer. undecimnotata* the absolute amount of **fat decreased to a** mere **quarter** (Hodek & Cerkasov 1963).

This decrease is much smaller in some Heteroptera and Coleoptera (Hodek 1996, p. 248) which hibernate in soil, isolated from temperature changes. The proportions of the initial fat content consumed throughout the whole hibernation period in *C. septempunctata*, *A. bipunctata* and *P. quatuordecimpunctata* were, for males and females, respectively, as follows: 49 and 61, 75 and 87, and 71 and 60% (Hariri 1966). *Chil. rubidus* catabolized 32% of the initial amount of fat during dormancy (Pantyukhov 1968b).

The rate of decrease in fat reserves in ***Cer. undecimnotata*** is related to the **impact of ambient temperature**. The rapid decrease in fat content commences in late summer and early autumn. In September 1957 it was 0.082 mg of lipids per day, and in October 1958 0.022 mg per day. During the cold period of November to March the decrease of fat was 0.009 mg per day. In April and early May the utilization of fat reserves again increased, and varied in the three springs studied due to different temperatures: the decrease of fat in *Cer. undecimnotata* males amounted to 0.018 mg, 0.045 mg and 0.027 mg per day in 1957, 1958 and 1959, respectively (Hodek & Cerkasov 1963). Findings by Hariri (1966) and (Pantyukhov 1968a, b) agree with the above.

Also in the small ***Aphidecta obliterata*** fat reserves decreased in early diapause from 0.75 to 0.5 mg per beetle, then remained unchanged during hibernation and decreased again in spring from April to May to 0.3 mg per female (Parry 1980). Even taking into account that adults of *A. obliterata* are small, the fat content is still rather low. In *A. bipunctata* adults, the initial fat content of 3.3 mg per beetle (54% dry weight in August and September) was reduced to 1.2 mg (30% dry weight in April). The average daily consumption was 0.01 mg fat per day (Mills 1981). This rate of decrease was very similar to 0.009 mg fat per day calculated for *Cer. undecimnotata* above (Hodek & Cerkasov 1963).

A laboratory experiment established the effect of six temperatures on the decrease in lipid reserves and survival in diapausing adults of ***Col. maculata lengi*** (Jean et al. 1990). At the start of the experiment in November, lipid reserves averaged 1.9 mg per insect and they diminished rapidly as the temperature increased. The rate of lipid decrease was significantly higher at 20, 10, 4 (and strangely also at −10°C) than at −0.5 and −4°C. The relationship between temperature and daily lipid consumption was stable between −0.5 and −4°C and exponential between 0 and 20°C (Jean et al. 1990).

Just before emerging from hibernation, *Cer. undecimnotata* males contained 17–19% and females 18–22% dry weight as fat (Hodek & Cerkasov 1963), *A. bipunctata* 14%, *P. quatuordecimpunctata* 19% and *C. septempunctata* 23% dry weight (Hariri 1966).

Sexual differences

On most occasions in the study by Hodek and Cerkasov (1963), *Cer. undecimnotata* females had a higher absolute fat content (by 0.1–1.2 mg) than males. Taking the amount of fat in males as 100%, then that in females ranged between 100 and 160%. In the three species studied by Hariri (1966), females contained 1.4 to 1.7 times more fat than males at the start of hibernation.

In addition to recording fat content, Pantyukhov (1968b) also observed changes in the iodine number during hibernation of *Chil. rubidus*. Although the **'iodine number'** before the onset of frosts in September and October amounted to 88–89, in January and February this index of the proportion of unsaturated fatty acids in fat rose to 93–94. As the unsaturated acids have a lower freezing point than the saturated ones, their rise would increase cold resistance. In *Chil. renipustulatus* (Pantyukhov 1965) similar increases in the iodine number from autumn (59–60) to winter (62–64) was observed.

6.4.2.2 Glycogen

Like lipids, glycogen reserves are accumulated during pre-diapause. However, glycogen is unstable in comparison with the more stable fat; thus both absolute and relative glycogen content greatly fluctuated in the course of dormancy in *Cer. undecimnotata* (Hodek & Cerkasov 1963).

The amount of glycogen in dormant coccinellids was roughly 10 times less than the amount of fat (for a comparison among four species see Hodek 1996, p. 251). In late August ***Cer. undecimnotata*** adults had about 0.4 mg of glycogen per beetle which represented about **2–3% of dry weight** (Hodek & Cerkasov 1963; Hodek 1996, table 7.03 therein). Values ascertained by Hariri (1966) for *A. bipunctata* in mid-October are one-tenth of those for *Cer. undecimnotata* – 0.04 mg of glycogen per beetle. In contrast to the above aphidophagous coccinellids, the coccidophagous ladybirds of the genus ***Chilocorus*** are reported to contain much more glycogen (**10–12% of dry weight**) at the beginning of diapause (Pantyukhov 1965, 1968a, b).

Rate of decrease

In all coccinellid species studied, glycogen content decreased during dormancy. In *Cer. undecimnotata* the rate of decrease (as for fat content) depended on temperature. In late summer and early autumn the decrease was **very steep** so that the **daily decrease** averaged 2.5 μg in males and 2.8 μg in females. During the 8 months of dormancy in *Cer. undecimnotata*, glycogen decreased in males to about 22% and in females to 25% of the initial amount (Hodek & Cerkasov 1963).

Before emergence from the dormancy site, *Cer. undecimnotata* contained 50–85 µg and 90–105 µg of glycogen in males and females respectively, which equalled 0.7–1.3% of dry weight. In *A. bipunctata* it was 5–8 µg in males and 6–9 µg in females and equalled about 0.2% of dry weight (Hodek & Cerkasov 1963).

Sexual differences

Markedly higher absolute glycogen content was found in females than in males of *Cer. undecimnotata*: if the content in males is taken as 100%, then the amount of glycogen in females represented 100–200% (Hodek & Cerkasov 1963). At the start of hibernation, *C. septempunctata* and *A. bipunctata* females again contained more glycogen than did the males, whereas both genders of *P. quatuordecimpunctata* contained almost equal amounts (Hariri 1966).

Since glycerol and other polyols are responsible for frost resistance in some insects, both fat and glycogen must be considered not only as a source of energy, but also as precursors for polyols (6.4.4).

6.4.2.3 Water

Water content in monthly samples of *Hip. convergens* taken from Sierra Nevada aggregations remained remarkably constant (Hagen 1962). Also in *Cer. undecimnotata* there was no general tendency for a decrease or increase in **absolute water content** during the 8 months of dormancy (Hodek & Cerkasov 1963). The water content usually amounted to **10–12 mg** per male and to **11–14 mg** per female. The continual increase in the **relative water content** from about 50–55% to about 60–63% which has been observed during dormancy is simply caused by the fall in dry weight, particularly of fat. The same applies for the increase to 64 and 68% from January to April in *Chil. renipustulatus* and *Chil. rubidus* (Pantyukhov 1965, 1968a, b).

Beetles may increase their body water content by **drinking** or by the production of **metabolic water** when splitting fat. The loss of body water by transpiration is affected by air humidity. Hagen (1962) assumes, for *Hip. convergens*, that the water balance is maintained by imbibing water. If the beetles from aggregations are kept in a refrigerator for a month or so in the absence of litter, a distinct water loss results. When these beetles are then exposed to water, they drink avidly. In field samples of *Cer. undecimnotata*, increases in water content usually coincided with rainfall and high humidity of the air, while decreases coincided with drought (Hodek & Cerkasov 1963).

6.4.2.4 Metabolic rate

Consistent with one of the adaptive roles of diapause (6.1), its onset is invariably associated with a striking drop in the metabolic level (Tauber et al. 1986, Danks 1987), e.g. in the chrysomelid *Leptinotarsa decemlineata* a drop to 15–20% of the normal respiratory rate was observed in dormant beetles (De Wilde 1969).

A **decrease in oxygen consumption** has also been demonstrated in diapausing coccinellids. When measuring the respiratory rate at 18°C in diapausing *Hip. convergens*, Stewart et al. (1967) obtained the value of 12 µl O_2/beetle/h immediately after the arrival at the aestivation sites in the Pinnacle mountains (Arkansas, USA) in late June. From July to February, oxygen consumption fluctuated between 5 and 9 µl, while in reproducing beetles in the lowlands it was 29 µl in March. During diapause, the level of metabolism was thus reduced to about 30 to 15%.

In diapausing *Col. maculata lengi* from Vermont (USA) the metabolic rate was low (0.18 µl O_2/mg body weight/15 min), but was about twice higher in summer samples (0.34 µl O_2/mg /15 min; Parker et al. 1977).

In two diapausing ladybirds, *Hip. convergens* and *Col. maculata*, Lee (1980b) compared the **effects of cold acclimation** at 6°C and **warm acclimation** at 20°C on the oxygen consumption within a range of five temperatures from 0 to 20°C. Within this temperature range the respiration rate in cold acclimated *Hip. convergens* increased from 0.1 to 0.75 µl O_2/mg/h and in warm acclimated beetles the values at all five temperatures were slightly but significantly lower by 0.05–0.2 µl O_2/mg/h. In *Col. maculata* this effect was reversed: the five oxygen consumption levels were lower in cold acclimated beetles. Lee (1989a) attributes these reversed responses to different acclimation temperatures associated with the different type of hibernation sites of the two species: *Hip. convergens* is more exposed to ambient temperatures (6.3.2.7), while *Col. maculata* hibernates in leaf litter under a snow cover (6.3.2.4). In a later experiment, *Hip. convergens* was collected in January and held for 6 weeks in the dark and at 4°C, and the metabolic rate was measured after exposure to 20°C and 12L:12D; within 14 days the oxygen consumption decreased to 40% of the initial value, from 1.13 to about 0.5 µl O_2/mg/h (Bennett & Lee 1989).

Pantyukhov (1968a, b) similarly recorded a much lower oxygen consumption in diapausing *Chil. rubidus* (0.1 µl O_2/mg/h) than in active adults (0.8 µl O_2/mg/h) (Pantyukhov 1968a), and similar difference between diapausing *Chil. renipustulatus* (0.52 µl O_2/mg/h) and active adults (1.25 µl O_2/mg/h) (Pantyukhov 1968b). Oxygen consumption by diapausing *Chil. bipustulatus* females reared in the laboratory was only a little more than half that of non-diapausing individuals (Tadmor & Applebaum 1971).

In Japan, Sakurai (1969) ascertained a decrease in respiration rate from 2–3 µl O_2/mg/h in active beetles to 0.4–1.3 µl O_2 in *C. s. brucki* during aestivation diapause (measured at 30°C). Hibernating *Cer. undecimnotata* have a respiration rate around 0.8 µl O_2/mg/h (measured at 25°C) but, if re-activated in the laboratory, the beetles show a doubled oxygen consumption, so that in ovipositing females it amounts to 1.6 (1.1–2.0) µl O_2/mg/h (Hodek & Cerkasov 1958, Hodek 1970). In populations of *A. obliterata* from eastern Scotland the lowest level of respiration (1.43 µl O_2/mg/h, measured at 20°C) was recorded in diapausing adults in mid-February, while the metabolic rate of post-hibernating beetles in early May was almost three times higher at 3.63 µl O_2/mg/h (Parry 1980).

6.4.3 *Corpora allata* and regulation of vitellogenesis

As in other insects, reproduction and adult diapause of coccinellids are regulated by the neuroendocrine system (see also 6.1.4). While evidence for the transfer of environmental signals to the neurosecretory cells of the *pars intercerebralis* of the brain and for the secretion and function of the brain hormone is still missing in coccinellids, there are some data on the subsequent pathways of the neuroendocrine regulation, i.e. on the action of the **juvenile hormone** (JH) secreted by the *corpora allata* (CA). In reproducing males, JH stimulates the development of the accessory glands and, in the maturation of the ovaries, JH is necessary for the synthesis of vitellogenins which are deposited in the developing oocytes as **yolk proteins** (vitellins). Although the **active inhibition of the CA** found in a heteropteran, *Pyrrhocoris apterus* (Hodková 1976, Hodková et al. 2001), may be a general feature in adult diapause of insects, we still lack similar evidence for coccinellids. Most data are concerned only with the so-called **'passive inhibition' due to the inactivity of the CA** or the very low titre of JH during adult diapause. All data have come from only two coccinellid species: *C. septempunctata* and *Cer. undecimnotata*.

The **volume of the CA** is the usual measure of their activity, but this has been questioned. In the bivoltine *C. s. brucki*, the size of the CA in hibernating beetles is similar to that in active beetles, but more than twice as large as during aestivation (Sakurai et al. 1981b, 1983). This has been interpreted as one of the important indications that aestivating adults of this subspecies enter diapause, while hibernation is a mere quiescence. Also during the hibernation diapause of the central European population of *C. s. septempunctata,* the onset of diapause was accompanied by a decrease in the size of the CA. In spring, the CA increased in size again with the resumption of activity and with the progress of ovarian maturation (Okuda 1984, Okuda et al. 1986). In the same region the same tendency was observed in *Cer. undecimnotata* (Okuda 1984, Okuda & Hodek 1989).

One of the ways in which the function of JH in the reproductive activity of females can be demonstrated is the monitoring of **electrophoretic patterns** of **haemolymph proteins**. Topical application of a JH-analogue (methoprene) to aestivating (i.e. diapausing) females of *C. s. brucki* caused the disappearance of bands specific for diapause, while a band appeared which was presumed to be vitellogenin. That band also appeared in late May and in mid-October, i.e. during, respectively, the spring and autumnal reproduction periods of young adults. It remains to be established why a distinct band of vitellogenin was not found after aestivation in late September though it was very distinct after hibernation in mid-April (Sakurai et al. 1987b).

Okuda and Chinzei (1988) studied the synthesis of **vitellogenin**, the yolk-protein precursor in *C. s. brucki*. While in pre-diapause the vitellogenin level was negligible and not detectable during aestivation diapause, it did occur after diapause, but then only after feeding.

Electrophoretic methods were also used to investigate the synthesis of vitellogenin in individual organs in *C. septempunctata*. Synthesis was observed in the **fat body** and to a lesser extent in the **ovaries** of mature females. Vitellogenin synthesis was not detectable in the brain or the thoracic muscles of mature females, or in the fat body of males (Zhai et al. 1984). When the coccinellids were reared on a substitute diet (raw pig liver, honey and sugar; Chapter 5.2.10) very low vitellogenin synthesis occurred; this was consistent with the observation that the oocytes in most females developed only to the pre-vitellogenic stage (Zhai et al. 1984). The synthesis of vitellogenin in the fat body of non-reproductive females fed on a substitute diet was induced by a JH-analogue (Zhai & Zhang 1984, Zhang & Zhai 1985).

Quantitative changes of vitellogenin synthesis in the fat body and the ovary were studied by **radioimmunoassay** in *C. septempunctata*. The fat body secreted more than 90% of the vitellogenin newly synthesized there, whereas the ovaries retained most of the vitellogenin they synthesized. The vitellogenin synthesized in the ovaries of active vitellogenic females was about 20% of that produced in the fat body (Zhai et al. 1985).

The correlation between the activity of the CA (i.e. synthesis of JH) and the development of oocytes was documented also in *C. septempunctata*, with both a **bioassay** using the wax moth, *Galleria mellonella* (Fu & Chen 1984) and a short-term radiochemical assay (Guan & Chen 1986).

6.4.4 Cold-hardiness

Studies focussing on the effect of low temperatures on coccinellids are of practical importance when a species has been introduced to a different climate for biological control, and the potential for survival in the new region needs to be estimated. This was done for *Har. axyridis* after invasion to the Nearctic region (see below).

The level of cold-hardiness achieved at the onset of diapause as a result of the **diapause syndrome** is usually increased through **cold acclimation** under the influence of decreasing temperatures in winter. Paradoxically, this process may continue after the winter solstice, after the end of diapause (Hodková & Hodek 2004). As the temperature falls, **supercooling** occurs which is followed by the freezing of the tissues accompanied by a rapid rise in internal temperature. The external temperature at which this occurs is defined as the **supercooling point** (SCP). For the general theory of cold-hardiness and definitions of terms see Salt (1961, 1964), Lee and Denlinger (1991), Leather et al. (1993) and Hodková and Hodek (2004). The results obtained for coccinellids are similar to those for most insects. Resistance to temperatures below zero is rather high in the middle of dormancy, after cold acclimation resulting from the gradual decrease in temperature. Early or late frosts, however, can be damaging. The degree of cold-hardiness varies naturally in different species and is related to their type of hibernation. **Subnivean** species which hibernate in litter, and are usually covered by snow, are certainly more sensitive to freezing than the more exposed **supranivean** species which hibernate in crevices of bark or rocks.

Early evidence for cold-hardiness in coccinellids came from the studies by Pantyukhov on two *Chilocorus* species. The common *Chil. bipustulatus* has low cold-hardiness. Most individuals have their supercooling point at −8 to −9°C, the limit for survival being −10 to −12°C (Pantyukhov 1965). A prolonged decrease in temperature down to −5 to −6°C at the soil surface in the hibernation sites causes rather high mortality. Considerable mortality is to be expected where there is a thin snow cover or where the spring temperature fluctuates. A considerable increase of cold-hardiness in *Chil. rubidus* was recorded between September and January (Pantyukhov 1968a). In spring the cold-hardiness decreased again. Similar changes have been noticed in measurements of the supercooling points (Table 6.19). The lowest temperature that could still be survived by a considerable number of beetles (40%) in January was −13.5°C for 2 days. *Chil. rubidus* hibernates in the litter and, if this is additionally covered by a sufficient layer of snow, this subnivean beetle is not exposed to extreme low temperatures. In the Petersburg region, Pantyukhov (1968b) measured the minimum **temperature** on the ground **under** the **snow**. In the winter of 1964/65 this was −2.5°C, and −7°C the next winter; the survival of the beetles was 67–82% and 52–70%, respectively. By contrast, in Alma-Ata, Kazakhstan, where the minimum temperature (without snow) was −10°C, only 12% of the beetles survived. In the Far East region of Russia the survival was 21% (Pantyukhov 1968b).

In the **freezing-intolerant** *Aphidecta obliterata* (overwintering beneath bark, the supercooling points of adults without visible **gut contents** were much lower (in late autumn from −28.0 to −30.2°C) than those of the more abundant adults with guts containing food material (from −13.7 to −13.0°C) (Parry 1980). The difference is evidently caused by gut contents forming nucleating agents for freezing.

Table 6.19 Supercooling point and freezing point in *Chilocorus rubidus* (Pantyukhov 1968a).

		Supercooling point (°C)			Freezing point (°C)		
Months	***n***	**aver.**	**min.**	**max.**	**aver.**	**min.**	**max.**
Sep.	41	–8.2	–2.7	–14.2	–2.6	–0.4	–5.2
Dec.	57	–13.2	–4.9	–17.4	–3.2	–0.7	–6.8
Jan.	50	–12.9	–4.3	–17.2	–3.2	–0.8	–6.7
Feb.	40	–13.5	–5.6	–17.8	–3.4	–1.1	–6.9
April	32	–3.4	–0.2	–5.5	–0.8	–0.1	–2.7

Overwintering with the digestive tract containing food material is unusual in coccinellids, and may be associated with the mild climate of eastern Scotland where the study was carried out. The highest mean supercooling point in spring was –7.5°C. *Aphidecta obliterata* contains no polyols.

Although warm winds may melt the snow in southern Alberta, Canada, *Hip. quinquesignata* can be considered as a **subnivean hibernator**. The supercooling points in late autumn and winter averaged between –18.5 and –22.2°C, with maximum supercooling capacity around –27°C. In spring, before dispersal, the supercooling point was higher, with means between –12 and –13°C (Harper & Lilly 1982). In outdoor beetles, the supercooling point correlated well with the content of glycerol, but the beetles reared in the laboratory and containing no glycerol or sorbitol, still supercooled to –18.5°C; supercooling is evidently also due to other factors.

Adalia bipunctata showed a much higher resistance to low moisture and larger extremes of temperature than the coccinellid species that hibernate in litter (Novák & Grenarová 1967). When placed under a roof or into the crown of *Picea* trees, *A. bipunctata* suffered only 13 to 40% mortality, while *C. septempunctata*, *C. quinquepunctata* and *Exochomus quadripustulatus* died out completely or by more than 90% within 4.5 months (mid-October to early March). There were no differences, however, in the winter survival in the grass environment among the four species tested. The higher tolerance of *A. bipunctata* is obviously connected with its usual hibernation site in the drier microhabitat of bark crevices where it is not protected from ambient extreme conditions.

The low survival of the litter-hibernator *E. quadripustulatus* may be considered surprising as very low values of the supercooling point (SCP) were ascertained in hibernating individuals (Parry 1986, Nedvěd 1993). Parry emphasized the difference in the supercooling point in late October between *A. obliterata* (hibernating in bark crevices (SCP, –14.3°C) and *E. quadripustulatus* (SCP, –19.4°C); this difference was the reverse of expectation. The samples had, however, different sources: *A. obliterata* adults were not collected in bark crevices but from the needles of Douglas fir. This means that *A. obliterata* was still feeding and would have contained food material in the gut (see above in this chapter), while *E. quadripustulatus* was inactive in the litter.

A detailed comparison has been undertaken between *Hip. convergens* and *Col. maculata lengi*, which are both freezing-intolerant insects. The diapausing adults collected from hibernation sites were stored for one month in screened field cages covered with snow. In February, the SCPs were similar for both species: –18°C for *Col. m. lengi* and –15°C for *Hip. convergens*. However, during an exposure of five days to 25°C, the SCP for *Col. m. lengi* adults increased to –9°C, while it remained low and constant for *Hip. convergens* (Lee 1980b). The difference could again be related to the condition of the hibernation sites. In Lee's **supranivean** hibernacula, diapausing *Hip. convergens* are in nature exposed to the direct influence of ambient temperature and insolation (which may have raised the body temperature) and have evolved an adaptation keeping cold-hardiness resistant to temperature impact. In contrast, *Col. m. lengi* hibernates in **subnivean** hibernacula and thus lacks such adaptation (Lee 1980b).

In central Europe a comparison was made between a **subnivean** hibernator *C. septempunctata* (mostly in grass tussocks), and *Cer. undecimnotata*, hibernating predominantly in **supranivean** sites (rock crevices). When the SCP is measured in samples of beetles taken from the field in the coldest period, the values for

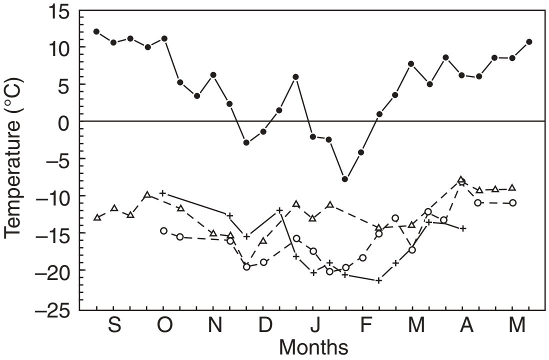

Figure 6.14 Seasonal changes in supercooling point in dormant coccinellid adults (below) and temperature means (above). *Coccinella septempunctata* collected in natural insulated hibernacula (grass tussocks, triangles); *C. septempunctata* exposed in outdoor insectary (crosses); *Ceratomegilla undecimnotata* exposed in outdoor insectary (open circles) (from Nedvěd 1993).

C. septempunctata were much higher than for *Cer. undecimnotata* (Fig. 6.14). The lower SCP for *Cer. undecimnotata* was evidently induced by the exposure to ambient temperature. When the adults of *C. septempunctata* were transferred from their insulated hibernaculum to a field-cage (where they were exposed to ambient temperature), their SCP decreased to the level of *Cer. undecimnotata* (Nedvěd 1993).

The importance of snow cover for the overwintering survival was also reported in the Texas High Plains for seven imported coccinellid species (Michels et al. 1997). The beetles were caged on native grasses during four winter periods. Survival ranged from 59% for *Hip. tredecimpunctata* to only 3% for *Scymnus frontalis* (Table 6.20). Higher survival was recorded in colder years with snow cover. An absence of snow cover combined with cold temperature, particularly in April and May, was probably detrimental in 1993, the last year.

Table 6.20 Overwintering survival in caged coccinellid adults (Bushland, Texas, USA) (Michels et al. 1997, modified).

	Percentage survival by year			
Species	**1990**	**1991**	**1992**	**1993**
Snow cover (cm)	45.7	36.0	57.2	17.8
Cycloneda ancoralis	—	—	12.9	7.8
Eriopis connexa	—	—	31.1	2.7
Hippodamia tredecimpunctata	58.6	—	27.4	—
Hippodamia variegata	29.4	20.4	17.9	4.3
Oenopia conglobata	—	—	14.1	10.9
Propylea quatuordecimpunctata	33.9	25.6	16.5	—
Scymnus frontalis	2.4	5.1	3.9	—

The SCP is not always a good indicator of cold-hardiness. In *Hip. convergens* adults exposed at the end of diapause to 20°C for 20 days (Bennett & Lee 1989), the SCP increased only slightly from −16 to −12.9°C, while the survival at −5°C for 2 hours decreased strongly from almost 100% to only 50%. The adaptive maintenance of supercooling discussed in an earlier paper by Lee (1980b) may thus have a lower value for survival than expected. With *Cer. undecimnotata*, however, the survival of a 24 hour exposure to low temperature is a good match to changes in the SCP (Nedvěd 1993). Also in *Har. axyridis*, the SCP was found to be a good indicator of cold-hardiness for summer and winter samples from Georgia and Minnesota, USA. It decreased by about 12°C from August to December (Koch et al. 2004).

Watanabe (2002) studied *Har. axyridis* in a **region with mild winters**, Tsukuba, Japan (36°N). The seasonal change of SCP and the 50% lower lethal temperature were both at their minimum in winter between December and February. The seasonal change in the polyol myo-inositol was well and negatively correlated with the changes in the SCP and lower lethal temperature (Fig. 6.15). Dynamics of SCP during hibernation indoor and outdoor was compared in *Har. axyridis* adults (Berkvens et al. 2010; Fig. 6.16).

While in central Japan the conditions are suitable for winter survival of *Har. axyridis*, there are harder winters in **cold regions of North America**. Although the SCP of *Har. axyridis* was there lower in winter (-23°C) (it was only −18°C in Japan), this evidently was not enough for survival as the minimum air temperatures were below −23°C. Therefore survival of extreme winter conditions depends on finding overwintering sites that provide adequate insulation (Carrillo et al. 2004). Koch et al. (2004) recorded 100% mortality after 24 hours at −20°C. Outside buildings, Labrie et al. (2008) did not observe any survival in

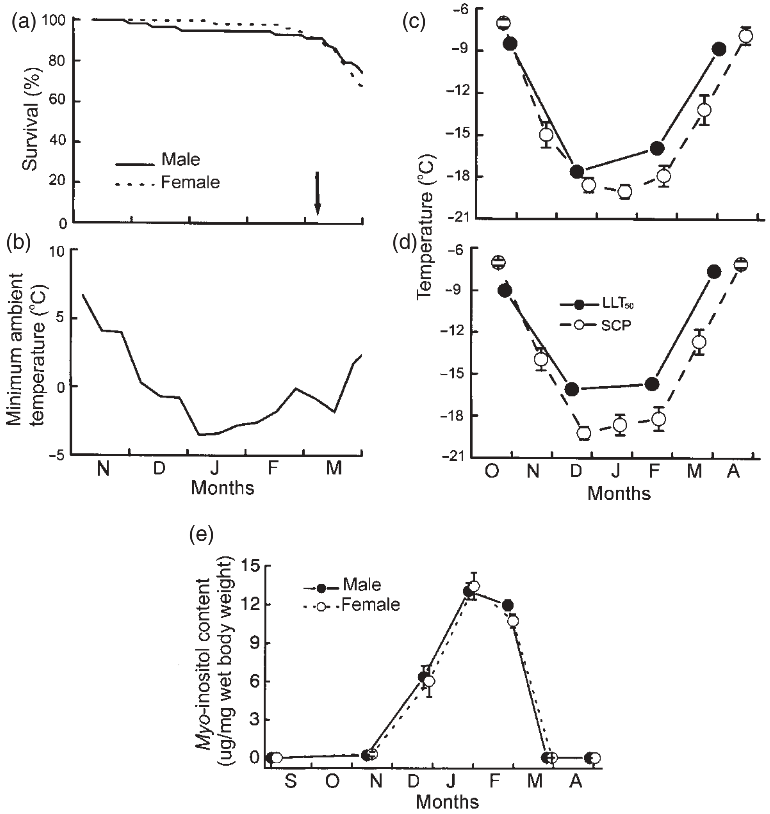

Figure 6.15 Seasonal changes of survival rate in *Harmonia axyridis* adults (a) and 10-day-minimum temperatures in an artificial hibernaculum (b). Arrow, date of first observed mating. Seasonal changes of supercooling point (SCP, dashed line with open circles) and 50 % lower lethal temperature (LLT$_{50}$, solid line with closed circles) in male (c) and female (d) collected outdoors. (e) Seasonal changes of *myo*-inositol content in adults (all from Watanabe 2002).

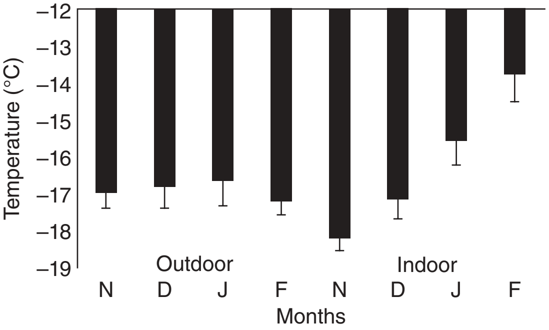

Figure 6.16 Supercooling point (mean ± SE) of outdoor and indoor overwintering *Harmonia axyridis* adults (from Berkvens et al. 2010).

the winter 2003/2004 in northeast Canada, when the temperature ranged between 5 and −25°C. Adults hibernating on hill tops near Montreal (71–73° W, 45° N) were dead at the end of winter (G. Labrie, unpublished)

Size and melanism can both affect winter survival, as shown by Osawa (2001) for *Har. axyridis*. In the cold winter of 1987/88 (with a great temperature range) a size-related mortality probably occurred in melanic females, with melanic females in spring 1988 being on average larger than in spring 1987. Small melanic females may have had lower survival because of lower reserves, with the reserves exhausted more easily in melanics because of their greater heat absorption during spells of warm sunny weather (Osawa 2001). Ohgushi (1986) showed that it was sex- and/or size-dependent mortality that resulted in larger adults and a higher proportion of females after overwintering in the herbivorous *Henosepilachna niponica*.

Some studies have aimed at finding the **storage temperature** for the developmental stages of ladybirds. Survival of eggs, larvae and pupae of *C. undecimpunctata* was quite high (65–85%) after 7-day storage at 6.0°C. Survival of adults was longer after 5–10 days of feeding on aphids at 28°C with a 14L:10D photoperiod (50%), than when the adults were exposed to cold immediately after emergence (30–40%) (Abdel-Salam & Abdel-Baky 2000). No attempt was made to induce diapause, which would have increased the survival (Iperti & Hodek 1974; 6.2.8). Survival of the second and third instars of *Col. maculata lengi* at 4 and 8°C was close to 100% for the first two weeks, but decreased drastically to 60% after 3 weeks and to 0% after 5 weeks. Development was resumed without negative effects when the 2-week larvae were returned to 24°C (Gagne & Coderre 2001).

6.5 CONCLUSIONS AND LACUNAE IN KNOWLEDGE

Diapause is an important component of ladybirds' life history. Knowledge of its timing (particularly of its end) is undispensable in improving biological control. Although the once popular method – using ladybirds from hibernation aggregations in gardens or crop fields – has almost been abandoned because of the subsequent fast dispersal of the released ladybirds, ecological methods of biological control and IPM depend on understanding **when and why** the **ladybirds arrive in crops**. This is particularly important when the crops have to be treated beforehand, e.g. by the application of alternative foods or other attractants (see Chapters 5 and 11).

Similarly, knowledge about **voltinism and its regulation** is needed for rational support of ladybird activity in the field or for rearing. Without insight into the role of photoperiod in diapause induction, one may make unrealistic high estimates of voltinism when they are based only on the effect of temperature on development.

Apart from its applied aspects, diapause of coccinellids is an interesting and useful **model for research** in several fields. The important critical remark by Sloggett (2005), that our research is focused on **only a few coccinellid species,** is highly applicable to diapause studies. Only lately, when *Har. axyridis* invaded both America and Europe, has this species begun to compete for research interest with *C. septempunctata*. Surprisingly diapause studies have not focussed on *A. bipunctata*, popular in other studies. Not only have **most species** been **ignored**, but even in monospecific studies, numerous for *C. septempunctata*, intraspecific variation has rarely been considered. Comparative studies on **diapause intensity** or **overwintering survival**, such as by Hodek et al. (1977) and Zhou et al. (1995), have remained rare and should be extended.

Also, **geographic variation in diapause** expression (which has often attracted interest in other insect groups) has been a rare topic even with common coccinellid species, such as *C. septempunctata.* Yet there are clearly regions where local authors could make interesting findings (Mediterranean Europe, India) because of the wide temperature and photoperiod ranges there. 'One-off' visits by foreign workers can produce only fragmentary results.

Still only partly solved is the problem why migrating coccinellids often **fly to prominent structures** and repeatedly visit the same hibernation sites. What is the contribution of passive transport by air currents versus active flight together with hypsotactic orientation? Similarly open is the role of various taxes versus pheromones in the formation of massive **aggregations** (also Chapter 9).

Another problem, belonging partly to the sphere of population genetics, is the **inheritance of** tendencies to the **expression of univoltinism and multivoltinism** (6.2.16). Another task for population genetics is how the seemingly deleterious (at least from the view of optimal foraging theory (OFT)) trait to **overproduction of offspring** in some species is perpetuated. Is an increased variance a satisfactory benefit in the trade-off between the 'OFT economy' and the overproduction of offspring?

One of important adaptations, essential for the aphidophagous coccinellids, but analyzed rather exceptionally (Osawa 2005), is the **postponement of egg laying** and reversible **resorption of eggs**.

It is a pity that the frequency of studies focussed on diapause decreased in the last two decades. Hopefully, the above-mentioned topics show that such research is rewarding.

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