INSECT DIAPAUSE

David L. Denlinger

Insect Diapause

Our highly seasonal world restricts insect activity to brief portions of the year. This feature necessitates a sophisticated interpretation of seasonal changes and enactment of mechanisms for bringing development to a halt and then reinitiating it when the inimical season is past. The dormant state of diapause serves to bridge the unfavorable seasons, and its timing provides a powerful mechanism for synchronizing insect development. This book explores how seasonal signals are monitored and used by insects to enact specific molecular pathways that generate the diapause phenotype. The broad perspective offered here scales from the ecological to the molecular and thus provides a comprehensive view of this exciting and vibrant research field, offering insights on topics ranging from pest management, evolution, speciation, climate change, and disease transmission, to human health, as well as analogies with other forms of invertebrate dormancy and mammalian hibernation.

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To Else, Esben, Solomon, Jude, and Liv

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Preface

> To every thing there is a season
>
> Ecclesiastes 3:1

The surge of new plant growth in the spring is followed quickly by a flush of insects that exploit those plants for food. And then, just as quickly, as summer wanes and winter approaches, plants die back and insects disappear until the cycle repeats the following year. Ours is a highly seasonal world. The tilt of the Earth as it circles the Sun dictates the intensity of heat impinging on the Earth's surface, thus driving the striking seasonal rhythms of life. Temperature contrasts between winter and summer are dramatic, and even in the tropics, where temperatures remain more constant, wet and dry seasons pose a conspicuous seasonal pattern. The impact this has on all life forms is consequential, but the challenge is especially pronounced for insects and other arthropods that, as ectotherms, can perform only within a narrow seasonal window. Escaping in time thus becomes an essential and crucial feature of the insect life cycle, allowing insects to survive seasons that are too cold, too dry, or lacking essential food resources required for development and reproduction.

Diapause, a dormant stage akin to mammalian hibernation, thus emerges as a key feature that may encompass the major portion of an insect's life. Development is shut down or dramatically retarded, allowing the insect to bridge inimical seasons. The ability of insects to avoid such unfavorable seasons by entering diapause has made it possible for this group of animals to not only invade all seven continents but also become the dominant life form in most of Earth's terrestrial habitats. Insects, of course, are not alone in their ability to respond to seasonal changes in the environment, but the diversity of insects, our ability to experimentally manipulate their environment, and our growing understanding of insect molecular processes have made insects a particularly rich taxon for probing seasonal responses. How an insect knows when bad times are coming, why and how it responds to evoke the diapause response, and the consequences of diapause for post-diapause development is the story I hope to relay.

Several wonderful reviews greatly influenced my understanding of diapause down through the years. My first encounters as a student with the diapause literature were delightful treatises by A.D. Lees (1955) entitled *The Physiology of Diapause in Arthropods* and by A.S. Danilevskii (1965 translation from Russian) entitled

Photoperiodism and Seasonal Development of Insects. This was followed by books that I eagerly devoured by S.D. Beck (*Insect Photoperiodism*, 1968); D.S. Saunders (*Insect Clocks*, 1976 [2002]); M.J. Tauber, C.A. Tauber, and S. Masaki (*Seasonal Adaptations of Insects*, 1986); and H.V. Danks (*Insect Dormancy: An Ecological Perspective*, 1987). The quality of these remarkable books makes it a bit daunting to consider adding my own perspective, but do so, I will! These previous books on insect diapause contain great background information on diapause as well as lists of diapausing insects that I will not repeat here, but instead, I hope to highlight major features of diapause with special emphasis on recent developments in the field.

In spite of the diversity of insects, quite a few common themes unite diapause responses observed across a range of species. I offer some hints on the diversity of responses, but I err on the side of giving more depth to some of the best-studied models at the expense of portraying all the variants. I place special emphasis on flies and moths, a bias that comes from my life experiences with these two prominent groups.

I am enormously grateful to my mentors who introduced me to diapause and encouraged me along the way. My graduate advisors, Gottfried Fraenkel and Judith Willis, introduced me to flesh fly diapause and the broader realm of diapause physiology; my postdoctoral advisor Jan de Wilde shared his diapause expertise and dared to send this Pennsylvania Dutchman to Kenya as a Dutch representative to explore diapause in the tropics; and Carroll M. Williams provided an engaging laboratory, replete with a rich history of diapause experimentation, when I returned to North America. I am also enormously grateful to all the students, postdocs, and visiting scholars who have been through my laboratory at Ohio State and contributed so much to my own understanding of diapause. While I often got the credit, it was their hard work that allowed us to deepen our understanding of diapause mechanisms. And, more broadly, the entire diapause community has attracted a great cadre of people with whom I have enjoyed interacting. Thanks for all you have taught me. I hope I have captured your insights correctly.

Thanks to Dominic Lewis, Aleksandra Serocka, and Jenny van der Meijden from Cambridge University Press for encouraging me to embark on this project and for providing guidance along the way. Select portions of the book benefited from thoughtful comments provided by David Saunders (University of Edinburgh), Peter Cherbas (Indiana University), Dan Hahn and Clancy Short (University of Florida), William Bradshaw and Christina Holzapfel (University of Oregon), Peter Armbruster (Georgetown University), Mariana Wolfner (Cornell University), and Megan Meuti (The Ohio State University). Their helpful critiques, especially when adopted, resulted in a better book. Thanks to Jonathan Denlinger for assistance in preparing several figures, and a special thanks to my wife, Judy, for supporting my devotion to the preparation of this book over the past few years.

1 Confronting the Challenges of a Seasonal Environment

Our Earth imposes challenging seasonal obstacles for insect development, with few sites capable of continuously sustaining activity throughout the year. Low winter temperatures at temperate and polar latitudes are a conspicuous obstacle to continuous growth and reproduction. Not only is the cold a significant threat to insect survival, but the accompanying disappearance of plants, on which most insects depend for food, generates a seasonally depauperated environment not suitable for continuous development. Equally challenging are tropical dry seasons that lack critical food and water resources.

But, the advantages of diapause go well beyond avoidance of adverse seasons. A finely tuned diapause is critical for the precise timing of adult emergence in the spring. The fact that many insects are host specialists means that diapause is an essential element for coordinating the timing of adult emergence with the growth or flowering of favored host plants. By becoming dormant, insects also mitigate risks of predation, and periodic disappearance into diapause may help reduce biotic challenges from parasites and microbes.

Diapause occurring during the winter has the advantage of concomitant low temperatures that, for an ectotherm, help suppress metabolic rate and conserve energy reserves. Summer diapauses and diapauses in tropical insects lack this advantage and thus confront the extra challenge of conserving energy reserves in a warm environment. The ability to suppress metabolism without the aid of low temperature thus becomes a special challenge in select environments.

High latitudes pose yet another different challenge. Short growing seasons restrict development to narrow seasonal windows that may be inadequate for the completion of a single generation. Such conditions may require multiple years for completion of the life cycle, thus making it necessary for more than one developmental stage to enter diapause. In some cases, a developmental arrest may be repeatedly induced and broken to capitalize on brief favorable seasons.

Just as seasons have different profiles in different regions of the Earth, the role of diapause, the environmental regulators that are essential for coordinating the response, and the features of the diapause response can be expected to vary across the globe. Diapause thus emerges as a fascinating and plastic life-history trait whose evolution reflects adaptations essential for confronting challenges of life in a wide range of specific environments as well as life in a changing environment.

1.1 What Is Diapause?

1.1.1 Origin of the Term Diapause

The Greek word "diapause" means rest or interruption of work. The usage of this word has an interesting history (Lees 1955). As first used by Wheeler (1893) in his work with the grasshopper *Xiphidium ensiferum*, diapause defined the brief halt in development and embryonic transition between anatrepsis (when the embryo of a hemimetabolous insect moves tail-first through the yolk, away from the pole) and katatrepsis (when the embryo then migrates to either the ventral or dorsal surface, depending on the species). Its meaning was expanded by Henneguy (1904) to refer not to a *stage* of morphogenesis but the *condition* of arrested development, but Henneguy failed to make a distinction between the simple arrest prompted by cold and the type of arrest that is stage-specific and developmentally programmed. Shelford (1929) recognized the need to make such a distinction and used the term quiescence for the interruption of growth due directly to an unfavorable condition, while he used the term diapause to refer to "spontaneous arrest," an arrest that we now refer to as being developmentally programmed and stage-specific. This same sort of distinction was implied in the terms "diapause vrai" and "pseudodiapause" used by Roubaud (1930). Numerous and subtle distinctions can be made when arrested development is described for diverse species (Danks 2002), and distinctions between species have led to rather complex classifications of insect dormancy (e.g., Müller 1970, Mansingh 1971, Ushatinskaya 1976a), resulting in the introduction of terms such as oligopause, parapause, pluvipause, and others to describe developmental arrests with slightly different features. My own bias is that the terms diapause and quiescence are useful distinctions, but I see no need for further subdivisions, assuming we recognize that some features of diapause and quiescence may vary among species.

The term diapause extends beyond the insect literature and includes numerous examples among other arthropods, especially ticks and mites (e.g., Belozerov 2008, Lohmeyer et al. 2009, Bryon et al. 2017a). Diapause in Crustacea is well documented among copepods and brine shrimp (Marcus and Scheef 2010, Hand et al. 2016, Baumgartner and Tarrant 2017) and is prevalent in rotifers (Garcia-Rogers et al. 2019, Tarazona et al. 2020), as well as a few species of mollusks (Numata and Udaka 2010). Tardigrades, invertebrates best known for their capacity to enter a cryptobiotic state, also include some species that diapause as embryos (Guidetti et al. 2011). Diapause is also widely used to refer to delayed implantation of embryos in marsupials, polar bears, badgers, mink, anteaters, mice, gerbils, and over 130 other species of mammals (Renfree and Fenelon 2017) and is routinely used to refer to embryonic dormancies noted in non-mammalian taxa ranging from lizards to some of the bony fish species (Rafferty and Reina 2012), sharks and rays (Waltrick et al. 2012), and some of the bony fish species such as annual killifish (Martin and Podrabsky 2017, Hu et al. 2020).

Although developmental arrest in nematodes is a similar form of dormancy, the arrest in nematodes is more commonly referred to as the **dauer state** (Riddle 1997). Mammalian **hibernation** shares many features with insect diapause (Andrews 2019), but the unique physiological features of endotherms and ectotherms justify making a distinction between these two forms of dormancy. Though we may use different terms for these forms of animal dormancy, there is growing evidence that certain common themes operate across the animal kingdom and much can be gained from a quest for universal principles.

In most cases diapause is one of two developmental alternatives produced by the same genotype, thus it fits the classic definition of **polyphenism**, coined by Ernst Mayr (1963). Insects of the same genotype can either enter diapause or develop without diapause, a developmental decision that depends on the seasonal environmental cues received. The term polyphenism is thus distinct from the common usage of polymorphism, a term usually restricted to differences that have a genetic basis. The features of diapause, in which the environment dictates the phenotype (diapause or nondiapause), conform to the classic definition of **phenotypic plasticity** or a plastic response to environmental conditions.

1.1.2 Diapause Is a Programmed Event

Diapause is a programmed, stage-specific arrest or retardation of development commonly used to circumvent an adverse season. Upon entry into diapause, insects remain in this arrested state for some time, even if prevailing environmental conditions are favorable for development.

"Programmed" implies that it is not an immediate response to environmental adversity but a response to environmental signals that have been received in advance of the actual onset of diapause or, alternatively, is hard-wired genetically to occur at a specific stage. The distinction between an environmentally programmed diapause and one that is hard-wired genetically is captured in the terms "facultative" and "obligate" diapause. **Facultative diapause** implies a plastic response dependent upon receipt of specific environmental cues commonly received well in advance of diapause onset. By contrast, **obligate diapause** refers to a genetically programmed diapause that occurs at a specific stage regardless of the prevailing environmental input. While an insect with an obligate diapause is likely to complete only one generation a year, an insect with a facultative diapause has the flexibility to complete multiple generations, commonly several nondiapausing generations during the summer, followed by an overwintering generation that enters diapause.

Both obligate and facultative diapauses can sometimes be seen within the same family, such as the stink bugs Pentatomidae (Musolin and Saulich 2018). Certain trends exist within taxa such as hymenopteran parasitoids: parasitoids that attack univoltine (one generation/year) hosts tend to have an obligate diapause, while those attacking polyvoltine (multiple generations/year) hosts usually have a facultative diapause (Polgar and Hardie 2000). Although rare, one species may exhibit both an obligate and facultative diapause: certain populations of the European spruce bark beetle *Ips typographus* harbor both of these diapause phenotypes (Schebeck et al. 2017). Some species that appear to have an obligate diapause may, under close scrutiny, avert diapause under specific circumstances. For example, the saturniid moth

Hyalophora cecropia, a species that always enters pupal diapause when reared outside, can actually be enticed to develop without entering diapause when exposed to artificially long daylengths (Waldbauer 1996); hence this species, long thought to be an iconic example of a species with an obligate diapause, is more correctly recognized as a species with a facultative diapause, albeit a species that normally completes only a single generation each year and appears to seldom exploit its potential to skip diapause.

The beauty of the programming aspect of diapause is that it provides a preparatory phase that allows sequestration of additional energy reserves, augmentation of cuticular waterproofing, changes in color to match the winter or dry season habitat, migration, and selection of a well-protected hibernaculum (overwintering refuge).

1.1.3 Making a Distinction between Diapause and Quiescence

The programmed feature of diapause is in contrast to **quiescence**, a form of dormancy that is an immediate response to adversity and includes no preparatory phase. Unlike diapause, quiescence is broken immediately when favorable conditions return. Quiescence offers the capacity to quickly stop and restart development multiple times and at any stage in response to certain environmental challenges. For example, an insect placed in a refrigerator will halt development and become inactive but will resume development almost immediately when retrieved from the cold environment. Similarly, an insect denied food may enter an arrest that is terminated immediately when food again becomes available. This sort of rapid entry into and recovery from a dormant state distinguishes quiescence from diapause. Distinct endocrine signatures underlie these two forms of developmental arrest, as noted in adult females of the linden bug *Pyrrhocoris apterus* (Hodková and Okuda 2019). The corpora allata (CA) cease producing the juvenile hormone needed for vitellogenesis in both starvation-induced quiescence and diapause, and when food again becomes available the CA is immediately activated in starved bugs but not in diapausing bugs.

One of the interesting current debates is whether the well-studied adult dormancy in *Drosophila melanogaster* is a programmed arrest (i.e., a diapause) or a simple quiescence that is temperature-induced. Although the term diapause is frequently used in this context (e.g., Zonato et al. 2017), strong arguments counter that quiescence is a more appropriate term for the dormancy of *D. melanogaster* (e.g., Emerson et al. 2009b,c, Saunders 2020b) due to the immediacy of the response. One strong argument that the dormancy of *D. melanogaster* should not be regarded as diapause is the fact that egg chambers of the female initiate yolk uptake but then, in response to the low temperatures or other stresses that evoke the dormancy response, degenerate, an energy-consuming event not normally observed in a diapausing insect (Lirakis et al. 2018). The fact that this same response can be evoked in tropical populations of *D. melanogaster* has been used to suggest that the dormancy is not a diapause, but many tropical species do indeed have a diapause (see Section 2.3), thus that argument is not compelling. That egg chambers degenerate, that other stresses such as starvation elicit an identical response, and that the response is so volatile support the argument that dormancy noted in *D. melanogaster* represents a general stress response in this species, rather than a strictly binary trait. Yet, an examination of latitudinal clines suggests that certain populations of *D. melanogaster* do indeed possess a photoperiodically programmed diapause while others have features more akin to quiescence (Tatar et al. 2001, Anduaga et al. 2018).

A distinction between diapause and quiescence can sometimes be challenging to discern in other species as well. The invasive species *Drosophila suzukii* seems to show a slight photoperiodic response but, like *D. melanogaster*, its winter dormancy is more akin to quiescence (Everman et al. 2018). The mountain pine beetle *Dendroctonus ponderosae* was initially reported to overwinter in quiescence, but more recent experimental evidence shows a period of developmental latency, suggesting that the overwintering stage of the larvae can more accurately be described as diapause (Bentz and Hansen 2017). It is still unclear whether the midsummer disappearance of the yellow dung fly *Scathophaga stercoraria* in Central Europe can be attributed to diapause or quiescence (Blanckenhorn et al. 2001). Although less common, quiescence is also a viable option for overwintering, as noted in the diamondback moth *Plutella xylostella*, a species found in various life stages during winter in eastern North America (Dancau et al. 2018). Perhaps as a bet-hedging strategy, the European water strider *Velia caprai* can overwinter both as an adult in quiescence and as an embryo in diapause (Ditrich and Koštál 2011).

Though diapause, with its opportunity for preparation, would appear to be the ideal option for bridging unfavorable seasons, there are certain advantages to quiescence. Not only is it an option available to any developmental stage, but it also offers flexibility for rapidly responding to environmental conditions. For example, when temporary rock pools inhabited by the midge *Polypedilum vanderplanki* dry up in Nigeria, midge larvae enter a cryptobiotic state, a form of quiescence that is quickly broken when the rains return (Hinton 1951, Cornette and Kikawada 2011). This form of dormancy offers extreme flexibility, for both time of entry as well as exit from the dormant state. Quiescence may also be the preferable state for aquatic insects residing in cool, high-latitude streams (Danks 1987). Unlike the habitat of the African midge, cool streams represent a stable habitat, and quiescence enables swift transition between an active and inactive lifestyle, making it possible to maximally exploit periods when temperatures exceed a certain threshold.

1.1.4 Stage Specificity

The adjective "stage-specific" implies that diapause capacity is restricted to a single stage of development for each species. Although diapause can occur in embryos, larvae, pupae, or adults, for any one species the capacity for diapause is usually restricted to a single stage. There are, however, good examples of species, especially those confronting short growing seasons at high latitudes, that have the capacity for diapause at two stages of development. Northern populations of the spruce budworm

Choristoneura biennis first overwinter in a second-instar larval diapause and spend the second winter in a final larval-instar diapause (Nealis 2005), northern populations of the blow fly *Calliphora vicina* diapause both as third-instar larvae and as adults (Vinogradova and Reznik 2013), and the bruchid beetle *Bruchidius dorsalis* diapauses in cooler areas as a final-instar larva (Kurota and Shimada 2003a) and as an adult in warmer regions (Kurota and Shimada 2003b). Larval diapause in the longicorn beetle *Psacothea hilaris* is unusual in that it can occur across a wide developmental range from the fifth to ninth instar, although it most commonly occurs in the sixth or seventh instar (Asano et al. 2004). In central Sweden, the butterfly *Parage aegeria* diapauses as a pupa, but both larval and pupal diapause are common in southern Sweden (van Dyck and Wiklund 2002). *Lycaena hippothoe*, a butterfly with a wide Eurasian range, is capable of entering larval diapause as either a third or fourth instar (Fischer and Fiedler 2002). However, in the majority of insect species, the capacity for diapause resides in only one stage. This sort of stage specificity is not true for quiescence. Insects can become quiescent in any developmental stage, although the threshold for entering quiescence may differ among stages.

1.1.5 Diapause Development

In the strictest sense, development is not completely arrested during diapause. A slow progression of developmental processes occurs during diapause, culminating eventually in termination of diapause, a progression that Andrewartha (1952) referred to as **diapause development**. Diapause is best viewed not as a “stop/start” event but as a dynamic developmental trajectory operating on a much slower time scale than the developmental events observed in nondiapausing insects. Although covert molecular events are occurring as diapause progresses in all species, in most cases development appears at a standstill. In a few cases, however, overt changes can be noted during diapause, generating a response more akin to a slowing of the developmental rate rather than a complete developmental arrest. For example, during adult diapause in females of the mosquito *Culex pipiens*, ovarian follicles progressively enlarge, but it takes more than 20 weeks for the follicles to attain the same size attained by a nondiapausing female in 3 days (Readio et al. 1999). Similarly, the sexually produced embryos of the pea aphid *Acyrthosiphon pisum* overwinter in a diapause that can best be described as extremely slow, albeit progressive development during the interval between anatrepsis and katatrepsis (Shingleton et al. 2003). Legs and body organs continue to grow but at a rate that is largely temperature-independent, a contrast to the temperature-dependent growth rate observed after katatrepsis. A similar slow progression of development is noted for diapausing embryos of aphids *Cinara cupressi* and *Cinara juniperi* (Durak et al. 2020): Mitotic cell division continues at a slow pace, resulting in a doubling of body size from day 16 to day 70 after oviposition. In some Orthoptera, for example the cricket *Modicogryllus siamensis*, diapause is manifested not as a complete halt in development but as a much slower rate of development that incorporates additional nymphal molts (Miki et al. 2020).

1.2 Who Does It and in What Stage?

Diapause has evolved numerous times, as discussed in Chapter 11. In some taxa, diapause characteristically occurs at one certain stage, but that is not universally true. In some families and genera, the stage of diapause is not highly conserved. Among the many species of flesh flies (family Sarcophagidae) that have been examined from around the world, those from South America appear to lack the capacity for diapause (Denlinger et al. 1988a), but all species examined from North America, Europe, Asia, and Africa have a pupal diapause. Ladybird beetles (family Coccinellidae), due to their importance as biological control agents, have been examined extensively, and reports on species from Europe, North America, and Asia consistently reveal the capacity for an adult diapause (Hodek 2012).

At the other extreme, the genus *Drosophila* contains species that diapause as larvae (*D. deflexa*), pupae (*D. obscura*), or adults (e.g., *D. virilis* and *D. obscura* groups) (Lumme 1978). The diapause stage differs in two closely related Swiss dung flies that occupy a similar niche: *Scathophaga stercoraria* diapauses as a pupa, while *Sepsis cynipsea* diapauses as an adult (Blanckenhorn 1998a). Scandinavian butterflies within the Nymphalini tribe show remarkably different overwintering strategies (Wiklund et al. 2019). One of the nymphalids enters pupal diapause, two migrate south for the winter, four are univoltine and have an obligate adult diapause, and three have a partial second generation (two species have a facultative adult diapause determined by larval daylength, and one species shunts half the population into an obligate adult diapause while the other half enters a facultative adult diapause dependent on larval daylength). Stink bugs, family Pentatomidae, are known to diapause as embryos, nymphs, and adults (Musolin and Saulich 2018). Most bruchid beetles diapause as adults but at least two temperate species, *Kytorhinus sharpianus* and *Callosobruchus ademptus*, diapause as larvae (Kurota and Shimada 2001). Two closely related weevils differ considerably in their diapause attributes: *Exapion ulicis* diapauses as an adult and lays eggs in the spring, while *Exapion lemovicinum* lays eggs in the autumn and diapauses overwinter as a larva, responses driven largely by different fruiting times of their host plants (Barat et al. 2010). Among mosquitoes, certain trends emerge (Denlinger and Armbruster 2016, Diniz et al. 2017), as shown in Figure 1.1. While embryonic and larval stages predominate as the diapause stage in the genus *Aedes*, most mosquitoes in the genus *Culex* diapause as adults. In other genera, such as *Anopheles*, diapause is reported in embryonic, larval, and adult stages. No mosquitoes appear to use the pupal stage for diapause. Among crickets, embryonic diapause is the most prevalent stage of diapause, represented by over 80% of the species in Japan and the eastern United States (Masaki and Walker 1987). Among 200 species of mirid bugs from the Netherlands, 85% diapause as embryos (Cobben 1968), and among 14 species representing 5 genera of plant bugs in the subfamily Mirinae, 4 genera consistently diapause as embryos and members of another genus, *Lygus*, consistently diapause as adults (Saulich and Musolin 2020). So, some trends are noted within taxonomic

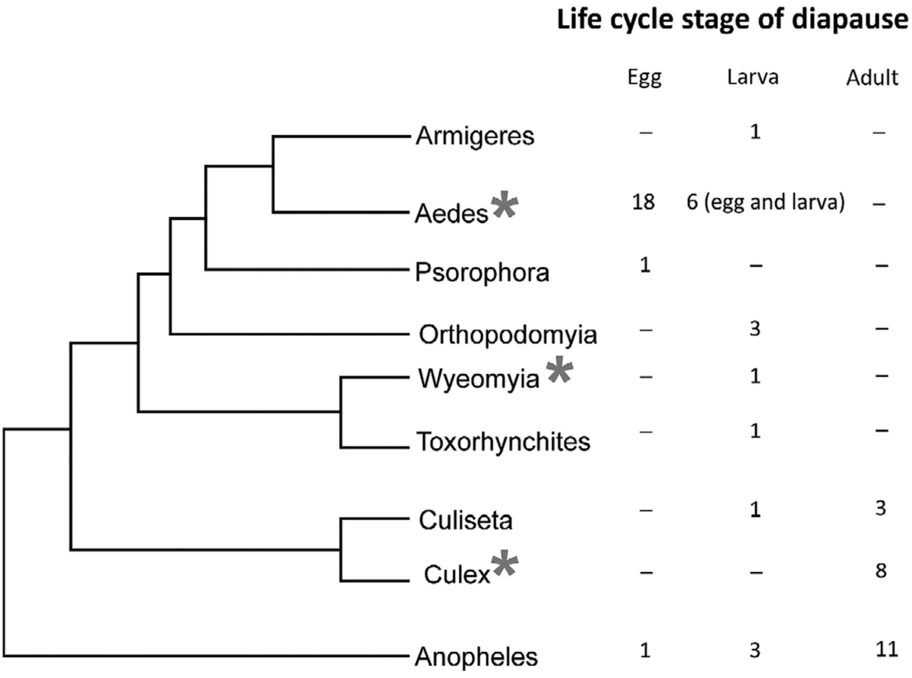

Figure 1.1 Phylogenetic distribution of life cycle stages of diapause in nine mosquito genera. * indicates genera containing at least one species for which considerable information exists on the transcriptional basis for diapause.
From Denlinger and Armbruster (2016).

groups, but many exceptions are evident. Diapause is a highly variable trait, not only among closely related species but also among populations, as further discussed in Chapter 3.

1.2.1 Embryonic Diapause

Embryonic diapause is especially well documented in Orthoptera (crickets, grasshoppers, walking sticks), true bugs in the order Heteroptera, and butterflies and moths in the order Lepidoptera, and among the lower Diptera, such as mosquitoes and midges. It occurs in some Coleoptera but appears to be less common in that order. Diapause is not restricted to a specific stage of embryonic development but has been noted across a wide range of embryonic stages. During embryonic development, the zygote divides (cleavage), forms a layer of cells surrounding the yolk (blastoderm formation), and in hemimetabolous insects, the blastoderm then invaginates slightly and migrates (blastokinesis). The embryo first migrates posteriorly (anatrepsis) and then dorsally (katatrepsis), movements that are somewhat simplified in holometabolous insects (Panfilio 2008). Diapause can apparently intercede at almost any point during embryogenesis, from the early stages of blastoderm formation through to the completion of pharate larval development (at which point the first-instar larva is completely formed but has not yet hatched). A study on the crane fly *Tipula simplex* suggests that diapause in this species occurs prior to cleavage (Hartman and Hynes 1980), although additional studies at the electron microscope level would be helpful in validating this report. One particularly well-documented case of a species with an early-stage diapause is the band-legged ground cricket *Dianemobius nigrofasciatus* (Tanigawa et al. 2009). This diapause occurs prior to germ band formation when the embryo is merely a cellular blastoderm, a continuous layer of single cells. Diverse stages of embryonic diapauses have been documented for other species. Diapause occurs in a pre-anatrepsis stage in the Australian plague locust *Chortoicetes terminifera* (Deveson and Woodman 2014) and balsam twig aphid *Mindarus abietinus* (Doherty et al. 2018). The well-studied commercial silk moth Bombyx mori also enters diapause quite early, when the blastoderm is in the form of an unsegmented dumbbell (Yamashita and Hasegawa 1985). Embryonic diapause in the pea aphid *A. pisum* occurs at a slightly later stage when the embryo is fully segmented and the limb buds are clearly defined (Shingleton et al. 2003). Embryonic development in a Tibetan population of *Locusta migratoria* is classified into 27 stages, with diapause interceding at stage 19, a stage prior to katatrepsis, occurring 9 days after oviposition at 30°C (Su et al. 2019, Wang et al. 2021). Two Japanese cicadas diapause at distinctly different developmental stages prior to katatrepsis: *Cryptotympana facialis* diapauses upon completion of body segmentation when the antennal buds are segregated from the cephalic lobes, while *Graptopsaltria nigrofuscata* diapauses at a slightly later stage when the antennae, maxillary buds, and thoracic appendages have already differentiated (Moriyama and Numata 2008). At the other extreme, the katydid *Eobiana engelhardti subtropica* enters diapause as an almost fully developed embryo (Higaki and Ando 2005), and many Lepidoptera, such as the gypsy moth *Lymantria dispar*, enter diapause at the completion of embryogenesis when the embryo is more appropriately termed a pharate first instar (Leonard 1968). When diapause in the gypsy moth is broken, the fully developed pharate first-instar larva consumes the final remaining yolk, breaks through the chorion, and begins its life as a first-instar larva.

Different stages of embryonic diapause may exist in closely related species. The Heteroptera present a fascinating array of diapausing embryonic stages (Cobben 1968). Nearly all conceivable stages of embryonic development, ranging from the early blastoderm through to pharate first-instar nymph, are used by this taxon as the diapausing stage (Figure 1.2). This observation underscores the idea that natural selection can capture different embryonic stages among closely related species. One caveat is that the array of diapause stages depicted in Figure 1.2 is based on single snapshots in time, thus any sort of progression as noted in pea aphid embryos (Shingleton et al. 2003) would be obscured.

Early studies forming the foundation for embryonic diapause include extensive work on *B. mori,* initiated by Fukuda (1951) and Hasegawa (1951), followed by beautiful studies from Yamashita (1996) and his colleagues, experiments on the gypsy moth *L. dispar* (Leonard 1968, Hoy 1977), detailed series of experiments in Japan on diapause in several species of crickets by Masaki (1967) and his students, and experiments with French populations of *L. migratoria* (Le Berre 1953). More recently,

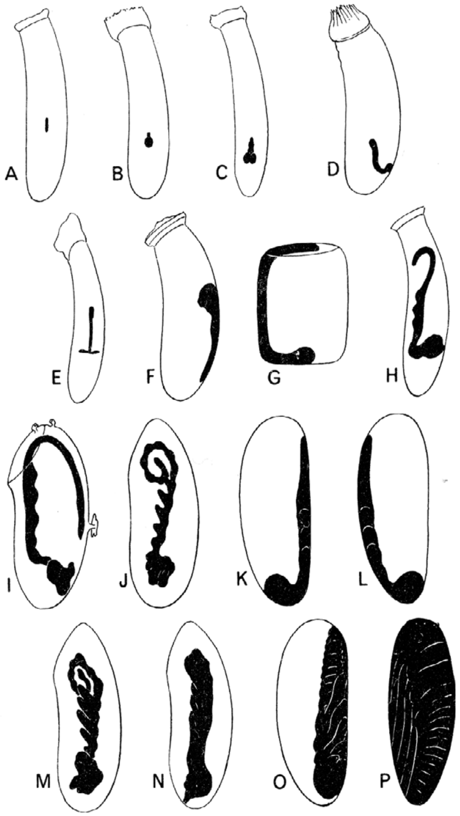

Figure 1.2 Stages of embryonic diapause in different species of Heteroptera. A, *Plagiognathus arbustorum*; B, *Megalocoleus molliculus*; C, *Pantilius tunicatus*; D, *Loricula elegantula*; E, *Leptopterna ferrugata*; F, *Coranus subapterus*; G, *Picromerus bidens*; H, *Himacerus apterus*; I, *Myrmus miriformis*; J, *Chiloxanthus pilosus*; K, *Notonecta lutea*; L, *Notonecta maculata*; M, *Chiloxanthus pilosus*; N, *Salda littoralis*; O, *Notonecta reuteri*; P, *Nysius thymi*. From Cobben (1968), with permission from Wageningen University.

embryonic diapause of the mosquito *Aedes albopictus* is attracting considerable attention as it invades new territories in Europe and North America (Armbruster 2016).

1.2.2 Larval Diapause

Larval (or nymphal) diapause is common among Lepidoptera, certain groups of Diptera, Hymenoptera, Coleoptera, Neuroptera, Odonata, Orthoptera, Hemiptera, and Plecoptera. Among the Lepidoptera, some species diapause in early larval instars, such as the first overwintering generation of the northern population of the spruce budworm *Choristonerura biennis* (Nealis 2005). But, more commonly diapause is entered at the end of the final larval instar when larvae have attained their full size and have ceased feeding. Although less common, a few Lepidoptera diapause midway through larval development. For example, the copper butterfly *Lycaena tityrus* diapauses as a third-instar larva before molting in the spring to a final, fourth-instar larval stage (Fischer and Fiedler 2001). *L. hippothoe*, a species with five larval instars, overwinters in either a third or fourth larval instar (Fischer and Fiedler 2002), and the large copper butterfly *L. dispar batavus*, diapauses as a second instar (Nicholls and Pullin 2003). The European corn borer *Ostrinia nubilalis* (Beck and Hanec 1960) and the southwestern corn borer *Diatraea grandiosella* (Chippendale and Reddy 1972) are among the best-studied examples providing early insights into larval diapause in Lepidoptera.

Among the lower Diptera, the pitcher plant mosquito *Wyeomyia smithii* offers the most robust and extensive body of literature on larval diapause, thanks mainly to the dedicated work from the Bradshaw-Holzapfel Laboratory and that of their colleagues (e.g., Bradshaw and Lounibos 1972). Larval diapause of *W. smithii* can intercede during either the third or fourth instar in certain populations (Lounibos and Bradshaw 1975), but a third-instar diapause is most prevalent. The mosquito *Anopheles barberi* most commonly enters diapause in the second instar, but some individuals diapause in the third instar as well (Copeland and Craig 1989).

Among the higher Diptera, I am not aware of larval diapause occurring in stages earlier than the fully grown, post-feeding third (final) larval instar, also known as the wandering stage. A well-studied example of such a third-instar fly diapause is the blow fly *C. vicina* (Vinogradova and Zinovjeva 1972, Saunders 2000). More recently, the drosophilid *Chymomyza costata* (Shimada and Riihimaa 1990, Koštál et al. 2011) joins *C. vicina* as a powerful model for larval diapause in Diptera. The final larval instar also dominates as the diapausing stage for larval diapause in the Hymenoptera. Diapause in the ectoparasitoid *Nasonia vitripennis* is perhaps the most thoroughly studied larval diapause among the Hymenoptera, prompted by several key early papers (Schneiderman and Horwitz 1958, Saunders 1965). More recent studies on the fifth-instar larval diapause of the alfalfa leafcutting bee *Megachile rotundata* adds this economically important species to the list of well-studied examples of larval diapause among the Hymenoptera (Yocum et al. 2006).

1.2.3 Pupal Diapause

Pupal diapause, best known for Lepidoptera and Diptera, is also noted in a few Hymenoptera but appears to be completely absent in Coleoptera, the largest insect order. The arrest usually occurs in the true pupal stage, that is, before the onset of adult differentiation. However, in a few species of moths (Sahota et al. 1982, 1985, Monro 1972), diapause occurs in pharate adults, that is, when adult development has been completed but the insect is still within the pupal cuticle. Thus, development can be halted either before differentiation begins, the most common stopping point, or after it is completed, but not at some midpoint between these two extremes. This is in marked contrast to embryonic diapauses that can intercede at nearly any point during the course of embryonic development.

Pioneering studies on the saturniid moth *H. cecropia* (Williams 1946), the tobacco hornworm *Manduca sexta* (Rabb 1966), the flesh fly *Sarcophaga argyrostoma*

(Fraenkel and Hsiao 1968), and fruit flies in the genus *Rhagoletis* (Bush 1969) provide the backdrop for much recent work on pupal diapause and for understanding seasonal patterns of distribution. Experiments using species from the agriculturally important *Heliothis/Helicoverpa* complex of moths add a more recent model for understanding pupal diapause (Meola and Adkisson 1977, Xu and Denlinger 2003).

1.2.4 Adult Diapause

Adult diapause is characterized by the arrested development of the ovaries, testes, accessory glands and related reproductive structures, suppressed feeding, locomotor and mating behavior, and in some cases, degeneration of the flight muscles. Adult diapause is common among Coleoptera, Lepidoptera, Diptera, Hemiptera, Orthoptera, Neuroptera, Trichoptera, Thysanoptera, as well as the Acarina. It is most frequently manifested in newly emerged adults that are not yet reproductively active, and characteristically the adults remain reproductively inactive until diapause is terminated. But, there are exceptions where diapause is entered following a bout of reproductive activity. For example, the leaf beetle *Diorhabda elongata* when transferred from long days to short days, can switch to diapause after a period of egg-laying (Bean et al. 2007a), and before entering summer diapause, females of the Colorado potato beetle *Leptinotarsa decemlineata* may lay a few eggs (Tauber et al. 1988). As discussed in Section 3.2, a few species exit and enter adult diapause multiple times.

A short period of **feeding** by newly emerged adults is sometimes essential for entry into diapause. Without feeding the twospotted spider mite *Tetranychus urticae* fails to assume the bright orange coloration of diapause and fewer individuals enter diapause (Kawaguchi et al. 2016). Although feeding may continue in some diapausing adults, feeding intensity is greatly suppressed, as reported for several species of *Drosophila* (Matsunaga et al. 1995), the blow fly *Phormia regina* (Stoffolano 1975), and the ladybird beetle *Harmonia axyridis* (Gao et al. 2019), among others. In *P. regina*, like other flies, the proboscis is extended when tarsi of the adult detect a food source. Interestingly, the threshold for this tarsal response differs little between diapausing and nondiapausing flies, suggesting that the feeding inhibition during diapause is controlled by the central nervous system rather than the peripheral system (Stoffolano 1975). Distinctions may also occur in the food source used by diapausing and nondiapausing adults. This is especially evident in the northern house mosquito *Culex pipiens*. While nondiapausing females are avid blood-feeders, females programmed for diapause feed exclusively on nectar (Bowen 1992). Only at the completion of diapause do females regain an interest in seeking a blood meal from their avian hosts (Faraji and Gaugler 2015).

Diapause in males has been examined less extensively than in females (Pener 1992), but in many cases, the same sort of reproductive shut-down is seen in both sexes (Kubrak et al. 2016, Urbanová et al. 2016, Ala-Honkola et al. 2018). **Mating behavior** is curtailed. Accessory glands remain undeveloped. Spermatogenesis ceases and most undifferentiated sperm degenerate. The only cells within the testes that appear to be affected by diapause in larvae of the waxworm *Galleria mellonella* are spermatocytes in proximal regions of the testicular follicles, cells that undergo apoptotic degeneration (Bebas et al. 2018). In diapausing males of the leaf beetle *Gastrophysa atrocyanea*, the testes slowly increase in size during the first two to three months of diapause and then regress and remain small for the remaining three to four months of diapause (Ojima et al. 2015). During diapause in the nymphalid butterfly *Polygonia c-aureum*, and presumably in other Lepidoptera that characteristically produce both nucleated eupyrene sperm (fertile) and anucleated apyrene sperm (infertile), production of both types of sperm is synchronously reinitiated at diapause termination (Hiroyoshi et al. 2017).

Diapausing females are characteristically not attractive to either diapausing or nondiapausing males, and diapausing males usually will not attempt to mate with sexually attractive females. Males of *Drosophila montana* that are reproductively active will court reproducing, post-diapause females but completely ignore diapausing females (Ala-Honkola et al. 2018). The same is true for *D. melanogaster* (Kubrak et al. 2016). Diapausing males of the grasshopper *Anacridium aegyptium* fail to display mating behavior until several months after reaching the adult stage (Greenfield and Pener 1992). Late in diapause, males of the fungus beetle *Stenotarsus rotundus* will attempt to mate if they are physically handled, but mating is not normally observed until the environmental signal for diapause termination has been received (Tanaka et al. 1987b).

Foundational experiments on the Colorado potato beetle *L. decemlineata* (de Wilde and Bonga 1958), the grasshopper *Oedipoda miniata* (Pener and Broza 1971), linden bug *P. apterus* (Hodek 1968), lacewing *Chrysopa carnea* (Tauber and Tauber 1970a,b), and mosquito *Culex pipiens* (Spielman and Wong 1973) offer a rich launching point for more recent studies on adult diapause. Though *D. melanogaster* has a rather weak adult dormancy (Saunders et al. 1989), probably most correctly termed a quiescence (Section 1.1.3), the powerful genetic tools available for this model species have prompted a surge of recent interest in this species, as well as in other members of the genus.

In some cases, **only adult females** enter diapause. Males of the northern house mosquito *Culex pipiens* inseminate females in the autumn and then die without entering diapause, leaving only inseminated, diapausing females, to survive the winter (Spielman 1964). Similarly, only females of the twospotted spider mite *Tetranychus urticae* (Veerman 1985, Suzuki and Takeda 2009), anthocorid bugs in the genus *Orius* (Musolin and Ito 2008), eastern yellow-jacket *Vespula maculifrons* (Kovacs and Goodisman 2012), bumble bees *Bombus impatiens* (Amsalem et al. 2015), and *B. terrestris* (Colgan et al. 2019), among others, overwinter in diapause, after mating in the autumn. In the autumn, overwintering females of the hover fly *Episyrphus balteatus* accumulate impressive fat reserves, while males fail to do so and all males succumb by the end of December, leaving only the inseminated females to survive until spring in northern Germany (Hondelmann and Poehling 2007). In tropical Australia, only females of the nymphalid butterfly *Hypolimnas bolina* appear to use diapause to bridge the dry season (Pieloor and Seymour 2001). Males of this species survive the dry season and have sperm present throughout the year, suggesting they are consistently able to take advantage of unpredictable female activity.

In other species such as the ladybird beetle *Coccinella septempunctata* (Hodek 2012), the Colorado potato beetle *L. decemlineata* (de Wilde et al. 1959), and the handsome fungus beetle *S. rotundus* (Wolda and Denlinger 1984) both sexes diapause and mating occurs only when diapause is terminated. But, there are exceptions. A high proportion (40–60%) of central European populations of *C. septempunctata* mate prior to entering diapause, while *Ceratomegilla undecimnotata*, a ladybird beetle that hibernates in the same area, delays mating until spring (Hodek and Ceryngier 2000). Most females of the linden bug *P. apterus* mate in the spring after diapause has been completed, but a few females (up to 7% in South Bohemia) mate prior to overwintering and retain viable sperm throughout the winter (Socha 2010). Similarly, both sexes of the kudzu bug *Megacopta cribraria* overwinter in adult diapause, and most females delay mating until spring, but approximately 15% of overwintering females mate before the onset of diapause and thus store sperm for up to seven months (Golec and Hu 2015). Female dung flies (family Sepsidae) store sperm over winter, but fertility increases when they also have an opportunity to mate in the spring (Zeender et al. 2019). It seems unlikely there are cases in which only adult males diapause.

The fate and survival of stored sperm within diapausing females have received little attention in insects. However, in the marine copepod *Neocalanus flemingeri*, sperm within fertilized females remain quiescent during diapause and complete maturation inside the female, as indicated by upregulation of spermatogenesis toward the end of diapause (Roncalli et al. 2018). Similar scenarios may operate in insects, but such results have not been documented.

Mating prior to diapause sometimes alters the overwintering female's physiology through unexpected mechanisms. Pre-diapause mating boosts the abundance of antimicrobial hemolymph peptides in the buff-tailed bumble bee *B. terrestris*, an effect that is sustained throughout diapause and likely enhances survival of the diapausing queen (Colgan et al. 2019).

Little is known about the physiology of males that die rather than entering diapause. Do the environmental factors that trigger diapause in females also impact male physiology? Sperm produced by the male are transferred to the female's spermatheca in the autumn and remained stored there until the following spring. Do they have special properties or are they indistinguishable from sperm produced by their nondiapausing male counterparts that mature under spring or summer conditions?

1.3 Do Colonies of Social Insects Diapause?

Overwintering survival of many social insects is vested in a queen that overwinters and restarts the colony again in the spring, but many social insects including honey bees, termites, and ants maintain their colony structure throughout the winter. Are these overwintering colonies in diapause? The literature usually avoids this terminology, and instead opts for terms such as **summer bees and winter bees**. Yet, when viewed as a superorganism, the winter phases of colony life share many attributes of diapause.

Ant colonies that Kipyatkov and his colleagues (e.g., Kipyatkov and Lopatina 1999, Kipyatkov 2006) have studied in northern Russia are considered to be in diapause during the northern winter. Like most diapausing insects, ants in several genera have been documented to respond to short daylength by entering a nonreproductive phase. In some species, only adults and workers are capable of entering diapause (tribe Formicini), but in several other species, larvae also have the capacity for diapause. Which larval stage is used for diapause varies with genus: Diapause occurs in early instars (1–3) in *Lepisiota*, *Plagiolepis*, *Tapinoma*, and some *Camponotus*, in middle instars (2–4) in some *Camponotus*, in late instars (3–4) in *Harpagoxenus*, *Leptothorax*, *Temnothorax* and *Messor*, and in the final larval instar in *Manica*, *Diplorhoptrum*, *Leptanilla*, *Monomorium*, *Myrmica* and *Tetramorium*, and in all six instars in *Aphaenogaster, Crematogaster, Lasius, Paratrechina*, and some species of *Camponotus*. In southern Russia, larvae overwinter only once, but as the growing season shortens in the north, more and more larvae overwinter twice or more. In many of these taxa, overwintering larvae give rise to alates (winged adults that are reproductively active) that emerge in the spring to establish new colonies. Few physiological properties have been evaluated in these overwintering colonies, but the cessation of reproduction, the response to daylength, and the stage specificity of the overwintering stage share many of the attributes one expects to see in diapause. In the fire ant, *Solenopsis invicta*, a species recently introduced into the southern portion of the United States, clear behavioral and physiological attributes distinguish summer and winter colonies (Tschinkel 1993, Cook et al. 2016): Summer colonies have faster turnover rates, higher rates of metabolism, and nutrient utilization patterns that are distinct from patterns noted in winter colonies. What prompts these seasonal shifts, however, remains undefined and can be challenging to uncover in this and other social species.

In **honey bees** (*Apis mellifera*), winter workers tend the queen and continue to regulate hive temperatures, but they remain rather idle, they no longer forage and have reduced duties in brood rearing. There is a striking difference in their longevity compared to summer bees. Although summer bees live for 15–38 days, the lifespans of winter bees average 140 days, a tenfold increase over summer bees (Winston 1987). In addition, winter bees have a more extensively developed fat body than their summer counterparts and display a more robust immune response (Dostálková et al. 2021). Brood-rearing activity declines in response to short days and is accelerated by long days (Kefuss 1978). Thus, the winter bee is in many ways similar to other insects in diapause. The transcriptional profile of winter bees, based on 14 candidate genes, differs from that of summer bees: Abdominal tissues resemble profiles of nurse bees and thoracic tissues resemble foragers (Bresnahan et al. 2021), thus the winter bee appears to be a distinct phenotype. Thermoregulatory activities that invoke the use of thoracic flight muscles in winter bees are presumably the functional link with summer-active forager bees. A full transcriptomic analysis will be useful in searching more broadly for expression patterns that may be shared with other insects in diapause, especially bumble bee queens and other Hymenoptera that overwinter in diapause. It is unclear which seasonal environmental features are responsible for the switch to the production of winter bees. Whether the winter bee should be considered to be in diapause is a semantic issue. Although it is reasonable to view winter bees as being in diapause, the more important issue is to at least recognize that winter bees and diapausing insects do share some common attributes.

The same can be said for many species of **termites.** Workers of the eastern subterranean termite *Reticulitermes flavipes* survive low temperatures better when subjected to a decreasing thermoperiod and photoperiod prior to the onset of winter, a response that appears to drive the workers to a greater depth where temperatures are not as severe (Cabrera and Kamble 2001). Activity levels remain low during winter, but workers do not appear to sequester additional fat reserves. During the dry season in the African savanna, termite densities are low and activity is suppressed (Davies et al. 2015), but when the rains arrive, the next generation of reproductives, the alates, emerge from the ground in enormous numbers to initiate the next cycle of nest founding and reproduction. The pause in development that precedes the emergence of reproductive adults is in many ways akin to post-diapause quiescence. The alates are fully capable of progressing to the next phase of their life cycle, but they fail to do so until the proper environmental stimulus (rain) arrives. Again, termite researchers do not refer to this waiting period as diapause and post-diapause quiescence, but this life cycle does indeed share features in common with diapause.

1.4 Phases of Diapause

The impact of diapause on the insect life cycle is profound, extending from the perception of environmental signals used to program diapause, through diapause itself, to alteration of traits manifested after diapause has ended. In some cases the influence is also transgenerational, initiated by the parents and extending to subsequent generations. It is this pervasive impact of diapause across a broad temporal and developmental scale that justifies identifying diapause as an **alternative life cycle**, a syndrome that encompasses more than just the diapausing stage.

Although numerous terms have been used to define different phases of diapause in the older literature, the terminology championed by Koštál (2006) and articulated also by others (e.g., Tauber and Tauber 1976, Tauber et al. 1986, Danks 1987, Denlinger 2002) broadly divides diapause into three phases: pre-diapause, diapause, and post-diapause (Figure 1.3).

Pre-diapause includes the environmental programming events (also known as diapause induction) as well as the preparative steps that the insect undergoes prior to diapause. These two phases of pre-diapause are usually separate in time but may overlap, depending on the species. Diapause itself encompasses the total period when the insect is refractory to the progression of development, even though favorable environmental conditions are present. Diapause can be divided into three distinct phases, including **diapause initiation** (or onset), **maintenance**, and **termination**. It is during the initiation phase that development ceases or dramatically slows, and the metabolic rate usually drops, although some feeding and accumulation of energy

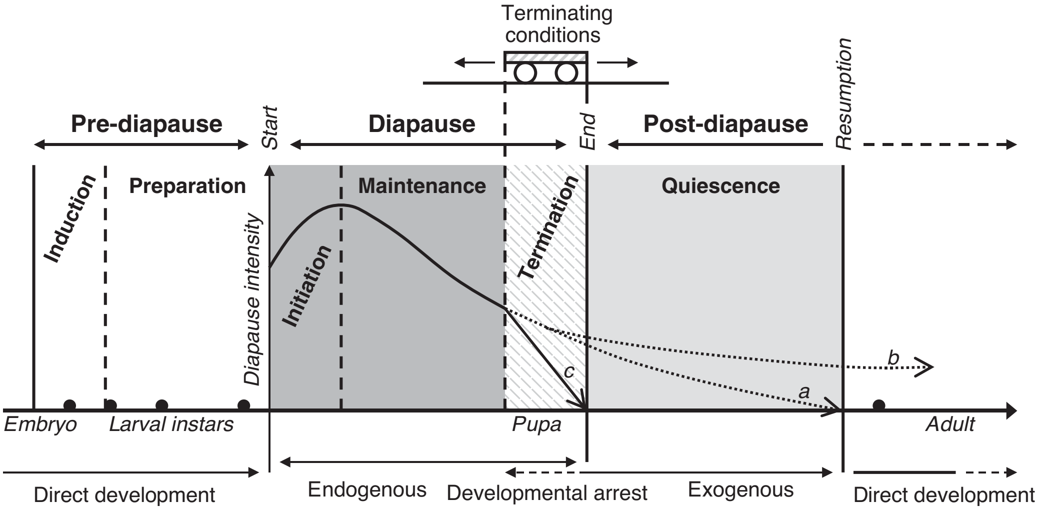

Figure 1.3 Schematic depiction of three major phases of diapause (pre-diapause, diapause, post-diapause) and finer subphases including environmental induction, preparation, initiation, maintenance, termination, and post-diapause quiescence. Changes in diapause intensity are represented by dotted lines (a and b) under two hypothetical constant conditions, while the solid line (c) indicates a change in environmental conditions that favors diapause termination. From Košťál (2006), with permission from Elsevier.

stores may continue. The intensity of diapause usually increases during this period, that is, it becomes increasingly more difficult to reverse the diapause decision by external stimuli.

The period of diapause maintenance is the longest, extending from diapause initiation to diapause termination. It is during this period that the insect gradually acquires the capacity to bring diapause to an end. Diapause termination marks the time when the actual diapause period is completed and the insect can resume development if the correct conditions are present. This point marks a rapid developmental transition defining the end of diapause. Diapause termination usually occurs long before the insect actually resumes development.

Post-diapause includes all developmental events that ensue following diapause termination. **Post-diapause quiescence** refers to the period between diapause termination and the actual resumption of development, an interval that frequently encompasses many months in temperate latitudes. For example, in northern latitudes, diapause may be terminated in early winter, but development may not be reinitiated until the arrival of warm days in spring because cold conditions in late winter are not permissive for development. The distinction between diapause and post-diapause quiescence is frequently overlooked because the two phases are morphologically indistinguishable. The only obvious distinction is whether the insect is capable of reinitiating development if favorable conditions are present. However, from a developmental perspective and for projecting the timing of spring emergence, the distinction between diapause and post-diapause quiescence is extremely meaningful. Following a period of post-diapause quiescence, development resumes when favorable conditions are present, and the ensuing development follows a developmental trajectory that usually closely matches that of its nondiapausing counterparts. It is not uncommon for post-diapausing insects to exhibit fitness consequences that result from having experienced diapause.

In subsequent chapters, I use this framework to discuss more fully the environmental programming events involved in diapause induction (Chapter 5), preparation (Chapter 6), diapause (Chapter 7), termination of diapause, and resumption of development (Chapter 8), as well as consequences of diapause (Chapter 4).

2 Which Seasons Are Being Avoided?

Seasonal patterns of temperature and rainfall, largely dictated by geography, define annual periods suitable for continuous insect growth and development and conversely define times that are unsuitable or suboptimal. Winter has received the most attention as the season for diapause, but summer diapauses are also well documented, and diapause is common in the tropics as well. Receiving less attention are the dormancy patterns operating at high latitudes where seasonal patterns are most extreme. This chapter introduces distinctions noted in diapauses used for different seasons. **Aestivation** is commonly used to describe dormancy that bridges dry seasons, but the aestivation literature often does not distinguish the programmed diapause response from the nonprogrammed response of quiescence. Aestivation can be found in both summer diapauses that occur in temperate latitudes and tropical diapauses, and not all summer and tropical diapauses are driven by dry seasons. I have thus elected to incorporate the aestivation literature within the categories of summer and tropical diapause, rather than as a distinct category.

2.1 Winter Diapause

Winter is an obvious obstacle for most insects living in temperate latitudes, and indeed the overwhelming body of diapause literature focuses on diapause that is used to bridge this unfavorable season. This book also reflects that popular winter bias, and hence features of winter diapause, from the ecological to the molecular, prevail throughout the text and will be given less specific attention in this section.

Insect performance curves plotting activity, growth, development, or reproduction as a function of temperature clearly show optimal temperatures, as well as the extremes that limit activity. Some insects have performance curves shifted to maximize activity at low temperatures, but in most cases, optimal activity curves do not include the sorts of temperatures typically encountered during the winter months. As ectotherms with limited ability to physiologically control their body temperature, insects are forced to escape in time or space, and usually, this means becoming dormant to circumvent the impact of winter. Diapause by itself does not imply cold hardiness as will be discussed later (Section 7.13.6), but it brings a halt to development and sets the stage for evoking cold hardening mechanisms.

But, low temperatures are likely only one of the driving evolutionary forces leading to diapause in temperate latitudes. The onset of winter brings with it the demise of host plants exploited by insects for food. Without a food source, insects cannot continue to develop and reproduce. The lack of host plants has a cascading effect, also impacting the life cycles of insect predators and parasitoids. When the food resource is gone, the insect must adjust its seasonal cycle to accommodate the lack of food. For most insects that confront winter, diapause is the answer. The active phase of the life cycle coincides with the favorable summer season (Figure 2.1A and B).

As discussed in Chapter 5, the overwhelming majority of insects that enter a facultative winter diapause do so in response to short daylengths that prevail in late summer and early autumn, a response that is frequently enhanced by low temperatures. Yet, as reported later, diapause at other times of the year and in regions beyond temperate latitudes is common, albeit understudied.

2.2 Summer Diapause

It may seem a bit counterintuitive that an insect would enter diapause in summer, a season when temperatures are clearly favorable for development, yet the presence of summer diapause underscores the fact that diapause is not just about winter survival but has much to do with the synchrony of insects with their food sources, as Masaki (1980) and Saulich and Musolin (2018) nicely outline in their reviews of summer diapause. Escape from parasitoids can be another good reason to avoid activity during the summer, as proposed for the white butterfly *Pieris brassicae* (Spieth et al. 2011). In the early literature, summer diapause was thought to be restricted to arid or Mediterranean climates, but Masaki’s elegant review makes it clear that summer diapause is widely distributed geographically, from Finland, Alaska, and Canada to the lower latitudes and has been documented in nearly all major insect orders and mites (Ridsdill–Smith et al. 2005).

Many insects that overwinter in diapause enter diapause in mid-to-late summer (Figure 2.1B) or early autumn (Figure 2.1A), but this situation is not referred to as a summer diapause because that diapause persists through the winter. By contrast, a summer diapause is frequently terminated in the cooling days of autumn (Figure 2.1C). Summer diapause, which occurs during the hottest time of the year, can also be distinguished from tropical diapause that commonly is used at low latitudes to coordinate activity with predictable alternation between seasonal dry and wet seasons.

A common pattern of summer diapause is for the insect to be **active in spring**, enabling it to feed on buds, flowers, young shoots and leaves, or to prey on other insects if it is a predatory species and then become dormant during the summer. As temperatures drop and/or photoperiod declines in the autumn, summer diapause is commonly broken and the insect again becomes active (Figure 2.1C). The reproductive phase may occur either before or after summer diapause or both times. The insect may then enter a winter diapause, often in a different developmental stage, or remain

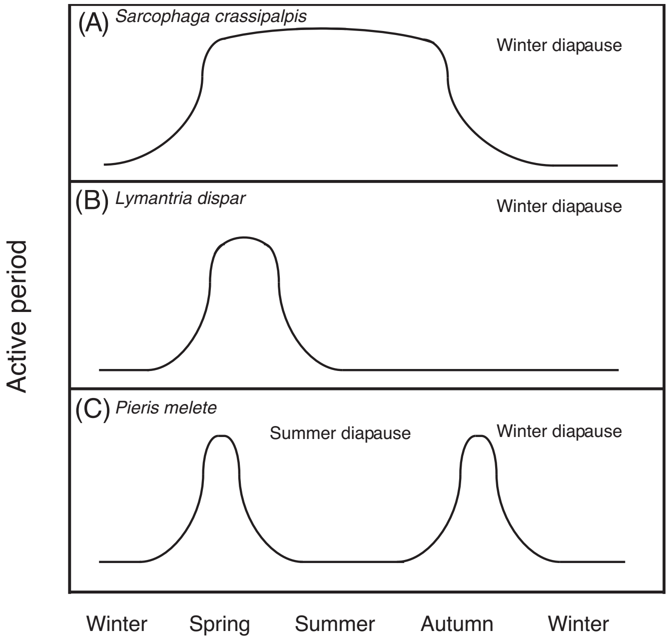

Figure 2.1 Schematic representation of the active (nondiapause) life phases of (A) an insect that overwinters in diapause and is active during most of the summer (e.g., *Sarcophaga crassipalpis*); (B) an insect that is active for a short interval in early summer but is in diapause for the remainder of the year (e.g., *Lymantria dispar*); and (C) an insect that is active in spring and autumn but enters both a summer and winter diapause (e.g., *Pieris melete*).

active during the winter. For example, the bug *Poecilocoris lewisi* enters an overwintering diapause as a fifth-instar nymph in response to short daylengths but then responds to long daylength in the spring by entering a summer adult diapause. This summer diapause and delay in adult activity is used to synchronize adult activity with the July appearance of seeds from its dogwood host *Cornus controversa* (Tanaka et al. 2002). In Greece, adults of the ladybird beetle *Hippodamia undecimnotata* feed voraciously on aphids in the spring and early summer in the lowlands, but in June, they head for the mountains where they enter a summer diapause in large aggregations (Katsoyannos et al. 2005). The beetles display classic attributes of diapause, failing to reproduce for several months, even when provided an optimal environment. Though they remain in the mountains throughout the winter, by late autumn they are capable of reproducing when collected and returned to optimal conditions in the lowlands. Thus, what starts as a summer diapause transitions into post-diapause quiescence during autumn, and the beetles remain in that stage until they return to the lowlands in the spring and again become reproductively active.

Environmental cues programming summer diapause are frequently a mirror image of cues used to program winter diapause, as illustrated for summer and winter pupal diapauses of the noctuid *Mamestra brassicae* in Figure 2.2 (Grüner and Masaki 1994). Rather than using short days and low temperatures to enter diapause, common signals for winter diapause, insects entering summer diapause frequently do so in response to long days and high temperatures. Interestingly, critical daylengths for both summer and winter diapauses in *P. lewisi* are between 14 and 15 hours of light/day, but the winter nymphal diapause requires daylengths shorter than 14–15 hours for diapause induction, while the summer adult diapause requires daylengths greater than 14–15 hours (Tanaka et al. 2002). In *M. brassicae*, winter diapause is induced by a range of daylengths shorter than 13 hours, while summer diapause is induced by daylengths that exceed 14.5 hours (Grüner and Masaki 1994). Nondiapausing pupae are generated only within the narrow range between 13 and 14.5 hours of daylength (Figure 2.2). A similar response is observed in the cabbage butterfly *Pieris melete*: Long daylengths prompt a summer pupal diapause, while short daylengths lead to a winter pupal diapause, and most pupae develop without diapause at intermediate daylengths between 11.5 and 13.5 hours of light (Xue et al. 1997, Xiao et al. 2008a). In the moth *Pseudopidorus fasciata*, an overwintering prepupal diapause is triggered by daylengths exceeding 14 hours of light, but a small portion of the population enters summer diapause during the fourth instar in response to daylengths shorter than 13.5 hours (Wu et al. 2006). In the leaf beetle *Phaedon brassicae*, long days result in an adult summer diapause, while short days elicit an adult winter diapause (Wang et al. 2007a). The winter embryonic diapause of the stink bug *Picromerus bidens* is obligate, but summer adult diapause in this species is facultative and relies on the long-day signals of early summer (Musolin and Saulich 2000). Daylength may impact not only the decision to enter summer diapause but also diapause duration, as in the Mediterranean tiger moth *Cymbalophora pudica* (Koštál and Hodek 1997). This species enters summer diapause under both long and short daylengths, but switching from one daylength to the other either shortens or lengthens diapause duration, demonstrating that the duration of diapause is responsive to change in daylength, rather than absolute daylength.

In the cabbage beetle *Colaphellus bowringi*, photoperiod seems not to be particularly important (Xue et al. 2002): Low temperatures in early spring induce summer diapause, high temperatures of summer promote nondiapause, and low temperatures of autumn induce winter diapause. The onion maggot *Delia antiqua* enters summer diapause in response to temperature (>24°C), but its winter diapause is evoked by both temperature (<18°C) and photoperiod (Ishikawa et al. 2000). Long daylength, coupled with high temperature, prompts summer diapause in the burying beetle *Nicrophorus quadripunctatus*, but high temperature by itself will also bring about a reproductive halt (Nisimura et al. 2002). Summer diapause in larvae of the hemlock woolly adelgid *Adelges tsugae* also seems to be evoked strictly by high temperature (Sussky and Elkinton 2015).

A single species may use summer diapause in some portions of its range and winter diapause in others. In Spain and Northern Africa, populations of *Pieris brassicae* respond to long daylengths by entering a summer pupal diapause, while relying on short daylength and low temperature to program diapause at higher latitudes of France and Denmark (Held and Spieth 1999, Spieth 2002). Likewise, within Japan, adults of

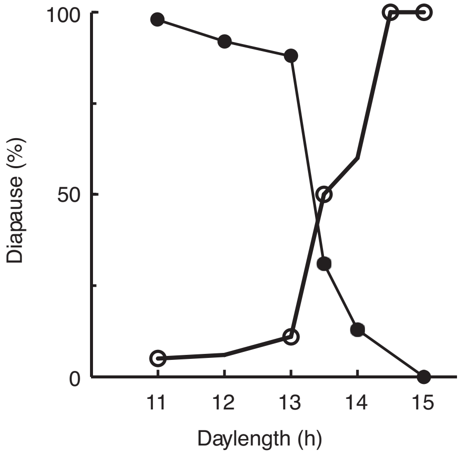

Figure 2.2 Photoperiodic response curves for induction of winter diapause (solid circles) and summer diapause (open circles) in pupae of a southern Japanese strain of *Mamestra brassicae.* Adapted from Grüner and Masaki (1994), with permission from Kluwer Academic.

the coccinellid beetle *Epilachna admirabilis* have a summer diapause in warm Kyoto (35° N) but not in much cooler Sapporo (43° N) (Imai 2004). Adults of the coccinellid *Coccinella septempunctata* fail to enter summer diapause in the north of Japan but do so in central and southern Japan, although the incidence of summer diapause varies not only geographically but annually as well, presumably a consequence of annual temperature variation; the incidence of summer diapause is high when July temperatures are high (Ohashi et al. 2003). As these few examples suggest, the incidence of summer diapause does indeed seem to be greater at lower, warmer latitudes, but numerous examples demonstrate summer diapause at high latitudes as well.

Although limitations of a **seasonal food resource** are likely the most common driving force for the evolution of summer diapause, related features of competition for a dwindling food source may also contribute. Experiments by Tougeron et al. (2018, 2019) suggest that increased competition between the braconid parasitoids *Aphidius avenae* and *A. rhopalosiphi* affects diapause incidence in the parasitoid's progeny. Reducing the abundance of host aphids does not induce the summer diapause, but increasing parasitoid abundance leads to a boost in summer diapause incidence. How such a fascinating mechanism works remains unknown. In populations of the white butterfly *Pieris brassicae* from the southern Iberian Peninsula, summer pupal diapause is possibly driven by an evolutionary attempt to escape parasitism (Spieth and Schwarzer 2001). While nearly all individuals of *P. brassicae* enter a three- to four-month diapause in the summer, the major parasitoid that attacks this butterfly, *Cotesia glomerata*, lacks the capacity for summer diapause and is forced to switch to less suitable hosts for the summer months, thus diminishing its reproductive success.

Physiological features of summer diapause have not been examined as extensively as winter diapause, but there seem to be no major differences. Body mass and energy resources such as lipid and glycogen reserves are commonly elevated in both summer and winter diapause, and metabolic activity is depressed in both cases (Ding et al. 2003, Liu et al. 2006, 2016, Weyda et al. 2015, Saulich and Musolin 2018). Net transpiration rates and critical transition temperatures, a reflection of cuticular hydrocarbon composition, are nearly identical for summer and winter diapauses in pupae of the Hessian fly *Mayetiola destructor* but differ considerably from values noted in nondiapausing fly pupae (Benoit et al. 2010b). Larvae of the stem borer *Chilo partellus* have a winter diapause in northern India but a summer diapause in southern India; one conspicuous difference in these larvae is that those in summer diapause are heavier than those in winter diapause (Dhillon et al. 2020). Another distinction apparent in some species such as the cabbage armyworm *M. brassicae*, is that pupae in winter diapause have a greater capacity for cold tolerance than their counterparts in summer diapause, a feature linked to higher trehalose levels evoked by winter diapause (Goto et al. 2001, Ding et al. 2003). Both summer and winter diapauses appear to have the same endocrine basis, as outlined in Chapter 9.

Molecular signatures of diapause in the onion maggot *Delia antiqua*, such as an elevated expression of genes encoding heat shock proteins, are evident in both summer and winter diapauses (Chen et al. 2005b, 2006, Ren et al. 2018). Genes involved in lipid metabolism, immune responses, and cytoskeletal modifications are also differentially expressed in both types of diapause in this species, but certain transcripts are more highly expressed in one or the other: Genes associated with lipid metabolism are more highly upregulated in winter diapause, while genes associated with immune responses are more highly upregulated in the summer diapause of *D. antiqua* (Hao et al. 2012). Though the transcript encoding a trypsin-like enzyme is upregulated in both summer and winter diapauses in *D. antiqua*, the level of upregulation is greater in winter diapause (Chen et al. 2005a). To date, *D. antiqua* is the only species in which both summer and winter pupal diapauses have been examined at the molecular level, and thus it is too early to be certain if differences noted in this species are common to other species as well. The fact that different temperatures are required to trigger summer and winter diapauses in *D. antiqua* makes it challenging to determine whether the molecular responses are due to temperature or the diapause program (Ren et al. 2018). Summer diapauses in adults of the chrysomelid beetle *Galeruca daurica* share many of the proteomic (Ma et al. 2019), microRNA (Duan et al. 2021), and endocrine (Ma et al. 2021) signatures seen in winter diapauses, suggesting that at the molecular level there is little to distinguish a summer diapause from a winter diapause.

2.3 Tropical Diapause

The common assumption that insects living in the tropics do not diapause is not well founded. A compilation of tropical insects with a diapause or at least some form of dormant state includes 73 species, spanning all major insect orders and representing diapause in all life stages (Denlinger 1986). A number of examples, primarily Hymenoptera from Hawaii, are also cited in a review (Nishida 1955). Since then, diapause in additional tropical species has been noted, as recorded for examples in Table 2.1.

Additional examples are abundant from subtropical areas, such as larvae of the braconid *Microplitis demolitor* (Seymour and Jones 2000) and adults of *Drosophila ananassae* (Lambhod et al. 2017). Circumstantial evidence for diapause can also be gleaned from natural history observations. For example, huge numbers of insects, representing 33 families in diverse orders, are reported during the dry season in ball moss, an epiphyte in the dry forest of Central Mexico (Luna–Cozar et al. 2020). The fact that most of the insects in the moss are inactive and in non-feeding stages suggests they are in diapause and are using the site as a dry season refuge. There is every reason to believe that diapause is widespread within the tropical and subtropical world, although some species clearly lack this capacity, as noted in a brief, but not exhaustive, listing of 16 tropical species that appear to lack a diapause response (Denlinger 1986).

Temperature variation, the conspicuous driver of seasonality in temperate latitudes, is evident in many tropical environments but is considerably more muted than at higher latitudes. Alternating **rainy and dry seasons** dictate the most obvious seasonal environmental pattern. Rainy seasons alternate with one or two dry seasons each year, depending on locality. Seasonal patterns of rainfall and other environmental variables range widely within the tropics, generating a variety of seasonal patterns of insect abundance in different tropical regions (Wolda 1988). Seasonal patterns of insects collected in traps have been well documented in the tropics, including Brazil (Davis 1945), Panama (Wolda 1978), and Kenya (Denlinger 1980). Pronounced **seasonality** is evident for most taxa examined. For example, a five-year study in the Nairobi National Park, Kenya (Denlinger 1980) reveals a huge single peak abundance of lacewings (Chrysopidae) each August, a peak of psocids (Psocidae) in November and December, a peak of horse flies (Tabanidae) in May, and bimodal patterns of abundance for robber flies (Asilidae) in November and April and wood nymph butterflies (Satyridae) in December and May. Nairobi annually experiences two rainy and two dry seasons, but the insect distribution pattern noted in Malaise trap catches indicates there is no single period universally favored by all taxa. Some are abundant in the rainy season, others in the dry season. A striking peak in abundance of adults of the seed bug *Jadera aeola* appears late in the dry season on Barro Colorado Island, Panama, but the bugs are nearly absent for the remainder of the year (Tanaka et al. 1987c). Even in regions of nearly constant temperature and rainfall, such as Fortuna, Panama, two species of dobsonflies (*Chloronia* sp. and *Platyneuromus* sp.) are highly seasonal, emerging during a two-month window each year (Wolda 1988).

Seasonal differences of insect abundance do not, of course, immediately imply diapause during the nadir of seasonal abundance. Tropical species such as the tsetse flies *Glossina morsitans* and *G. swynnertoni* display highly seasonal abundance patterns in trap catches in Tanzania, with peak abundance in July (Nnko 2017), yet,

Table 2.1 Recent examples of diapause in tropical insect species.

Species	Stage	Latitude	Country	Reference
Orthoptera				
Stictophaula armata	Embryo	19° N	Thailand	Ingrisch (1996)
Oedaleus senegalensis	Embryo	17° N	Mali	Gehrken and Doumbia (1996)
Hemiptera				
Deois flavopicta	Embryo	15° S	Brazil	Pires et al. (2000)
Encosternum delegorguei	Adult	22° S	S. Africa	Dzerefos et al. (2015)
Coleoptera				
Pachymerus nucleorum	Embryo	10° S	Brazil	Benton (2015)
Bruchidius atrolineatus	Adult	9° N	Togo	Glitho et al. (1996)
Callosobruchus rhodesianus	Adult	9° N	Togo	Amevoin et al. (2005)
C. subinnotatus	Adult	9° N	Ghana	Appleby and Credland (2007)
Lepidoptera				
Omphisa fuscidentalis	Embryo	19° N	Thailand	Singtripop et al. (1999)
Chlosyne lacinia	Embryo	16° S	Brazil	Moreira et al. (2012)
Coniesta ignefusalis	Embryo	11° N	Ghana	Tanzubil et al. (2002)
Papilio demoleus	Pupa	15° N	India	Singh (1993)
P. polytes	Pupa	15° N	India	Singh (1993)
Hypolimnas bolina	Adult	17° S	Australia	Kemp and Jones (2001)
Diptera				
Procontarinia mangiferae	Embryo	21° S	Réunion Isl.	Amouroux et al. (2014)
Anopheles coluzzii	Adult	14° N	Mali	Dao et al. (2014)
Hymenoptera				
Eurytoma sp.	Embryo	20° S	Brazil	Barbosa and Fernandes (2019)

despite my own extensive experimental attempts to find diapause in tsetse, there is no evidence for any such arrest. High seasonal mortality most likely explains the decline in tsetse populations during dry seasons. Local and long-distance **migration from drier to wetter sites** are presumed to account for many seasonal patterns of tropical insect abundance (Janzen and Schoener 1968), including the dramatic seasonal cycles noted in the malaria mosquitoes *Anopheles arabiensis* and *An. gambiae sensu stricto* in the Sahel (Dao et al. 2014). Remarkably, the dry season in the Sahelian region of Senegal generates extreme fluctuations in populations of *An. arabiensis*, yet there is no evidence for a dry season bottleneck, indicating that the mosquito maintains a large permanent deme spread across a broad geographic area (Simard et al. 2000). Likewise, the eastern highlands and coastal regions of Kenya appear to provide habitat for continuous breeding of the African armyworm *Spodoptera exempta*, thus offering a dry season refuge from the more seasonal grasslands (Dewhurst et al. 2001). An abundance of the Panamanian frog-biting midge (*Corethrella* spp.) is high during the rainy season but drops during the dry season, but again this seasonal difference does not appear to be linked to diapause but to the abundance of host frogs (Legett et al. 2018). During the rainy season these blood-feeding midges cue in on the calls of an abundant host, the túngara frog, but during the dry season, túngara frogs cease chorusing and midges then switch their response to identifying a less abundant host, the pug-nosed tree frog, a species that calls only during the dry season. However, in addition to differential seasonal mortality, migration, and host shifts, diapause is likely responsible for the seasonal patterns noted in many tropical species.

The striking differences between tropical diapauses and diapauses occurring at higher latitudes are the **environmental regulators** that preside over the diapause decision (see Section 5.14). In brief, the role of photoperiod, so dominant at high latitudes, is frequently supplanted near the equator by environmental regulators that rely on wet/dry season cues, including not only rainfall patterns but the downstream effects of rainfall on plant growth, flowering, seed set, and the nutritional status of food plants, as well as subtle seasonal changes in temperature (Denlinger 1986).

Another attribute of tropical seasons is that they are less predictable than winter in temperate latitudes. Timing of the onset and end of the rainy seasons can vary considerably, and in some years, the rains may not come at all. The inherent lack of seasonal predictability is possibly why only a portion of a population is likely to enter diapause. For example, approximately 18% of the population of the nymphalid butterfly *Hypolimnas bolina* fails to enter adult diapause even under optimal diapause conditions in tropical Australia (Pieloor and Seymour 2001). Similarly, among the sarcophagids from East Africa, low levels of diapause are consistently found in pupae, even when reared under the strongest diapause-inducing conditions (diapause incidences range from 7% to 79% among seven species under the extreme conditions of 12°C) (Denlinger 1979). In Nigeria, a portion of the maize stem borer *Busseola fusca* population enters larval diapause each month regardless of the condition of the maize host plant (Usua 1970).

As with summer diapause, diapause in the tropics requires an impressive mechanism for **suppressing metabolism** at high temperatures, a challenge not faced by insects overwintering at high latitudes. The stage of diapause (pupa) is the same for flesh flies (Sarcophagidae) from equatorial Africa as it is for their relatives living in temperate regions (Denlinger 1974, 1978). Similar levels of metabolic suppression, patterns of oxygen consumption cycles, the influence of water content in their food (higher water content yields higher diapause incidence), hormonal regulation by ecdysteroids and juvenile hormone, and the ability of the same select agents (e.g., hexane, acetone) to artificially break diapause all underscore the fact that the basic physiological properties of diapause in flesh flies from tropical Africa and temperate latitudes are remarkably similar (Denlinger 1979). The big differences in winter and tropical diapauses in flesh flies are the environmental features that characterize diapause: seasons of diapause (winter vs cool African dry season), duration of the dormancy (nine versus one to two months), and the triggers for diapause onset (photoperiod versus low daytime temperatures). Among other species that have been examined at the physiological level, few distinctions between tropical and temperate latitude diapauses are evident, such as tropical adult diapauses in both the beetle *Stenotarsus rotundus* and the seed bug *J. aeola* appear to be the consequence of a shut-down in juvenile hormone production, and both species degenerate their flight muscles and suppress gonadal development (Tanaka et al. 1987c), as noted in adult insects that diapause at temperate latitudes. Diapausing larvae of *C. partellus* and *C. orichalcociliella*, stem borers from the Kenyan coast, show similar levels of metabolic depression and reduction in the rate of the heartbeat as seen in temperate species during diapause (Scheltes 1978). Metabolic depression of approximately 28% is noted in diapausing adults of the Australian tropical butterflies *Euploea core* and *E. sylvester* (Canzano et al. 2006); this is a modest decline but not too different from that of temperate species that diapause as mobile adults. Like many temperate latitude species, diapausing adults of the tropical butterfly *H. bolina* are larger and darker than their nondiapausing counterparts (Kemp and Jones 2001). Enhanced desiccation resistance is associated with embryonic diapause in the grasshopper *Oedaleus senegalensis* from Mali (Gehrken and Doumbia 1996). Citrate synthase activity and other enzymes involved in oxidative metabolism are depressed in diapausing larvae of the tropical butterfly *Chlosyne lacinia* (Moreira et al. 2012), again suggesting that physiological features of tropical diapause, including metabolic suppression, are indistinguishable from features characterizing diapause in temperate species.

At the molecular level, many of the same patterns of gene expression observed for diapausing insects from temperate latitudes are also noted for diapause in tropical species, such as the tropical solitary bee *Tetrapedia diversipes* (Santos et al. 2018), further underscoring the fact that molecular and physiological attributes of diapause are shared in spite of differences in environmental regulation. A shared molecular underpinning for diapause in tropical and temperate species is also indicated by a large number of diapause-associated single nucleotide polymorphisms that are common to populations of *Drosophila melanogaster* from Zambia and populations from temperate latitudes (Erickson et al. 2020).

The more intriguing question is why insects would diapause in the tropics. Drought resistance is likely one of the major driving forces, especially in regions with pronounced dry seasons, but of parallel importance for many insects is the ability to coordinate development with highly seasonal patterns of plant development, growth, fruiting, and seed production. Like many tropical species, the butterfly *H. bolina* breaks diapause in tropical Australia at the onset of the rainy season to exploit new growth of its larval food plant, in this case, *Synedrella nodiflora* (Pieloor and Seymour 2001). Similarly, the onset of the rainy season in Panama terminates diapause and prompts a mating frenzy in the endomychid beetle *S. rotundus* (Wolda and Denlinger 1984, Denlinger 1994), enabling it to disperse from its diapause aggregation and lay eggs, presumably on fungi that appear only at that time of year. Seed production in the Sapindaceae is a dry season event in Panama, and accordingly, the seed bug *J. aeola* breaks its diapause late in the dry season in concordance with the mass production of seeds from its host plant (Tanaka et al. 1987c). The absence of suitable aquatic larval habitats during the dry season in the Sahel likely drives the cycle of adult dormancy in the mosquito *Anopheles coluzzii* (Yaro et al. 2012, Dao et al. 2014). Less obvious is why a portion of flesh fly (Sarcophagidae) populations living near the equator in Africa should enter pupal diapause during July and August, the coolest time of the year. Although temperatures at this high-altitude site (1,700 m) near Nairobi, Kenya are low by African standards (mean temperatures of 16–22°C during those two months), such temperatures are certainly not lethal and would not be assumed to significantly impair development. These flies are carrion and dung feeders, and such resources appear to be equally abundant throughout the year, yet some of the flies enter a short diapause each year at this time (Denlinger 1974, 1978). **Biotic factors** may be more important than abiotic factors in driving seasonality in certain species. Entering diapause to escape predators or parasites, avoiding entomophagous fungi or other pathogens during rainy seasons, avoiding competition, and other biotic features could be just as important driving forces for tropical insects as seasonal features of the abiotic world. Although one can imagine tropical diapause as a relict from the insect's evolutionary past, this seems unlikely. Diapause responses are lost quickly in laboratory cultures, and some species, including flesh flies, are thought to have evolved in the Old World tropics (Denlinger 1979).

Clearly, no single tropical season is bad for all species. Many tropical plants flower and bear fruit during the dry season, and thus provide seasonal resources for insect pollinators, frugivores, and seed predators. Not all features of the rainy season are positive: Rains can wash out stores of nectar and pollen, and destroy nesting and oviposition sites of soil-dwelling insects (Denlinger 1986). Despite the vagaries of tropical seasons and the generally mild conditions that prevail, there are still needs that can be met by diapause. Diapause remains a potent mechanism for tropical insects to synchronize mating, to optimize the timing of their development with a specialized and sometimes highly seasonal food source, and to mount protective measures for avoiding drought or other challenges of the unfavorable season. Despite the adaptive advantages of diapause, mortality can be high during diapause, such as in northern Ghana, mortality toward the end of the dry season exceeds 50% in diapausing larvae of the millet stem borer *Coniesta ignefusalis* (Tanzubil et al. 2002).

The fact that diapause is already present in many tropical species suggests that such species are primed to invade temperate latitudes. What is required is not the evolution of diapause capacity but simply the modification of the signaling system that responds to environmental cues. The sarcophagid flies, with a pupal diapause already intact in tropical Africa, appear to have moved from Africa to temperate regions of Europe, Asia, and North America, a migration that required a shift from temperature as the sole diapause determinant to the reliance of both photoperiod and temperature (Denlinger 1974, 1979). Similarly, as tropical and subtropical populations of the cotton bollworm *Helicoverpa armigera* invaded temperate regions of Japan, they intensified their reliance on both photoperiod and temperature as pupal diapause regulators (Shimizu and Fujisaki 2006), a response also reflected in populations of *D. melanogaster* that have migrated from Africa to Europe (Zonato et al. 2017).

2.4 What Happens at High Latitudes?

The challenges confronting insects during winter in temperate climates are exacerbated at high latitudes (Danks 2004a). Temperatures during winter can be incredibly low for an ectotherm, and even summer temperatures are cool by temperate zone standards. Impressive cold tolerance mechanisms are in place, and thermal thresholds for activity are adjusted downward. Humidity is typically low, with water resources frequently locked in a frozen state, making high latitude sites the equivalent of **polar deserts**. The seasonal cycle of daylength swings dramatically, yielding unusually short days during winter and long periods of continuous or nearly continuous daylight during the summer. The net effect is a brief, and sometimes highly variable, growing season for plant life, accompanied by a short season that can be exploited by insects for their development and reproduction. Despite these harsh conditions, an impressive number of insects thrive in the **Arctic**. As many as 2,200 species of insects and their relatives have been reported above the tree line in North America (Danks 2004a). Survival in such environments requires not only physiological and morphological adaptations to combat the cold, but behavioral responses to capitalize on the limited energy from sunlight, and temporal developmental patterns that allow the insect to maximize the short growing season. Insects living in high alpine regions at lower latitudes are confronted with many of these same constraints (Convey 2010).

These extreme challenges suggest that some form of dormancy is essential. Frequently, the favorable season is simply not sufficiently long to permit completion of the full life cycle, thus several years, with intermittent periods of dormancy, may be required for completion of a single generation. The brevity of the favorable season demands that the insect precisely synchronize its development to fully exploit the summer. But, is it diapause that is used to bridge these unfavorable times, or is it a form of quiescence that simply turns development on and off in direct response to the prevailing conditions? Very few species have actually been subjected to the type of rigorous investigation that enables us to distinguish between these two forms of dormancy. The synchronous response to favorable summer conditions could reflect either diapause or quiescence. Diapause is likely in at least some Arctic species, especially those such as the geometrid moth *Psychophora sabini* that molts each spring to the next instar and then halts development and overwinters at the end of the instar (Danks 2004a). Mitochondria in the lymantrid moth *Gynaephora groenlandica* degrade in winter (Levin et al. 2003), and overwintering larvae show metabolic depression characteristic of diapause (Bennett et al. 1999), but this feature alone does not distinguish diapause from quiescence. Diapauses occurring in larval, pupal, and adult stages have been nicely documented for species of drosophilids that live beyond 65° N (Lumme 1978). The malt fly *Drosophila montana* has been especially well studied (e.g., Tyukmaeva et al. 2011, Salminen et al. 2015). This species, found as far north as 67° N in Finland, exhibits a classic photoperiodically induced adult diapause, albeit a diapause that relies on an extremely long critical daylength (daily light:dark cycle of 19 hours:5 hours) for induction. Populations of the blow fly *Calliphora vicina* that live above the Arctic Circle (Lofoten Islands, northern Norway), retain responsiveness to photoperiod for induction of larval diapause, but some will enter diapause at low temperatures (4–10°C) regardless of photoperiod (Vinogradova and Reznik 2015). A population of the yellow dung fly *Scathophaga stercoraria* from Tromsø, Norway (69.7° N) enters pupal diapause at 12°C and will do so under either a short or long daylength, whereas more southerly populations enter diapause at that temperature only under short daylengths (Scharf et al. 2010).

Antarctica and the sub-Antarctic islands lack the species richness seen in the Arctic. In fact, only one insect species is endemic to the Antarctic continent, a flightless midge *Belgica antarctica* (Lee and Denlinger 2015). A few additional species have more recently invaded the northern tip of the Antarctic Peninsula, and a number of mites, ticks, and Collembola are common in this region and on the sub-Antarctic islands (Convey 2010). Little is known about the actual overwintering mechanisms used by these arthropods, but our experiments with *B. antarctica* are suggestive of diapause. Measurements of metabolic rate show a characteristic drop in metabolism in mid-February (near the end of the Antarctic summer), regardless of temperature, photoperiod, humidity, or other environmental signals to which they are exposed (Spacht et al. 2020). These results suggest an obligate diapause of some sort or, alternatively, that the environmental regulator has yet to be discovered. Laboratory conditions to which the larvae were exposed approximate summer conditions (4°C and long daylength), thus they should be able to continue developing, but they fail to do so. Larvae are capable of initiating metamorphosis and emerging as adults only after a few months at low temperatures have elapsed. A metabolic depression of this sort, occurring in the absence of any obvious environmental cue, is indicative of diapause, as is the synchrony of adult emergence the following summer. During the brief austral summer, the midge is continuously active, seemingly oblivious to the daily environmental cues that normally dictate activity patterns. Although the canonical clock genes are present, the cyclic patterns of expression seen in temperate species are not observed (Kobelkova et al. 2015). This lack of rhythmicity in the midge is similar to summer observations of reindeer (Lu et al. 2010) and Svalbard ptarmigan (Reierth and Stokkan 1998, but see Appenroth et al. 2020) living in the high Arctic.

From the limited data available, it is apparent that diapause does indeed occur in some species living at high latitudes, but dormancies that are more akin to quiescence are also likely to be prevalent. The quick response time for entering and exiting quiescence would seem to be an attractive option for an insect living in an extreme and highly variable environment. The flexibility of being able to rapidly become active when conditions are favorable and then just as quickly become quiescent at any life stage would appear to be a highly adaptive response to exploit the short intervals that favor growth and development at high latitudes.

3 Variation in the Diapause Response

Seldom is the diapause response absolute. This chapter explores variation in the response within and among populations, as well as some of the oddities that have evolved such as repeated diapause and prolonged diapause. Though most insects diapause only once during their life, some species do so repeatedly, and some have the capacity to extend their diapause for multiple years. This type of rich variation inherent in populations provides the grist for selection and thus is highly relevant for evolutionary change and adaptations, allowing a successful invasion of new niches.

Though there is usually, but not always, consistency in the stage used for diapause within a species, there can be a huge variation in the incidence of diapause within and between populations, as well as variation in the timing of diapause onset and termination. Individuals entering prolonged diapause frequently represent only a small portion of the overall population, and some species, such as flesh flies in the Old World tropics, consistently display a low incidence of diapause. Species with an obligate diapause are more uniform in their response, but even here it is not unusual for a few individuals to deviate from the norm by avoiding diapause. Diapause responses that appear fairly uniform under defined laboratory conditions can show considerable variation in the field (Danks 2007, Yocum et al. 2011).

3.1 Variation within and among Populations

Variation is especially pronounced in species with a facultative diapause. The incidence of diapause is rarely 100% under any given set of conditions. For example, the flesh fly *Sarcophaga bullata* reared under natural conditions in central Illinois yields a low incidence of diapause (<2%) in June and July, 20% diapause in August, and 98% diapause in September (Denlinger 1972a). What is striking about this result, and this is similar to what is observed with other species as well, is that a few individuals enter diapause during the peak summer season and, at the other extreme, a few pupae fail to enter diapause in late autumn. Likewise, under carefully controlled laboratory conditions *S. bullata* varies in its response to both photoperiod and temperature (Denlinger 1972b). Although a critical photoperiod of 13.5 hours of daylength can be calculated for the population, a few individuals enter diapause at daily light: dark cycle of 14 hours: 10 hours, while shortening the daylength to LD 13:9 is required to generate the maximum incidence of diapause. At 25°C, approximately 70% of the individuals enter diapause at short daylengths, but the diapause incidence jumps to nearly 100% when flies are reared at 17°C. A similar pattern of variability is noted for a population of blow flies *Calliphora vicina* monitored in the field in St. Petersburg, Russia (Vinogradova and Reznik 2013): A few larvae (20%) enter diapause in early August, but it is late September before nearly all larvae enter diapause. This is reflected in critical daylengths that vary from 16.5 L:D to 12:12 LD for different cohorts from the same location. In a population of the mosquito *Culex tarsalis* monitored in the Sacramento Valley of California, the incidence of adult diapause jumps from 41% in October to 90% in November (Nelms et al. 2013), again demonstrating differences in individual thresholds for diapause induction. The incidence of dormancy in adults of *Drosophila melanogaster* is remarkably variable throughout the season in North America (Erickson et al. 2020). Though the incidence of arrested ovarian development is highest in December and lowest in July, surprisingly high incidences of diapause-like ovaries are noted as well in flies collected during the intervening summer and autumn months.

Environmental conditions differ from year to year, impacting the proportion of individuals in the population entering diapause. This is evident from field observations with the hemlock woolly adelgid *Adelges tsugae*. This species most commonly completes two generations annually in western North America, but an unusually hot period coinciding with the settlement of the crawlers in April 2014 prompted entry into diapause, resulting in the completion of only a single generation that year (Weed et al. 2016). These sorts of differences in the diapause threshold or prevailing environmental conditions are also reflected in the seasonal appearance of molecular markers for diapause in field populations of the Colorado potato beetle *Leptinotarsa decemlineata* observed in the Red River Valley of North Dakota and Minnesota (Yocum et al. 2011). Such observations underscore the inherent variability of the diapause response within a single population. Even more variation is noted when populations from different geographic regions are compared, as discussed in Chapter 4.

Distinctions can also be noted in closely related populations that feed on **different host plants**. Rice and water-oat populations of the striped stem borer *Chilo suppressalis* from the same region of Wuhan, China differ in critical daylength and duration of the sensitive period that programs larval diapause (Zhou et al. 2018). Similarly, black-headed and red-headed forms of the moth *Hyphantria cunea*, sibling species from the same location in Missouri, feed on different host plants and have critical daylengths that differ by 30 minutes (Takeda 2005). Such differences are not necessarily innate but may be induced by feeding on different host plants. For example, when the ragweed beetle *Ophraella communa* feeds on *Ambrosia artemisiifolia*, it has a higher diapause incidence than when it feeds on a closely related species *A. trifida* (Tanaka and Murata 2016).

Variation is evident even **within a single clutch** of eggs. For example, most females of the Australian plague locust *Chortoicetes terminifera* lay eggs that are of one type, either diapause or nondiapause, but as many as 20–30% of egg pods contain a mix (Deveson and Woodman 2014), a situation likely to pose a challenge for nondiapausing hatchlings as they attempt to escape from an egg pod containing diapausing siblings that block their passage. Similar variation within a single clutch is evident in embryonic diapause of the cricket *Gryllus firmus*, a species in which the mix of the two phenotypes varies seasonally from less than 5% diapause in March–June to 50% in November–December (Walker 1980), in embryonic diapause of the striped ground cricket *Allonemobius socius* (Huestis and Marshall 2006), in larval diapauses of the pitcher plant mosquito *Wyeomyia smithii* (Bradshaw 1976) and the Swede midge *Contarinia nasturtii* (Des Marteaux and Hallett 2019), in pupal diapause of flesh flies in the genus *Sarcophaga* (Denlinger 1972b), as well as in many other species.

Among species with an obligate diapause, the incidence of diapause is commonly 100%, but even in such species, a few individuals may develop without diapause. For example, the vast majority of embryos of the gypsy moth *Lymantria dispar*, enter an obligate embryonic diapause, but a few eggs may hatch immediately without entering diapause (Hoy 1977), an aberrancy that has been exploited to establish a nondiapausing, laboratory strain of gypsy moths. Similarly, most embryos of the hemlock looper *Lambdina fiscellaria* enter an obligate overwintering diapause in eastern Canada, but a few individuals (8 out of 16,000) hatch in early autumn, suggesting they did not enter diapause or were arrested only briefly (Delisle et al. 2009). Most tephritid flies enter an obligate pupal diapause, but a few (<2%) develop without interruption, as noted in populations of *Rhagoletis cingulata* and *R. turpiniae* from high elevations in Central and Northeastern Mexico (Rull et al. 2017). A population of the eastern larch beetle *Dendroctonus simplex* was thought to overwinter in an obligate adult diapause, but closer examination reveals that at least a few females can reproduce without first entering diapause (McKee and Aukema 2015).

Distinct populations can be expected to differ in diapause attributes, as evidenced in the butterfly *Pieris napi*, a species that, in the warm climate of northern Spain, has a facultative pupal diapause that responds to photoperiod for induction, allowing it to complete three to four generations each year, while a population from northern Sweden, above the Arctic Circle, shows little response to photoperiod, suggesting an obligate diapause that allows completion of only a single generation annually, although a few individuals (<10%) from the northern population fail to diapause across a range of photoperiods (Pruisscher et al. 2017). Similarly, a southern subspecies of the eastern tiger swallowtail *Papilio glaucus* has a facultative pupal diapause, while diapause in the northern subspecies is obligate (Rockey et al. 1987). Or, diapause may be absent in some populations and present in others. For example, embryonic diapause in the mosquito *Aedes aegypti* is not seen in most geographic locations but is documented from Buenos Aires, Argentina (Fischer et al. 2019).

One aspect of variation that seems to differ between tropical and temperate latitude species is that, when strong diapause-inducing signals are presented, nearly all temperate latitude species display a high incidence of diapause, often near 100%, a feature that does not appear to be true for many tropical species. The capacity for diapause exists in **tropical species**, as noted in Chapter 2, but levels of diapause within a population frequently remain low. For example, among eight species of flesh flies (Sarcophagidae) originating from within 9° N or S of the equator in Africa, maximum diapause incidences observed under a range of laboratory environmental conditions ranges from 79% for *Poecilometopa spilogaster* to 7% for *Sarcophaga par* (Denlinger 1979), and under natural field conditions in Nairobi, Kenya (1° S), the highest pupal diapause incidence for *P. spilogaster* was a meager 4.7% (Denlinger 1978). Similar low diapause levels are noted for larvae of the tropical stem borers *Chilo zonellus* and *C. argyrolepia*: Maximum diapause incidences in three experimental fields on the Kenyan coast range between 20 and 60% (Scheltes 1976), although higher incidences of diapause likely occur in non-irrigated fields (Scheltes 1978). In another stem borer *Busseola fusca*, a few individuals enter larval diapause each generation in Nigeria, with incidences of diapause in field-collected larvae ranging from 3.4% in April to a high of 86.1% in December (Usua 1970). A low diapause incidence prevails among embryos of the Asian tiger mosquito *Aedes albopictus* in southern Florida (Lounibos et al. 2011, Urbanski et al. 2012), and individual females subjected to an unambiguous short-day photoperiod under laboratory conditions produce eggs with a highly variable diapause incidence, ranging from 0% to over 90% (Peter Armbruster, in preparation). On Reunion Island (20° 58′ S), a portion of the population of the mango blossom gall midge *Procontarinia mangiferae* can be found in larval diapause throughout the year, with incidences ranging from 3% (September) to 31% (February). Consequently, a large portion of the population is continuously active, a feature facilitated by the presence year-round of leaves and inflorescences of the mango tree (Amouroux et al. 2014). These observations suggest that levels of diapause in species living in moderate climates, where selective forces promoting diapause may not be as intense, still vary but oscillate at lower levels. But, there are exceptions, such as nearly all individuals of the tropical beetle *Stenotarsus rotundus* likely enter adult diapause (Denlinger 1994), presumably because the beetle feeds on a fungus that is restricted to a narrow seasonal window.

Natural variation is evident not only for the timing and incidence of diapause onset but also for **diapause duration** (Masaki 2002). Although the timing of spring emergence is notable for its amazing synchrony, as discussed in Chapter 8, there is again some variation in this response. A notable feature of diapause in the subtropical cockroach *Symploce japonica* is the high variability in the timing of diapause termination: While the majority of nymphs break diapause within a brief developmental window, a small portion remains in diapause much longer than their counterparts (Tanaka and Zhu 2003). Adult emergence of the silk moth *Hyalophora cecropia* from overwintering pupal diapause in central Illinois occurs over a broad and disjunct seasonal window, resulting in a bimodal emergence pattern (May 19–25 median emergence date over a nine-year period for the first emergence and June 17–26 for the second emergence) (Waldbauer 1978). Natural variation in the timing of diapause termination has led to distinct early and late-emerging genotypes of the cabbage root fly *Delia radicum* (Lepage et al. 2014) and the turnip root fly *D. floralis* (Biron et al. 2003), as well as the remarkable segregation of host races in fruit flies in the genus *Rhagoletis* (Lyons-Sobaski and Berlocher 2009, Egan et al. 2015, Meyers et al. 2016), as discussed further in Chapter 8**.** Variation is also evident in the timing of the **transition between diapause and post-diapause quiescence** (when the insect becomes competent to terminate diapause if exposed to suitable conditions).

A population of the wheat blossom midge *Sitodiplosis mosellana* from northern China requires a cold treatment of 60–90 days before making the transition, while a Canadian population requires greater than 112 days of chilling (Cheng et al. 2017). Under field conditions, a single population may show considerable variation in the timing of this transition, as demonstrated in overwintering larvae of the peach fruit moth, *Carposina sasakii* (Zhang et al. 2016a). Differences in diapause duration are evident even between individual orchards and among almond cultivars for the almond seed wasp, *Eurytoma amygdali* in northern Greece (Margaritopoulos and Tzanakakis 2006).

Though the **stage of diapause** is usually rather rigidly fixed within a species, that is not always so. The third instar is the predominant stage for larval diapause in the burnet moth *Zygaena trifolii*, but in natural populations diapause can also occur in later instars (Wipking 1988). Within the range of daylengths that are diapause-inductive for *Z. trifolii*, longer daylengths within this range shift the stage of diapause to later instars, and in a laboratory setting, it is possible to quickly select for a specific diapause stage (Wipking and Kurtz 2000). Larvae of the European butterfly *Lycaena hippothoe* diapause in either the third or fourth instar (Fischer and Fiedler 2002). Populations of the bruchid beetle *Bruchidius dorsalis* living in the cold, far northern region of Japan diapause mainly as fourth-instar larvae, populations in central Japan diapause both as fourth-instar larvae and adults, while those in even warmer regions overwinter not only as diapausing larvae or adults but also as nondiapausing larvae (Kurota and Shimada 2007).

Variation is also evident in **molecular profiles** observed during diapause (Yocum et al. 2018). Gene expression patterns observed during the course of diapause do not yield a simple ordered sequence. Instead, the environmental history of individual insects has a profound impact on the patterns observed, as noted in diapausing larvae of the alfalfa leafcutting bee *Megachile rotundata* reared under laboratory and various field conditions.

These few examples suggest there is sufficient variation within a natural population to allow selection to modify diverse features of the diapause response. This variation underscores the potential of diapause as a **bet-hedging** strategy if environmental conditions change. If not all individuals respond in exactly the same way, the ability of a species to respond through natural selection to shifting climates is maximized, and variation allows rare phenotypes to successfully invade new geographic areas or switch seasonal developmental patterns to accommodate changes in resource availability. The rich variations of the diapause response also offer powerful tools for probing the genetic basis of diapause, as discussed in Chapter 10.

3.2 Repeated Diapauses

Another variation in the diapause response is the ability to enter diapause more than once. The common pattern is for diapause to be experienced only once during an insect's life cycle, but there are clear exceptions. At higher latitudes where the