
Pitcher-plant communities as model systems for addressing fundamental questions in ecology and evolution

Thomas E. Miller, William E. Bradshaw, and Christina M. Holzapfel

24.1 Introduction

Many carnivorous plants have close associations with other species. The best studied of such plants is *Sarracenia purpurea*. The genus *Sarracenia* includes 11 species (Chapter 9), most of which have upright tubular leaves and occur across parts of the coastal plain of southeastern North America. Many of these species serve as a host for other obligate or facultative visitors (Juniper et al. 1989), referred to generally as “inquiline” species. *Sarracenia purpurea* (referring to the *S. purpurea* species complex, which includes *S. purpurea* ssp. *purpurea*, *S. purpurea* ssp. *venosa*, *S. purpurea* ssp. *venosa* var. *montana*, and *S. rosea*; Chapter 9) is unique in several regards. Its distribution is much greater than the other pitcher plants; it occurs in bogs and related habitats from Alabama and Florida to Canada, where it can be found growing from eastern British Columbia to the Maritime Provinces (Chapter 9, Figure 9.2). *Sarracenia purpurea* also has a more prostrate growth form and the pitcher mouth is open to precipitation, unlike any of the other species (*S. psittacina* is also prostrate, but has a well-covered leaf opening). Because of this morphology, *S. purpurea* is the only species in the genus that consistently holds liquid in its leaves and this liquid almost entirely is derived from precipitation.

Sarracenia purpurea also is unique in the genus in having a relatively large number of mostly invertebrate and microbial inquiline species that live in its water-filled leaves (Miller and Kneitel 2005). Juniper

et al. (1989) describes many of these species and their associations with *S. purpurea*; since 1989, over 200 papers have been published on *S. purpurea* or its inhabitants, including studies of interactions among different inquilines; local and biogeographic patterns in inquiline community structure; and studies of their genetic and evolutionary processes. The *S. purpurea* inquiline community has become a model experimental system for studying contemporary questions in ecology and evolution (Srivastava et al. 2004).

In this chapter, we review how studies of *Sarracenia* inquilines have answered fundamental questions in ecology and evolution. Much of the work focuses on *Sarracenia purpurea*, but where appropriate we will discuss studies using other *Sarracenia* species.

24.2 Natural history of *Sarracenia* and its inquilines

Sarracenia purpurea s.l. is a clonal, long-lived species that occurs primarily in bogs and savannas and other wet areas with generally acidic, low-nutrient conditions. It can be locally abundant but regionally scarce, due to its highly specialized habitat needs. Gene-based studies show that *S. purpurea* forms two major clades: a southern clade ranging from the Gulf Coast to mainland Maryland (ssp. *venosa*) and a northern clade (ssp. *purpurea*), ranging from the Eastern Shore of Maryland northwards (Stephens et al. 2015b; Chapter 9). The large geographic

distribution of *S. purpurea*, including both non- and postglacial populations, results in variation in evolutionary history and genetic architecture over identifiable time scales and environmental gradients. Although the plants can be long-lived, individual leaves seldom survive longer than a year; one study from a southern population found a median survival time for leaves of 40 weeks (Miller and terHorst 2012). The shape of the leaf minimizes desiccation and provides a stable habitat for aquatic species in the leaf (Kingsolver 1981).

The complex species interactions associated with *S. purpurea* have been studied for many years (Addicott 1974, Fish and Hall 1978, Bradshaw 1983). The species that occur within leaves are components of a detritivore-based or “brown” food web (Butler et al. 2008, Mouquet et al. 2008), with basal energy and resources derived from prey captured by the pitcher plant’s leaves. Most of the energy and nutrients are captured by the pitcher early in the life of each leaf (Fish and Hall 1978, Bradshaw 1983, Miller and terHorst 2012) although leaves may continue to capture a significant number of prey if they persist into a second year (Heard 1998).

24.2.1 Prey capture

Some studies suggest that *S. purpurea* uses nectar to attract ants, which make up a majority of its prey (Miller and terHorst 2012), whereas others have suggested a more generalized prey spectrum (Folkerts 1982, Cresswell 1991). The discrepancy may result from differences in prey capture with leaf age; at least one study in a southern population shows that leaves attract only ants, presumably with nectar, during the first 4–6 weeks of life (Miller and terHorst 2012), after which leaves may act as a less selective pitfall trap (Fish and Hall 1978, Cresswell 1991, Heard 1998). Although algae have been reported in pitcher plants, they generally are rare and restricted to older leaves.

24.2.2 Microbes

Captured prey are primarily broken down by a diverse microbial community, with energy and nutrients transferred on to higher trophic levels. It is unclear how much the rest of the community

contributes to the activity of the microbes. Consumers have the potential to affect microbial abundance and diversity (Heard 1994, Peterson et al. 2008, Hoekman et al. 2009), but Butler et al. (2008) found that the rest of the community had little effect on nutrients available to the plant itself. All *Sarracenia* species are known to have digestive glands that are embedded in the cell wall of the leaf and produce digestive enzymes (Juniper et al. 1989; Chapter 13). Digestive enzymes are produced by *S. purpurea* mostly in younger leaves or facultatively in older ones in response to prey (Gallie and Chang 1997), but the contribution of enzymes to prey digestion still needs exploration. Molecular assays of microbial communities in *S. purpurea* are revealing high densities and diversities of microbes, consisting largely of Proteobacteria and Bacteroidetes and a variety of yeasts (Gray et al. 2012, Paisie et al. 2014). The composition of the microbial community has been shown to change with different bacterivores (Paisie et al. 2014) and the presence of the pitcher-plant mosquito, *Wyeomyia smithii* (Peterson et al. 2008).

24.2.3 Bacterivores

The microbes are consumed by a suite of bacterivores, including protists and rotifers. The protists are thought to be mostly generalists, and include species from several different phyla (e.g., Ochrophyta, Euglenozoa, Ciliophora) and both flagellates and ciliates. Although several different rotifers have been also identified from pitcher plant leaves, the bdelloid *Habrotrocha rosa* is the most common rotifer throughout the range of *S. purpurea* (Buckley et al. 2010). This species may contribute to the host plant by excreting nitrogen and phosphorus into the leaf water (Błędzki and Ellison 1998). The specialist mite *Sarraceniopus gibsonii* is thought to be omnivorous, feeding on bacteria and protists, and can be quite common within leaves (Miller and terHorst 2012) and across populations (Buckley et al. 2010). Very little is known about its ecology, however.

24.2.4 *Wyeomyia smithii*

Many of the bacterivores are consumed in turn by filter-feeding larvae of the well-studied pitcher-plant mosquito, *Wyeomyia smithii* (Coq.). Females oviposit

directly into leaf-held water (Bradshaw 1983, Miller and terHorst 2012), where the eggs hatch, develop through four larval instars, and then pupate, eventually emerging as adults. *Wyeomyia smithii* are multivoltine in the southern range of *S. purpurea* but univoltine in northern Canada, where they overwinter in the frozen water in the leaf. *Wyeomyia* are omnivores, selectively feeding on larger protozoa and rotifers (Błędzki and Ellison 1998, Kneitel and Miller 2002, Kneitel 2012), while also directly consuming bacteria and detritus (Cochran-Stafira and von Ende 1998, Hoekman 2007).

Historically, *Wyeomyia smithii* was divided taxonomically into two species based on characteristics of the anal and ventral papillae: *W. smithii* ranging from New Jersey northwards, and *W. haynei* ranging from Maryland to the Carolina coastal plain (Bradshaw and Lounibos 1977). Bradshaw and Lounibos (1977) found that both northern and southern “species” of pitcher-plant mosquitoes were fully interfertile and that hybrids between a Massachusetts and an Alabama

population were indistinguishable from *W. haynei*. They concluded that there is one single species of *Wyeomyia* in pitcher plants, *W. smithii*, and that *W. haynei* represents an intermediate morph along a geographic cline.

More recent work has shown that *Wyeomyia smithii* is divided into southern and northern clades (Figure 24.1, Merz et al. 2013), which matches the genetic variation among populations of the host plant (Stephens et al. 2015b). Morphological, physiological, developmental, and molecular characters consistently show that *W. smithii* has diverged from south to north, starting along the Gulf Coast, then along the Atlantic coastal plain and post-glacially to the southern Appalachians and northward into Canada (Bradshaw and Lounibos 1977, Merz et al. 2013). The southern clade of *W. smithii* splits, as does *S. purpurea*, between Gulf and Atlantic coasts with the Atlantic coastal populations being derived. Deeper taxon sampling of *W. smithii* shows that the northern clade splits into at least four monophyletic lineages (Merz et al. 2013).

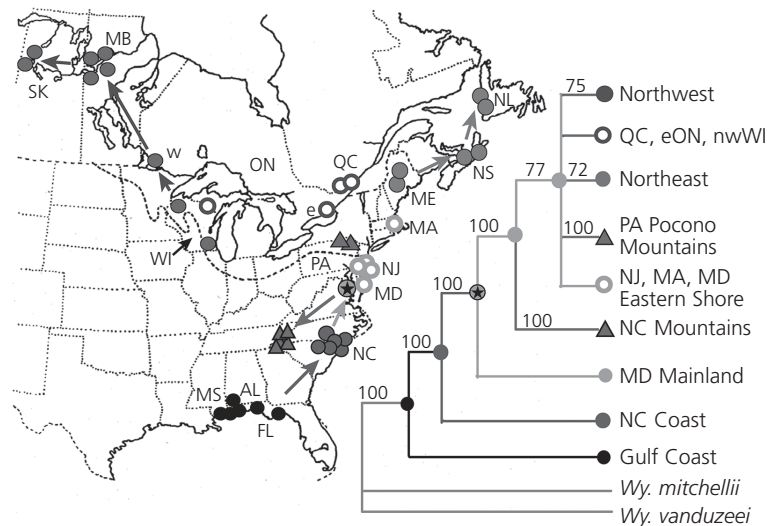


Figure 24.1 (Plate 16 on page P14) Phylogeography of *Wyeomyia smithii* based on the combined 46-population tree. Arrows indicate directions of range expansion. Maximum extent of the Laurentide Ice Sheet at the last glacial maximum is plotted as a dotted line. Two-letter abbreviations identify each state or province. USA states: AL—Alabama; FL—Florida; MA—Massachusetts; ME—Maine; MD—Maryland; MS—Mississippi; NC—North Carolina; NJ—New Jersey; PA—Pennsylvania; WI—Wisconsin. Canadian provinces: MB—Manitoba; NL—Newfoundland and Labrador; NS—Nova Scotia; ON—Ontario; QC—Quebec; SK—Saskatchewan. Triangles represent mountain populations in the southern Appalachians of North Carolina (southern triangles) or in the Pocono Mountains of Pennsylvania (northern triangles). The filled symbol with the star indicates the basal mainland Maryland population (MD2). Numbers associated with each node or branch tip represent maximum parsimony bootstrap support for that clade. There are no support numbers for the QC, eastern ON (eON) and northwest WI (nwWI), or the NJ, MA, and Eastern Shore MD populations because they do not constitute a monophyletic grouping. Adapted from Merz et al. (2013).

24.2.5 Other dipterans

The larvae of two other dipterans commonly occur in the leaves of *S. purpurea*. Larvae of the midge, *Metriocnemus knabi*, are shredders, and are thought to subsist on the prey carcasses that sink to the bottom of the leaves. In doing so, *M. knabi* larvae can increase resource availability for bacteria, which may have indirect positive effects on protozoa and mosquito larvae higher in the food chain in what Heard (1994) termed a “processing chain commensalism.” However, the role of *M. knabi* in the inquiline community is not clear and may vary among sites. Bradshaw (1983) found that *Wyeomyia smithii* can inhibit pupation success of *M. knabi*, whereas *M. knabi* can facilitate the rate of *W. smithii* development, especially at high larval densities. In some cases, midges may have direct negative effects on some bacterivores through predation (Hoekman et al. 2009).

Larvae of the flesh fly (*Fletcherimyia fletcheri*) also are common in *S. purpurea* leaves. The genus *Fletcherimyia* is tightly associated with *Sarracenia*: there are up to eight other species of *Fletcherimyia* that occur in the leaves of various *Sarracenia* (Dahlem and Naczi 2006). They all share a similar life history, including feeding on dead prey on the water surface, ovolarvipary, and cannibalism (Forsyth and Robertson 1975), except that flesh flies found in *S. purpurea* are necessarily more aquatic than those found in other *Sarracenia*. However, species in this group often are misidentified and some species of *Fletcherimyia* appear to live in more than one species of *Sarracenia* (Stephens and Folkerts 2012). The larvae complete three instars while consuming prey captured by the leaf (Forsyth and Robertson 1975). The larvae have unusual cup-like posterior spiracles, which, when spread out, allow the larvae to float at the surface of the water within the leaf. Cannibalism often results in one survivor per leaf (Rango 1999); the surviving instar crawls from the leaf and pupates in the moist vegetation around the plant. Adults of *F. fletcheri* also may act as a pollinator for *S. purpurea* (Ne’eman et al. 2006).

24.2.6 Inquiline dispersal

Leaves are generally colonized within days of opening, so significant dispersal appears to occur among

leaves. The mechanisms of dispersal of rotifers, protozoa, mites, and microbes such as bacteria and yeast are unknown, and their effects on the plant or the dipterans are rarely studied. Of the dipterans, *Wyeomyia smithii* are thought to be poor flyers, and generally lay 1–2 eggs per leaf in mostly young leaves. Oviposition by both *W. smithii* and *Metriocnemus knabi* is higher in larger leaves and higher local leaf densities (Trzcinski et al. 2003). Overall, dispersal remains an important gap in our understanding of the natural history of these communities.

24.2.7 Non-aquatic associates: moths

Several non-aquatic species are also associated with *Sarracenia*, including at least four noctuid moths that are obligate herbivores on *S. purpurea*: *Papaipema appassionata* and three species of *Exyra*. Little is known about the former species, except that it is a rhizome feeder that generally kills its host plant (Atwater et al. 2006). Species in the genus *Exyra* all use *Sarracenia* as hosts, including *E. fax* on *S. purpurea*, *E. ridingsii* on *S. flava*, and *E. semicrocea* on several different species of *Sarracenia* (Folkerts 1999). For all three *Exyra* species, adults lay eggs on the leaves, where they go through five instars, slowly filling leaves with frass. The larvae consume the inside of the leaf, leaving a thin membranous outer wall. They generally weave a fine web over the opening of the leaf, possibly to protect the larvae from predators. Larvae can move from leaf to leaf, but eventually move to a final leaf, often cut a hole in the bottom of the leaf to prevent drowning, skeletonize the leaf, and then develop into a pupa.

24.2.8 Pollinators

The common lore is that flowers of *Sarracenia* do not self-pollinate and require animal pollinators. For *Sarracenia* with larger flowers (including *S. purpurea*), it has often been assumed that natural pollinators include native bumblebees, whereas other species required smaller bees and flies. However, our current understanding of pollination in *Sarracenia* comes almost entirely from observations (e.g., Folkerts 1982, Ne’eman et al. 2006). Juniper et al. (1989: 271) noted that, “[a] critical comparative study of pollination processes in *Sarracenia* is

needed to determine how they relate to reproductive success and hybridization." This need still remains.

24.2.9 Spiders

Various spiders also often are found in association with carnivorous plants, where they may be prey, competitors, or even mutualists (Juniper et al. 1989). Some ground-foraging spiders are known to be occasional prey of *S. purpurea* (e.g., Cresswell 1991, Heard 1998). The Green Lynx spider, *Peucetia viridans*, often co-occurs with *Sarracenia* in the southeastern United States; these and other spiders may use leaves and flower heads for foraging or laying and guarding egg masses (Jennings et al. 2008). Because *Sarracenia* and spiders may have common insect prey, it has been suggested that they can be competitors (Cresswell 1991, Folkerts 1999), but further studies are required to demonstrate the implications of such interactions for either species.

24.3 *Sarracenia purpurea* and its associates as a model ecological system

Several features of *S. purpurea* and its inquilines make for a near-ideal experimental system in which to study questions about species interactions, species diversity, and community structure. Individual leaves create small and well-defined phytotelmata that can be easily manipulated. The communities found inside the leaves are transient and occur at well-defined spatial scales (e.g., host leaves, plants, and populations) and thus are ideal for studying dispersal, invasion, and succession. *Sarracenia purpurea* is highly constrained to specific habitats (bogs, seeps, and savannas), yet occurs over a very large geographic range from North Florida to northern Canada, providing significant variation in climate. The leaves host several obligate species over the entire range of the host plant. Further, both the plant and its inquilines can be maintained in growth chambers or greenhouses for more intensive study, enabling the evaluation of inquiline physiology, ecology, and evolution in their natural microhabitat under controlled conditions. The result is that many studies have been conducted with *S. purpurea* and its inquilines on a variety of questions.

Other pitcher plants share some of these characteristics (Adlassnig et al. 2011; Chapter 23). *Sarracenia alata* (e.g., Satler et al. 2016) and *Darlingtonia californica* (Naeem 1988) also host inquilines, but other related species do not (e.g., *S. psittacina*). In many cases, detailed studies of possible inquilines in other New World pitcher plants are simply lacking (e.g., *Heliamphora*; Adlassnig et al. 2011). Finally, other types of carnivorous plants also host inquilines, including those found in the bladder traps of *Utricularia* (Chapter 25). Unlike *S. purpurea* and its inquilines, however, most of these systems neither have been well described nor generally have been used to address broader questions in ecology and evolution.

24.3.1 Mutualism between *Sarracenia purpurea* and its aquatic inquilines

Sarracenia purpurea and its inquilines comprise a mutualistic, but not necessarily co-adapted relationship (Bradshaw and Creelman 1984). Decomposing prey provide the nutrient base for the inquilines community that respire CO₂ and excrete ammonium ions. Both CO₂ and ammonia production increase at higher temperatures while higher temperature and brighter light accelerate uptake of both molecules by the host plant. Ammonium ions are taken up directly by the glutamate cycle without further reduction and the concomitant reduction of CO₂ generates oxygen that is directly transferred from the leaves to the contained water. In essence, this is a feed-forward system: inquilines are producing maximum CO₂ and ammonium ion under exactly the same conditions that the plant is taking up these molecules and generating excess oxygen. There are two consequences. First, unless the system is overloaded by the capture of large prey, digestion remains aerobic. Second, pitcher plants are located within the boundary layer of their habitats and often exposed to direct sunlight. Water temperatures in leaves routinely range 40–50 °C in the south and 30–40 °C in the north (Bradshaw et al. 2004). At low levels of convection and high temperatures, carbon can become limiting for photosynthesis so that carnivory can provide a source of carbon as well as nitrogen (Bradshaw and Creelman 1984).

24.3.2 Consumer versus resource control of communities

Ecology has long grappled with understanding the roles of resource availability (competition), consumer abundance (predation), and their interactions (e.g., Hairston et al. 1960, Paine 1966). Theoretical work has suggested that both top-down and bottom-up forces can be important in communities (Powers 1992), but has also suggested that interactions among competition and predation can lead to more complex patterns (e.g., Paine 1966, Holt 1977). Experiments to disentangle resource and consumer effects can be complex, requiring manipulating both resources and predators in factorial designs.

Pitcher plants are ideal for such studies (Kitching 2001), as both the resources (dead prey) and top consumers (e.g., mosquitoes) are easily manipulated and community responses occur over relatively short timescales. One of the earliest experimental studies with *Sarracenia* demonstrated that mosquitoes suppressed protozoa without any “keystone effect” in increasing protozoa diversity (Addicott 1974, Cochran-Stafira and von Ende 1998, Peterson et al. 2008). Kneitel and Miller (2002) showed that effects of adding resources translate up through the food web to increase bacterial, protozoan, rotifer, and mite abundances, but increased predation affected only rotifers, with a weak trophic cascade on bacteria. Hoekman (2007, 2011) found similar patterns, although top predators were found to have different effects in Michigan and Florida populations that may be related to temperature-associated differences in microbial productivity.

The food webs in *S. purpurea* also have been used to demonstrate more complex interactions between competition and predation. Heard (1994) demonstrated that *M. knabi* larvae increase bacterial abundances, which can result in increased growth of *W. smithii* larvae further up the food web. Hoekman et al. (2009) found similar positive effects of *M. knabi* on bacteria, but found that it also suppressed rotifers and some protozoa, apparently through direct consumption. Finally, Kneitel and Miller (2003) demonstrated that moderate levels of dispersal among pitcher plant leaves can increase species richness, but only in the absence of the top predator, *W. smithii*. Collectively, these manipulative studies

of inquiline food webs show that entire communities are not dominated by only resource or only consumer effects and that omnivory can create more complex species associations in communities.

24.3.3 Testing theories of succession

Succession has remained a fundamental but frustrating concept in ecology, with longstanding questions about mechanisms and predictability (McIntosh 1999). Much of our understanding comes from plant succession and much of the evidence is indirect and obtained from chronosequences. Inquilines can be used to study succession in aquatic systems; entire successional sequences can be observed directly over a reasonable time frame.

The inquiline communities found within *S. purpurea* leaves are highly variable from leaf to leaf within and between plants in a single population. There have been several attempts to understand this variation; earlier studies identified specific leaf traits that are correlated with this variation, including leaf size (Cresswell 1993, Harvey and Miller 1996, Buckley et al. 2004) and density (Trzcinski et al. 2003). However, it appears that the primary factor determining variation among leaves is leaf age, and successional patterns of prey capture and *W. smithii* abundance. Fish and Hall (1978) documented dipteran abundances through succession in natural populations of *S. purpurea* in Massachusetts and prey capture rates in leaves of greenhouse-grown plants. They found that leaf age determines the strong resource and consumer drivers in inquiline communities. Most prey are captured in leaves < 5-wks old, when nectar is present, and most mosquitoes also occur in leaves < 7-wks old.

Miller and terHorst (2012) tested general theory associated with succession using a 28-month study of *S. purpurea* leaves in North Florida. Successional theory suggests that communities become more stable and converge with similarly aged communities during succession, and that species diversity peaks at some intermediate stage. Individual *S. purpurea* leaves survived for a median of 40 wks, with most of the successional changes occurring in the first 20 wks (Miller and terHorst 2012). Mosquitoes oviposit in young leaves (Figure 24.2a), except in the early spring before young leaves are available. At the

bottom of the food web, most ant prey are captured in the first few weeks, with virtually no ants captured after week ten (Figure 24.2c). Bacteria appear to be limited mostly by resources, whereas protozoa are limited by mosquito predation (Figure 24.2c and 24.2b, respectively). Communities did become more similar through time and stability peaked at intermediate levels, but diversity showed no consistent pattern with leaf age (Miller and terHorst 2012). The study demonstrated that understanding system-specific drivers of succession may be more important for understanding succession than general principles.

The pattern of succession in Miller and terHorst (2012) is very similar to that described by Fish and Hall (1978), despite having been studied in very

different locations and for a longer time. Both studies note that leaf age often is neglected in studies of *Sarracenia* inquilines and needs to be explicitly considered in future studies because of its strong effects on succession.

24.3.4 Dispersal and metacommunities

Dispersal among local habitats is known to affect species persistence and diversity in patchy habitats (Levin and Paine 1974) and islands (MacArthur and Wilson 1967). More recently, metacommunity theory has unified ideas about the role of dispersal, competition, and local habitat heterogeneity and stability (Leibold et al. 2004). This theory makes predictions about the maintenance of

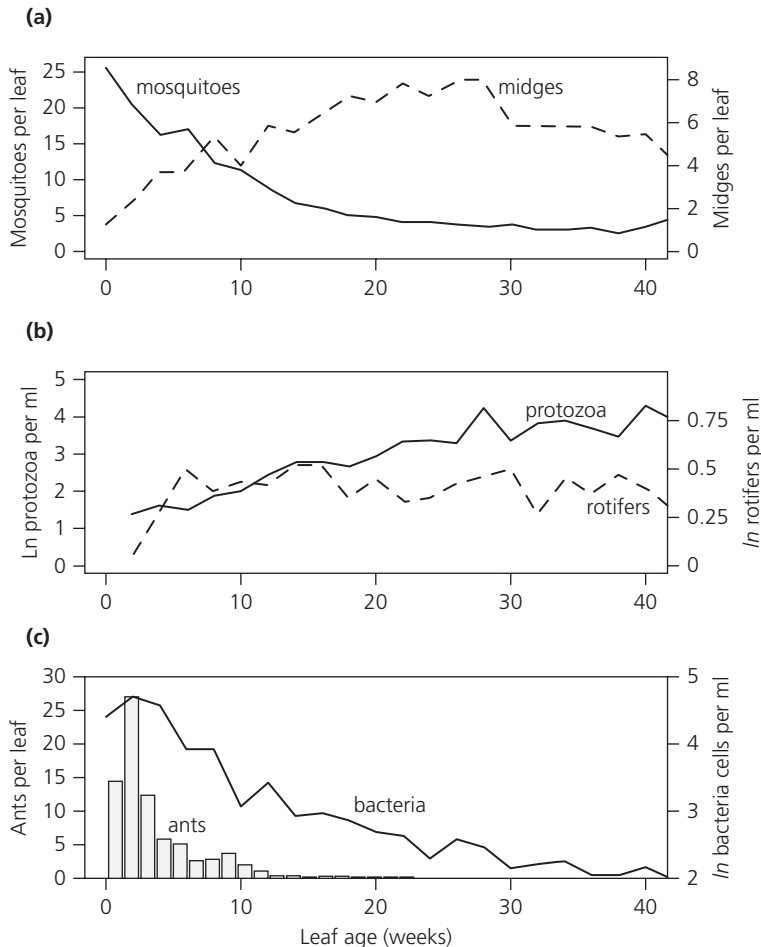


Figure 24.2 Successional patterns of selected inquilines from leaves followed from opening through the first 40 weeks. (a) Oviposition behavior by the Dipteran *W. smithii* (solid line) causes high abundances in younger leaves, with a gradual loss of individuals as they eclose. *Metriocnemus knabii* (dashed line) shows a much lower overall abundance and a more intermediate peak in abundance. (b) The bacterivores include various protozoa species (solid line) whose numbers increase as predation declines, and rotifers (dashed line) whose densities are less affected by leaf age. (c) Virtually all ant capture (bars) is by leaves <10 weeks of age, which generates a corresponding pattern in bacteria densities (dashed line). Adapted from Miller and terHorst (2012).

communities at different spatial scales, especially as a function of dispersal among local habitats. Testing such theory remains difficult, especially in natural communities.

Several studies have used patterns of inquiline species distributions among *S. purpurea* leaves to infer scales of dispersal (Harvey and Miller 1996, Trzcinski et al. 2003, Buckley et al. 2004). However, these studies generally explain only a small amount of the total variation in the composition of local communities, perhaps because they did not incorporate leaf age (§24.3.3). Baiser et al. (2013) analyzed inquiline data from a variety of spatial scales, and concluded that metacommunity models using either species-sorting or patch dynamics best predicted the variation in community structure. Studies also have used direct experiments to test if some inquiline species are limited by dispersal. Conditions for establishment success vary among protozoa species, with some species having higher success with higher resources, whereas others establish best when predators are absent (Miller et al. 2002). Finally, in the one study that varied rates of dispersal among leaves to test metacommunity theory, Kneitel and Miller (2003) demonstrated that dispersal can increase diversity of bacterivores when the mosquito predator is missing from the food web.

24.3.5 Biogeography at the scale of a community

An important goal of ecology is to understand the role of spatial and temporal scales in determining species abundances and community composition (Levin 1992). Reaching this goal could help to unify core concepts of population biology, evolutionary history, and ecosystem ecology. A unique characteristic of the *S. purpurea* inquiline community is its large range, which is ideal for biogeographic questions about diversity, stability, and coevolution. The host plant is similar across the entire range, although leaf morphology varies with temperature and precipitation (Ellison et al. 2004). Virtually all populations of *S. purpurea* have the same dipterans, rotifers, mites, and protozoa; although within-population variation in inquiline composition can be significant, among-population variation is relatively low (Buckley et al. 2003, Buckley et al. 2010).

Rasic and Keyghobadi (2012) used microsatellite loci for the midge, *Metriocnemus knabi*, in *S. purpurea* to suggest that bog size and plant density influence oviposition behavior, which in turn determines patterns of genetic differentiation within and across local populations. *Sarracenia purpurea* inquilines also have been used to address several questions in biogeography. For example, diversity increases with latitude (Buckley et al. 2003), contrary to the general pattern found in other communities, and species composition is correlated with climatic variables, including temperature and precipitation (Buckley et al. 2010). Variation in food-web structure among leaves within sites decreased with latitude (Baiser et al. 2012), perhaps because shorter growing seasons constrain variation in leaf age and conditions. However, despite large sample sizes and scale of sampling, only 40% of the variation in community structure among leaves could be explained by among-leaf, within-site, or among-site variables.

It is difficult to conduct experiments across the large scales necessary to test the factors determining biogeographic patterns for entire communities. Natural microhabitats, such as rock pools, tree holes, bromeliads, and pitcher plants are well suited for such studies (Srivastava et al. 2004). Hoekman (2007) found that bacterivores in Michigan and Florida responded differently to resource and consumer control, perhaps due to differences in temperature. Gray et al. (2016) investigated the importance of temperature, trophic structure, and local adaptation on ecosystem functioning. They collected leaf contents from five sites along a natural temperature gradient from Florida to Quebec, and then conducted a “reciprocal temperature” experiment using growth chambers. Communities from each site were maintained at a summer temperature appropriate for each of the five sites, with and without the top predator (mosquito larvae); ecosystem functions such as bacterial respiration, and ammonium and phosphorus production were monitored. Gray et al. (2016) found that temperature has a greater effect on ecosystem functioning than site of origin, and that top-down trophic regulation increases with temperature. Similar experiments may allow us to separate the effects of climate and host plant, and the contributions of

various trophic levels to large-scale community and ecosystem patterns (Parain et al. 2016).

24.3.6 Evolution in a community context

The realization that ecological and evolutionary changes can occur within similar time frames (Hairston et al. 2005) has led to questions about how evolution occurs in a multispecies context and how evolution can lead to feedback loops between ecological and evolutionary change (e.g., Kokko and López-Sepulcre 2007). Because pitcher-plant leaves develop from high prey capture and mosquito abundances to low prey and mosquito abundances (Figure 24.2), the conditions for bacterivores go from favoring predation tolerance (consumer control) to favoring competitive traits (resource control). Further, the relatively short generation times of pitcher-plant protozoa allow for “rapid” evolution of either predator tolerance (terHorst et al. 2010) or growth in competition (terHorst 2011). An investigation of the growth of protozoa from leaves of different ages does show that predator tolerance and interspecific competitive abilities evolve

(Miller et al. 2014). However, interspecific competitive ability evolves differently in subordinate and dominant competitors (Figure 24.3), perhaps because subordinate competitors experience largely interspecific competition, whereas dominant competitors experience intraspecific competition.

There are also several very interesting cases of coevolution of pitcher plants with other species. Different species of *Nepenthes*, for example, have apparently coevolved with ants, bats, and small mammals (Chapter 26). As obligates, many of the *Sarracenia* inquilines appear to be adapted to conditions in pitcher-plant leaves, but the reciprocal evolution of the plant to its inquilines is less clear (§24.3.1). The long shared history of pitcher plants and their inquilines over a large biogeographic scale means that they likely are interesting systems for studies of coevolution (e.g., Stephens et al. 2015b). *Sarracenia alata* has been shown to have population genetic structure that is congruent with several associated species, including mites and fungi (Satler et al. 2016). However, *S. alata* has a very restricted range; it would be very interesting to see similar work with the diverse community associated with *S. purpurea*.

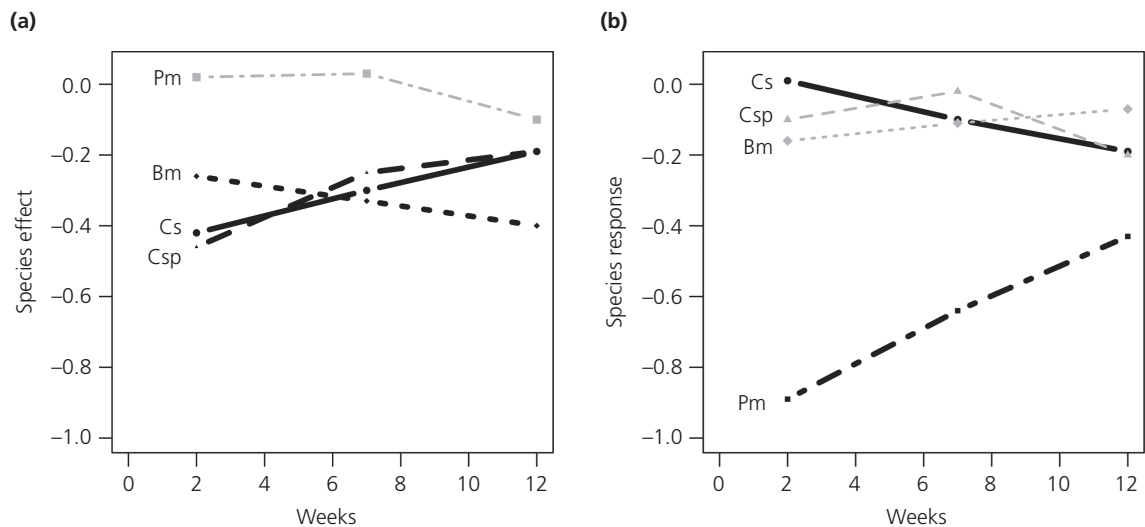


Figure 24.3 Average effect of protozoa species on other protozoans (a) and response to protozoa by other protozoans (b), using protozoa isolated from leaves of different ages (2, 7, and 12 wk old). Pm: *Poterioochromonas malhamensis*; Bm: *Bodo menges*; Cs: *Colpoda steinii*; Csp: an unidentified colpodid species. Values represent the growth of each species in mixture relative to its growth in monoculture; negative values indicate greater suppression of growth. Bold solid lines have significantly increasing effect or response with wk; bold dotted lines indicate significantly decreased effect or response with wk. Adapted from Miller et al. (2014).

24.4 *Wyeomyia* as a model system for inquiline species

A common characteristic of carnivory in plants is that they serve as habitats or hosts for unique and diverse assemblages of other species (Chapters 23–26), and many such associations remain to be described. Because inquilines are nearly ubiquitous, an understanding of these species may be necessary to fully understand the ecology and evolution of carnivorous plants themselves. Here we consider the biology of one inquiline in more detail, and illustrate that it can serve as a model for understanding the ecology of other inquilines worldwide.

The mosquito *Wyeomyia smithii* is the best studied inquiline among all carnivorous plants. The climates where different populations of *W. smithii* occur vary from relatively benign in the south where growth seasons are long and the dipterans are multivoltine, to harsh conditions in the north where there may be only a few months for the plants to grow and there is only one brood per year. These climatic differences have resulted in significant variation in the life history of *W. smithii* across its geographic range. For example, southern populations require a blood meal for their second and subsequent ovarian cycles, whereas northern populations do not bite. There are also latitudinal gradients in traits including number of egg batches per season, rates of egg production, and lifetime fecundity. This variation sets the stage for studies of the evolution of important life-history traits.

24.4.1 Density-dependent selection

The theory of r - and K -selection was developed by MacArthur (1962), who considered genetic models of how density-dependent selection should act. He concluded that in equilibrium populations, “the carrying capacity, K , replaces fitness (r) as the agent controlling the action of natural selection” (MacArthur 1962: 1897). This basic concept spawned a myriad of tests of MacArthur’s theory based on life-history traits presumably associated with r - and K -“strategies.” The ability to rear *W. smithii* in its natural microhabitats under controlled conditions provided the opportunity to test this theory in its most fundamental form: density-dependent selection imposed in nature directing the evolution of life histories, especially r and K .

Within pitchers, *W. smithii* larvae are limited primarily by intraspecific scramble competition for available resources, which in turn are determined by the prey captured by that leaf (Bradshaw 1983, Broberg and Bradshaw 1995). Mean crowding of *W. smithii* larvae per unit resource is negatively correlated with latitude and altitude (Bradshaw and Holzapfel 1986). This geographic gradient in mean crowding translates into a similar gradient of density-dependent development and pupation success. Southern populations experiencing a long growing season and mild winters exist near the carrying capacity of their environment much of the year (Miller and terHorst 2012). Density-dependent effects on growth and development then abate with increasing latitude and altitude and no density-dependent effects can be observed in some northern and mountain populations (Bradshaw and Holzapfel 1996, Bergland et al. 2005).

Bradshaw and Holzapfel (1989) sampled 12 populations from the Gulf of México to Canada that varied 25-fold in larval density and density-dependent development observed in the field. They varied larval density in the leaves of intact pitcher plants in computer-controlled climate rooms, with the daily light and temperature cycle programmed to that of a mid-latitude population during the summer. Larvae were fed freeze-dried *Drosophila* in a regimen mimicking the time-course of prey capture by leaves in nature (Bradshaw 1983). In these experiments fitness (capacity for increase, r_c) declined with increasing density. Regression of fitness on density then estimated r_{max} from the intercept on the y -axis at zero density and K on the x -axis when $r_c = 0$. Neither r_{max} nor K was correlated with density in nature, leading to the conclusion that r - and K -selection based on the effects of density alone are inadequate to explain the evolution of major demographic traits in *W. smithii* (Bradshaw and Holzapfel 1989) and casting doubt on the universality of MacArthur’s theory in natural populations.

24.4.2 Evolution of protandry

The emergence of males before females (protandry) in plants and animals usually has been interpreted as a consequence of sexual selection (Darwin 1871) on both sexes. Early emerging males have access to more females and females have immediate access to

mates. Selection on protandry should be especially strong in monogamous species such as mosquitoes that emerge synchronously in a seasonal environment and have non-overlapping generations. Selection on protandry should be enhanced even more when density-dependent effects result in larger early-emerging (more fecund) females. However, there is a tradeoff in the degree of protandry between competition for early-emerging females and survivorship of males from the time of male emergence to the time of female emergence. In addition, the optimal degree of protandry is dependent not only on female emergence time, but also on the degree of protandry of other males in the population (Holzapfel and Bradshaw 2002).

Protandry in *W. smithii* is a heritable trait that increases with larval density (Bradshaw et al. 1997). Larval densities and density-dependent development decrease with increasing latitude or altitude (Bradshaw and Holzapfel 1986, Buckley et al. 2003). Consequently, northern populations tend to have discrete summer generations whereas southern populations have overlapping generations. From the standpoint of sexual selection, protandry confers a fitness advantage only when female emergence is predictable in time, i.e., when generations are discrete. Sexual selection theory then predicts that protandry should be more pronounced in the north than in the south. Yet, over a geographic gradient from southern populations with multiple, overlapping generations to northern populations with a single synchronous generation, there is a non-significant trend toward greater protandry in southern than northern populations (Bradshaw et al. 1997). From the standpoint of natural, as compared to sexual, selection, female fitness represents a compromise between minimizing development time and maximizing size at pupation (fecundity). Male fitness is little affected by size (Benjamin and Bradshaw 1994) and males conserve development time while losing pupal mass with increasing larval density, thereby minimizing pre-adult development time with little cost on subsequent lifetime offspring sired. When confronted with variable larval resources, females and males then make different contributions to fitness: females through optimizing fecundity and development, and males through minimizing development, and therefore generation time. Hence, in southern populations with

overlapping generations, protandry is maintained through natural, not sexual selection. In contrast, sexual selection should become more important at higher latitudes and altitudes with lower larval densities and increasingly discrete generations.

24.4.3 The evolution of diapause and photoperiodism in *Wyeomyia smithii*

In temperate seasonal environments, selection should favor exploitation of the growing season, mitigation of the unfavorable season, and a means to switch from summer to winter lifestyles and back again in a timely manner. The optimal timing of development and diapause depends upon four geographic patterns of light and temperature (Figure 24.4). First, the climatic gradient in eastern and central North America is primarily one of winter cold, not summer heat. Second, with increasing latitude, spring arrives later and fall arrives earlier (e.g., 15 °C isotherm). Third, the length of the growing season declines with increasing latitude, leading to a decrease in the number of generations per year at higher latitudes. Fourth, the earlier arrival of winter at progressively higher latitudes imposes selection in response to day length.

At any specific spot on Earth, the day length today is the same as it was 10,000 years ago and will be 10,000 years into the future, regardless of temperature. Apart from equatorial latitudes, day-length cycles repeat with time of year (Figure 24.4b) and provide the most reliable predictive cue of any variable for future seasonal variation in the environment. At any given latitude, day length does not change with elevation and is the same in a coastal pine savanna as in a mountain bog at the same latitude. However, duration of summer and winter do change with elevation: a southern mountain bog has a similar seasonal climate as a coastal bog farther north.

Consequently, the optimal time to enter diapause at any locality depends upon the correct response to day length at that latitude and altitude.

Physiological response to day length is under selection and the selective force is the timing of the onset of winter, not day length itself. Entering diapause too early loses opportunity for reproduction and consumes nutritional reserves at warm temperatures; entering diapause too late exposes

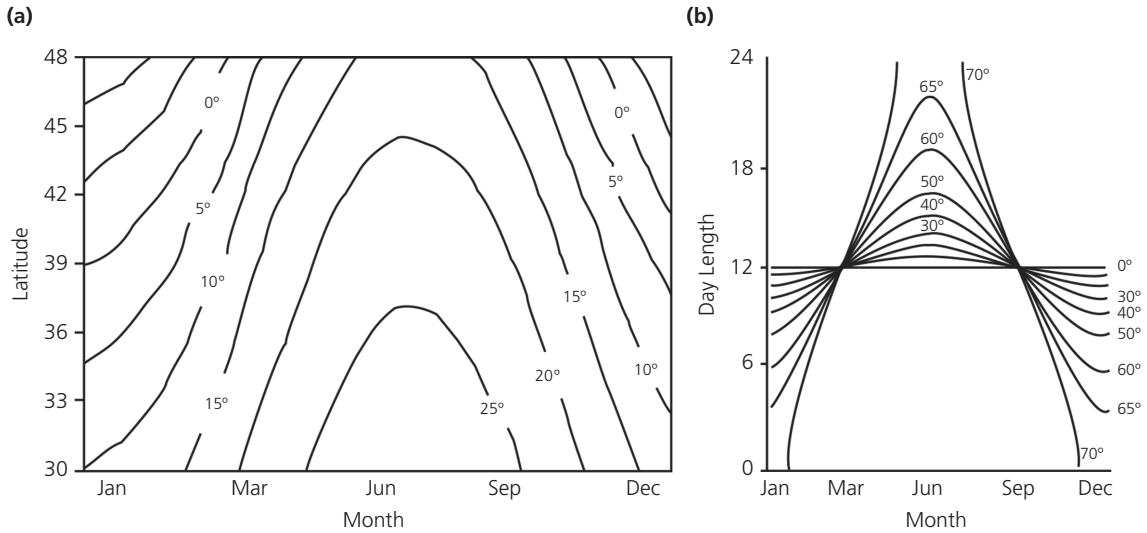


Figure 24.4 Geographic and seasonal variation in day length and temperature. (a) Isotherms for mean monthly temperature in central and eastern North America. The latitudinal variation in climate is less a matter of summer warmth (June isotherms are farther apart) than winter cold (January isotherms are closer together) and northern populations experience a shorter growing season than southern populations. Hence, changes in season length and the timing of spring and fall activities have a greater effect on animal populations than do the direct effects of temperature. (b) Seasonal patterns in day length (sunrise to sunset) at different latitudes ($^{\circ}$ N) in the northern hemisphere. Day length at temperate and polar latitudes predicts future seasons more reliably than any other environmental cue.

an individual to the lethal effects of winter. The optimal time to switch to diapause occurs at the point where there are insufficient thermal units remaining during the fall to support development through an entire generation (Taylor 1980). Therein lies a special relevance of day length as an environmental cue for anticipating future seasonal conditions: larvae of *Wyomyia smithii* (Lounibos and Bradshaw 1975; Bergland et al. 2005), like many other insects, are able to enter diapause at the optimal time of year even though current conditions are otherwise favorable for development and reproduction.

Adaptation to different climates requires genetic flexibility in a population's ability to use day length in the timing of seasonal events (photoperiodism). In *W. smithii*, the critical photoperiod has evolved 10 standard deviations between the Gulf of México and Canada (Lair et al. 1997) and is tightly correlated (R^2 repeatedly ≥ 0.92) with altitude as well as latitude of origin (Bradshaw and Holzapfel 2001). At higher latitudes, winter arrives earlier and when day lengths are longer (Figure 24.5); hence, switching from active development to diapause earlier in the year at higher latitudes involves reliance on

longer day lengths. With increasing altitude, the growing season also becomes shorter, winter arrives earlier, and *W. smithii* use progressively longer day lengths to cue the onset of diapause earlier in the year. Over altitudinal gradients at the same latitude, the seasonal environment changes while the annual fluctuation in day length is constant. Hence, the increase in critical photoperiod with increasing altitude illustrates the concept that photoperiodic response and critical photoperiod are adaptations to the timing of seasonal life-cycle events and not to day length itself.

The question still remains as to the relative importance of photoperiodic versus thermal adaptation over the climatic gradient of North America. To answer this question, Bradshaw et al. (2004) used processor-controlled climate rooms to replicate the year-long climates of the Gulf Coast (30° N), mid-latitudes (40° N), and northern latitudes (50° N). Experiments were run in the leaves of intact pitcher plants where four replicate *W. smithii* populations from each of these latitudinal regions were exposed to each annual climatic regimen in a fully crossed experiment. Fitness was assessed by the year-long

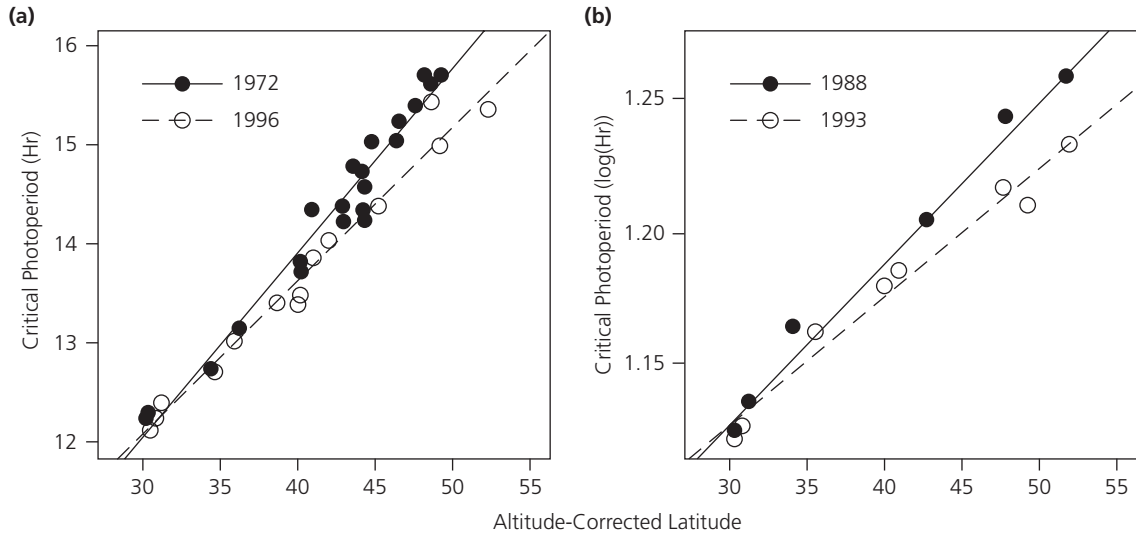


Figure 24.5 Critical photoperiods of *W. smithii* collected during the overwintering generation from 1972 to 1996 determined from (a) static (1972, 1996) or (b) changing (1988, 1993) photoperiods. Analysis of covariance indicated significantly steeper slopes for the earlier year in each comparison, meaning that shifts toward shorter critical photoperiods (more southern phenotypes) increased with latitude. Redrawn from Bradshaw and Holzapfel (2001).

cohort replacement rate integrated over all four seasons. Latitude of thermal year, region of mosquito origin, and their interaction all had significant effects on fitness. Along the climatic gradient of North America, there has been long-term thermal adaptation in *W. smithii*. However, fitness was highest in the moderate mid-latitude thermal year, indicating that extreme southern and northern climates imposed significant heat and cold stress, respectively, on mosquito populations living in pitcher plants at those latitudes.

This experiment allowed the effects of temperature and day-length to be disentangled (Bradshaw et al. 2004). When northern populations are “transplanted” to a mid-latitude thermal year, but kept at their native northern photic year, fitness increased 47% over the northern thermal-photic year. For northern populations, warmer is better. However, when northern populations were transplanted to the same benign mid-latitude thermal year, but exposed to the mid-latitude photic year, they lost 88% of fitness. Mid-latitude day lengths were simply too short to sustain non-diapause development and resulted in a negative rate of population growth, leading to rapid population decline and extinction

in an otherwise favorable thermal climate. Thus, the immediate target of selection in a rapidly changing temperate climate is photoperiodic adaptation, rather than the expected direct effect of warmer temperatures on thermal adaptation. These experiments also illustrate the power of the pitcher-plant system to dissect the causes of geographic variation in fitness in a temperate climate. The primary determinant of fitness is the correct timing of seasonal activities, i.e., being in the right stage of development at the right time of year mediated through the optimal, genetically programmed response to day length.

24.4.4 Climatic change as a selective force driving evolution

During recent rapid climatic change, two patterns of biotic response commonly have been observed. Organisms are expanding their ranges poleward and are altering the timing of their seasonal activities. Part of these seasonal alterations represents phenotypic plasticity, especially among ectothermic plants and animals. Even after accounting for these phenotypic patterns, the question remains as to

whether the observed effects of recent rapid climatic change also have a genetic basis. Long-term studies of photoperiodism in *W. smithii* over a 24-year period provide the first example of genetic shifts of any phenotype driven by recent rapid climatic change (Bradshaw and Holzapfel 2001). Larvae were collected from Florida to Canada and photoperiodic responses were quantified using rigorously controlled conditions in environmental chambers.

The results were conclusive (Figure 24.5). First, genetic change in response to selection imposed by climatic change occurred in a seasonal trait. Second, the genetic signature of climatic change can be detected in as little as five years (Figure 24.5b). Third, the genetic shift in critical photoperiod is greater at higher latitudes where the rate of climatic change is faster and genetic variation underlying photoperiodic response is higher than at lower latitudes. Fourth, there is always a concern when comparing phenotypes over time that identical environmental conditions were used over a period of years or decades. The southern populations (at 30 °N), where climatic change is proceeding more slowly and genetic variation is lower, provide an important between-year control. There is complete overlap in critical photoperiods over both a 24- and 5-yr interval in these southern latitudes (Figure 24.5a), demonstrating that exactly the same experimental conditions were imposed across the different ranges of years shown in Figure 24.5. Finally, documentation of genetic response to climatic change over a wide geographic range was possible because of the local nature of pitcher-plant populations and the long-lived nature of the plants themselves, enabling the establishment of a “library” of multiple, discrete, and readily resampled mosquito populations through decades of time.

In comparison with geographic variation in demographic traits, the five-year genetic shift in photoperiodic response of *W. smithii* in nature represents evolution at breakneck speed. Genetic shifts related to seasonality subsequently have been documented in a number of species, including plants, birds, and mammals (Bradshaw and Holzapfel 2006). The accumulating documentation of rapid genetic shifts in the timing of seasonal events in diverse organisms means that, when confronted with climatic change, the initial evolutionary response

involves seasonal adaptation, usually mediated by photoperiodism, and that thermal adaptation is a secondary physiological response.

24.4.5 Genetic architecture of adaptive evolution

Response to selection depends upon underlying genetic variation (additive genetic variation) whose expression is independent of alleles at the same (dominance) and other (epistasis) loci. Cryptic genetic variation exists in populations due to masking by dominance and epistasis. This cryptic genetic variation can be unmasked by selection on, or genetic drift resulting from, inbreeding following population collapse or isolated colonizing events. At the same time, standing genetic variation can be enhanced by alleles that have opposing effects on fitness (antagonistic pleiotropy). Hence, understanding genetic architectures within and among natural populations can provide insights into the rates and modes of evolutionary change.

Genetic variation among populations of *W. smithii*, as represented by average heterozygosity at protein-coding loci, remains high from the Gulf of México to the Mid-Atlantic Region, and then declines with increasing latitude northwards. This decline in heterozygosity occurs frequently in many organisms and is interpreted as the consequence of sequential founder events, isolation, and drift following recession of the Laurentide Ice Sheet about 20,000 years ago. By marked contrast, genetic variability (additive genetic variation) underlying photoperiodic response increases exponentially over the same range (Armbruster et al. 1998). Additional crosses showed that directional epistasis (gene-gene interaction) contributes to the differences in photoperiod response over latitudinal and altitudinal gradients (Hard et al. 1993, Lair et al. 1997).

Hard et al. (1993) proposed that the increase in genetic variability with latitude in post-glacial populations resulted from the release of hidden additive variation from epistatic variation. However, for there to be a release of additive variation from epistatic variation, there must have been epistatic variation for photoperiodic response in the ancestral population. This proposition was tested by selecting for divergent critical photoperiod in three

subpopulations collected from within a 200-m radius within the New Jersey Pine Barrens (40 °N) and then testing for differences due to epistatic effects in the diverged lines.

Hybrids between the long and short critical photoperiod lines revealed directional epistasis in all three cases (Bradshaw et al. 2005), demonstrating that there was epistatic variation within the three original subpopulations. Importantly, the “finger-print” of digenic epistasis (additive \times additive, dominance \times dominance, and additive \times dominance effects) differed among the three diverged lines. This result means either that there is fine-scale genetic structure even within a local habitat, or that response to selection involves separate, heritable genetic trajectories. In a separate study, quantitative trait loci (QTL) mapping revealed multiple regions of all three *W. smithii* chromosomes that contributed to the evolution of photoperiodic response (Bradshaw et al. 2012). The number and position of the QTL in the genome varied within a northern population, between populations within the northern clade, and between the northern and southern clades. Therefore, extensive genetic polymorphism and multiple alternative genetic pathways to climatic adaptation occur over the range of *W. smithii* and raise the question as to whether analogous genetic flexibility underlies geographic divergence in other inquilines or the pitcher plants themselves.

These results demonstrate that the genetic basis for adaptive evolution crucially depends on genetic background: the genetic context in which genes are expressed. Further, the genetic basis for adaptive evolution of photoperiodic response is highly variable within contemporary populations and between anciently diverged populations. Occupation of the pitcher-plant habitat and genetic flexibility underlying photoperiodic response has enabled *W. smithii*, a member of an otherwise tropical and subtropical genus, to invade and adapt to the climatic gradient of North America from the Gulf of México to northern Canada.

24.5 Future research

Communities living within the leaves of *Sarracenia*, especially *S. purpurea*, have provided a myriad of opportunities for addressing fundamental

questions in ecology and evolution. The inquilines in *S. purpurea* have become a model system, in large part because of the widespread distribution of the host plant, the discrete patches in which it lives, the constancy of a suite of dominant species inhabiting it, and the tractability of the community and ecosystem for experiments.

At the scale of individual leaves, experiments have demonstrated that the community is structured by both consumer (“top-down”) and resource-supply (“bottom-up”) forces and that consumer and resource effects vary predictably with the age of the community. However, the ecology of many species, such as mites and midges, remains relatively unexplored, as are the interactions between the plant and its inquiline community.

Because of the different spatial scales created by leaves, plants, and plant populations, *Sarracenia* inquilines have been ideal for studying questions about dispersal and the structure of metacommunities. The variation in community composition among leaves within a site is much greater than the variation among sites, likely because of the large contribution of leaf age and successional stage to community structure. Biogeographic and evolutionary constraints are difficult to quantify, but experimental studies with pitcher plants and their inquilines will continue to exemplify how to document the importance of evolutionary history for understanding population and community patterns.

Wyeomyia smithii also has become a model system for studying the evolution of complex life histories. This species has migrated northwards and shows an evolutionary progression in stage and depth of winter dormancy (diapause) by using an evolutionarily flexible, genetically programmed response to day length that optimizes the timing of seasonal diapause and development. *Wyeomyia smithii* encounters severe density-dependent development in the southernmost portion of its range and declining densities with increasing latitude and altitude. Yet, *r*- and *K*-selection theory based on density is without predictive power over a 25-fold range of density in nature. Whether this applies to other pitcher-plant inquilines, or to other species with similarly broad geographic ranges remain open questions.

Studies of pitcher-plant mosquitoes were the very first to demonstrate a genetic (evolutionary)

basis for phenotypic response driven by recent climatic change. Photoperiodic response in *W. smithii* shows a genetic shift in response to recent rapid climate change in as few as five years. Photoperiodic adaptation is a demonstrably more important evolutionary response to the climatic gradient of North America than has been thermal adaptation. This evolutionary flexibility is based on complex genetic architectures and QTL that vary in their position within the genome within as well as between populations. This variability highlights the importance of genetic background when evaluating the genetic foundations of evolutionary adaptation. Pitcher plants and their associates can provide the means to elucidate evolutionary patterns over multiple time scales, ranging from within-season evolution, over 5–25 years, post-glacial timescales of 20,000 years or less, and more ancient ancestral divergence over millennial time.

Scientists invested in a specific system often attempt to use that system for answering broader questions for which the system may be ill-suited. However, a large number of questions in modern biology are addressable with pitcher plants and their inquilines. The associated biota of pitcher plants range from microbes, protozoa, and rotifers to arthropods with complex life histories, and span multiple trophic levels, including herbivores of the host plant itself. Pitcher plants provide endless opportunities to address and resolve ever newer and developing broad questions in ecological, evolutionary, and genetic contexts at multiple levels of biological integration from molecules to ecosystems, and across a wide range of spatial and temporal scales. This microecosystem certainly will continue to be an important model for experimentally testing new concepts, theories, and questions in genetics, evolution, and ecology for the foreseeable future.